BREAST AND PROSTATE CANCER II

Organizers: Marc Lippman, Robert Dickson and John Isaacs

March 14 - 20, 1994; Lake Tahoe, California

Sponsored by Abbott Laboratories and OncQuest, Division of Specialty Laboratories, Inc.

Plenary Sessions *Page*

March 15
- Growth Factors I 220
- Growth Factors II 220

March 16
- Polypeptide Receptors 222
- Steroid Receptors 222

March 17
- Metastasis and Angiogenesis 223
- Cell Cycle/Paracrine Interactions 224

March 19
- Diagnosis, Treatment, and Prevention I 225
- Diagnosis, Treatment, and Prevention II 226

- Late Abstracts 227

Poster Sessions

March 15
- Growth Factors I and II (Y100-128) 228

March 16
- Polypeptide Receptors; Steroid Receptors (Y200-222) 235

March 17
- Metastases and Angiogenesis; Cell Cycle/Paracrine Interactions (Y300-318) 241

March 18
- Molecular Genetics (Y400-415) 246

March 19
- Diagnosis, Treatment, and Prevention I and II (Y500-513) 250

- Late Abstracts 254

Breast and Prostate Cancer II

Growth Factors I

Y 001 PROSTATIC CANCER CELL MOTILITY AND METASTASIS, James L. Mohler[1], Alan W. Partin[2], Yousuf Sharief[1], William E. Bakewell[3], Donald S. Coffey[2], and Gary J. Smith[3], [1]Division of Urology, Department of Surgery, [3]Department of Pathology and the UNC Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599 and [2]Brady Urological Institute, The Johns Hopkins University, Baltimore.

Metastases are clinically important because they prevent neoplasm ablation by operation or radiotherapy. Common to many steps of the metastatic process is a requirement for motility that cannot be appreciated by standard pathologic examination of fixed, dead tissues. The Dunning R-3327 rat prostatic adenocarcinoma model provides sublines whose biological behavior is not predictable by histology or numerous biochemical or morphological techniques. Visual grading of time-lapse videomicroscopic images distinguished in 96% of cases sublines of high and low metastatic capability when membrane ruffling, pseudopodal extension and retraction and cellular translation of single cells were analyzed. When prospectively tested in 11 Dunning sublines, highly metastatic cells were detected with a 94% sensitivity and 50% specificity. The methodology was applied successfully to incisional biopsies and fine needle aspirations of in vivo tumors. A more quantitative analysis of cancer cell motility was developed using complex fast fourier analysis. The motility of Dunning sublines tested was enhanced by application of autocrine motility factor or serum-free media conditioned by the highly motile, highly metastatic MAT-LyLu subline. However, of greater interest was the reduction in motility of MAT-LyLu cells by media conditioned by the non-motile, non-metastatic G subline. This activity was heat and protease labile and proved by dialysis due to non-reduced 50-100 kD proteins. G-conditioned serum-free media was separated into 100 fractions upon a DEAE-cellulose column eluted with 100 mM Tris at pH 8 with a 0-0.5 M NaCl gradient. Fractions 51-70 decreased the displacement of MAT-LyLu cells by 72%. Autoradiography of denaturing two-dimensional electrophoretic gels of media fractions conditioned by G cells fed radioactive sulfur-methionine containing serum-free media demonstrated that 90.3% of synthesized proteins were contained in fractions ≤ 50. Fractions 51-60 and 61-70 after superconcentration by desiccation and 3 weeks of exposure appeared to contain five "families" of proteins of 66, 52, 50, 44 and 38 kD. Isolation and identification of motility inhibitory protein may prove it the first substance discovered that is produced by a more differentiated component of a neoplasm that directly inhibits a metastasis-associated property.

Y 002 EPIDERMAL GROWTH FACTOR-RELATED PEPTIDES IN BREAST CANCER, David S. Salomon[1], Ralf Brandt[1], Fortunato Ciardiello[2], and Nicola Normanno[1,2], [1]Tumor Growth Factor Section, Laboratory of Tumor Immunology & Biology, NCI, Bethesda, MD 20892, [2]Oncologia Spermentale D, Instituto Nazionale per lo Studio e la Cura dei Tumori-Fondazione Pascale and Cattedra di Oncologia Medica, Facolta' di Medicina e Chirurgia, Universtita' degli Studi di Napoli Federico II, 80131 Napoli, Italy.

The epidermal growth factor (EGF) family includes a number of proteins that are structurally related and that include growth factors such as EGF, heparin-binding EGF (HB-EGF), transforming growth factor α (TGFα), amphiregulin (AR), betacellulin, heregulin α and β (HRG) and cripto-1 (CR-1). All of these growth factors with the exception of the HRGs and members of the CR-1 subfamily bind to and activate the EGF receptor (EGF-R). An autocrine role has been formally demonstrated for estrogen-inducible growth factors such as TGFα in several different human breast cancer cell lines and in c-Ha-*ras* transformed MCF-10A human mammary epithelial cells. TGFα can act as a dominant transforming gene in MCF-10A mammary epithelial cells. AR can function as an autocrine growth factor in the nontransformed human mammary epithelial cell line 184A1N4 (A1N4). An antisense phosphorothioate oligodeoxynucleotide (S-oligo) directed against the 5′ end of the human AR mRNA was able to significantly inhibit the growth of A1N4 cells. AR is expressed in a number of human breast cancer cell lines. ER positive cell lines generally express higher levels of AR when compared to the ER negative cell lines. In addition, estrogen treatment induces an ~ 15- to 20-fold increase in the expression of AR mRNA in MCF-7 breast cancer cells. AR is overexpressed in both c-Ha-*ras* and c-*erb* B2 transformed MCF-10A cells. AR mRNA expression is induced by EGF in both parental and oncogene transformed MCF-10A cells with the transformed cells showing levels of AR mRNA and protein ~ 15- to 30-fold higher than the parental cells. Inhibition of both anchorage-dependent and anchorage-independent growth of the oncogene transformed MCF-10A cells could be achieved by using an AR antisense S-oligo. AR protein is expressed in 77% of human primary breast carcinomas as detected by immunocytochemical analysis and in 43% of noninvolved mammary epithelium adjacent to carcinoma but at much lower levels when compared to the carcinomas. The pattern of staining was mostly cytoplasmic, but nuclear and occassionally nucleolar staining was observed. CR-1 can function as a transforming gene since overexpression of this protein in mouse NOG-8 or MCF-10A mammary epithelial cells leads to their ability to form colonies in soft agar. Both CR-1 mRNA and protein are expressed at a high level in a majority of primary human breast carcinomas and in several human breast cancer cell lines. The expression of CR-1 mRNA has been detected in MCF-7, ZR-75-1, T47-D, MDA-MB-231, and MDA-MB-468 human breast cancer cells. The CR-1 protein could be immunocytochemically detected in the same breast cancer cell lines that were expressing CR-1 mRNA. Within 68 breast carcinomas, 54 (79%) expressed TGFα and 56 (82%) expressed the CR-1 protein. CR-1 has been detected in only 3 cases (13%) of adjacent , noninvolved breast tissue while TGFα and AR are expressed in 26% and 43% of these tissues, repectively. These data suggest that the differential expression of CR-1 in malignant breast epithelial cells may serve as a potential tumor marker for breast cancer.

Growth Factors II

Y 003 Growth Factor-Sex Steroid Interactions in Breast Tumorigenesis, Robert B. Dickson[1], Laufey T. Amundadottir[1], Isabel Martinez-Lacaci[1], Michael D. Johnson[1], Gregory Plowman[2], Miguel Saceda[1], Nicola Normanno[3], Gilbert Smith[3], David S. Salomon[3], and Glenn T. Merlino[4], [1] Lombardi Cancer Research Center, Georgetown University, [2] Bristol Myers Squibb, Seattle, WA, [3] Lab. of Tumor Immunology and Biology, NCI, NIH, Bethesda, MD, [4] Lab. of Molecular Biology, NCI, NIH, Bethesda, MD

The steroid hormones estrogen and progesterone may interact on multiple levels with growth factors in the onset and progression of breast cancer. Initial observations that estrogen and progesterone modulate breast cancer cell growth in association with TGFα induction have now been extended to include the structurally related growth factor amphiregulin (AR). AR regulation appears to be similar to that of TGFα in MCF-7 breast cancer cells, but regulation is more marked: induction is 3-6-fold by estradiol and 6-8-fold by the tumor promoter TPA. Induction by both agents appears to be at least partly at the transcriptional level. Other investigators have shown that estrogen and progesterone also rapidly induce expression of c-myc protooncogene. Induction of TGFα and c-myc have been shown by others with antisense methodology to be at least partially necessary for estrogen-induced growth of MCF-7 cells. We have further analyzed the hypothesis that disregulated expression of TGFα and c-myc are sufficient to allow mammary tumorigenesis with a new bitransgenic mouse model of mammary cancer. We carried out a heterozygous cross of MT-100 mice (expressing a metallothioneine promoter driven TGFα construct) and MMTV-c-myc mice (expressing an MMTV-promoter driven c-myc gene, provided by P. Leder, Charles River and Dupont). We observed glandular hyperplasia and bilateral mammary tumors in 100% of both female and male offspring expressing both transgenes. Tumors occurred in the absence of prior pregnancy, had a latency of approximately 10 weeks, were rapidly progressing and appeared to be locally invasive. Thus, two sex steroid induced genes appeared to substitute for the steroids themselves in induction of hyperplastic growth and mammary tumors. In a second heterozygous cross we mated MT-100 mice with MMTV-neu, (expressing an MMTV-promoter driven neu gene, provided by P. Leder, Charles River and Dupont). In striking contrast to the results described above, no mammary tumors were observed in males or virgin females expressing both transgenes over a 6 month period. These data begin to define the scope of cooperation of growth factor receptors and nuclear oncogenes in mammary tumorigenesis.

Y 004 HB-EGF EXPRESSION IS DECREASED AND VEGF IS INCREASED IN THE PROGRESSION OF NORMAL TO TRANSFORMED PROSTATE EPITHELIA. Michael R. Freeman, Toshi Uchida, Shay Soker, Sy Blotnick, Gerhard Raab, and Michael Klagsbrun, Children's Hospital/Harvard Medical School, Boston, MA 02115.

Cytokines which bind to heparin or heparan sulfate proteoglycan (HSPG) with high affinity might act either as conventional soluble growth or regulatory factors or, alternatively, might be functional in the insoluble state by binding to extracellular matrix or being cell surface bound. Heparin-binding growth factors of the fibroblast growth factor (FGF) family have been implicated in mechanisms of autocrine and paracrine growth regulation and stromal-epithelial interaction in prostatic cells. We have studied two recently-described growth factors with affinity for immobilized heparin: heparin-binding EGF-like growth factor (HB-EGF) and vascular endothelial growth factor (VEGF). HB-EGF stimulates the EGF receptor and is a potent mitogen for smooth muscle cells, fibroblasts and epithelial cells. VEGF is a specific endothelial cell mitogen and an angiogenic factor which may be involved in the transition between the pre-vascular and vascular phases of tumor development. Both growth factors require binding to HSPG for optimal bioactivity. We have determined that cultured prostatic epithelial cells which exhibit a differentiated (barrier-forming) phenotype express high levels of a cell-associated form of HB-EGF. Secretion of biologically active HB-EGF can be rapidly induced in prostatic epithelial cells by protein kinase C (PKC) activation, suggesting that PKC pathways mediate proteolytic processing of membrane-bound proHB-EGF. Treatment of the cells with phorbol esters also induces HB-EGF mRNA expression dramatically over a two hour period, indicating that HB-EGF is regulated as an early response gene in normal prostatic epithelia. On the other hand, poorly differentiated prostatic tumor cells exhibit low or undetectable levels of HB-EGF protein, comparatively low levels of HB-EGF mRNA, and a poor response at the mRNA synthesis and secreted protein levels to PKC activation. These results suggest that malignant transformation of prostatic epithelial cells results in down-regulation of HB-EGF activity at multiple levels. In contrast, VEGF mRNA expression levels were found to be low in differentiated prostatic epithelial cells and extremely high in invasive, poorly differentiated prostatic tumor cells. Accordingly, we hypothesize that HB-EGF is expressed primarily in the membrane-bound precursor form in differentiated or non-transformed epithelial cells where it might act in a juxtacrine fashion to maintain or promote differentiated epithelial function. Loss of cell-surface HB-EGF may consequently de-stabilize the epithelial phenotype. In contrast, VEGF expression occurs in an inverse fashion to HB-EGF. It is increased with malignant transformation and might be secreted, potentially at high levels, by malignant cells. Therefore, coordinate changes in HB-EGF and VEGF expression in prostatic carcinoma cells might promote the loss of epithelial properties such as cell-cell adhesion, and simultaneously, increase the likelihood of capillary ingrowth and vascularization of prostatic tumors.

Y 005 GROWTH FACTORS IN THE PROSTATE: AN *IN VITRO* MODEL, Donna M. Peehl[1], Pinchas Cohen[2], and Ron G. Rosenfeld[3], [1]Stanford University, Stanford, [2]University of Pennsylvania, Philadelphia and [3]Oregon Health Science Center, Portland.

The human prostate differs considerably from the prostates of most animals in embryologic development, adult anatomy, and spectrum of disease. Therefore, we have emphasized the study of human material, and cell culture is perhaps our most versatile tool for analyzing biological parameters of growth and differentiation. During the past 10 years, significant improvements in cell culture methods for adult human prostatic cells have occurred such that it is now possible to readily culture pure populations of epithelial or stromal cells from normal or malignant tissues. The availability of an optimized culture system has permitted rigorous quantitative and qualitative analyses that were formerly impossible, and has provided numerous experimental advantages. We have used this culture system to identify autocrine, paracrine, and endocrine factors involved in regulation of growth and differentiation of prostatic stromal and epithelial cells. Factors recognized for their growth-inhibitory effects on prostatic epithelial cells include transforming growth factor-β, vitamin D, and retinoic acid. Of these, retinoic acid is perhaps unique in its ability to promote differentiation in conjunction with growth inhibition. Growth-stimulatory factors include members of the epidermal growth factor (EGF) family and fibroblast growth factors (FGF). EGF-like factors appear to be important autocrine factors in the epithelial compartment of the prostate, whereas a member of the FGF family, keratinocyte growth factor, is a unique androgen-regulated mediator of paracrine interaction between the prostatic stroma and epithelium. Recently, we have begun to address the role of another important system of growth-regulatory factors, the insulin-like growth factor (IGF) system, in the prostate. We have characterized the expression of the peptide factors, IGF-I and -II; the IGF-binding proteins (IGF-BP); the IGF type-I and -II receptors; and IGF-BP proteases in stromal and epithelial cell populations. An intricate system of potential autocrine, paracrine, and systemic interactions has been revealed, and a hypothesis regarding the role of the IGF system in metastatic prostate cancer will be presented.

Y 006 NEU DIFFERENTIATION FACTORS (HEREGULINS): FROM AN ONCOGENE TO A FAMILY OF NEURONAL AND MESENCHYMAL FACTORS, Yosef Yarden, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

The *neu*/HER-2 gene (also called *erb*B-2) is overexpressed in certain human adenocarcinomas and appears to contribute to their malignancy. The gene encodes a transmembrane receptor tyrosine kinase that belongs to the epidermal growth factor (EGF) receptor family. A rodent oncogenic form of the receptor contains a point mutation, that results in constitutive activation of the kinase and a consequent permanent coupling to cytoplasmic signaling pathways. This is mediated by five tyrosine autophosphorylation sites, out of which the most carboxy terminal (Tyr_{1253}) is sufficient to confer oncogenicity by coupling to an intracellular pathway that includes phospholipase C_γ, MAP-kinase and transactivation of c-Jun. The search for ligands that activate tyrosine phosphorylation of Neu led to the isolation and molecular cloning of a 44-kDa glycoprotein, termed Neu differentiation factor (NDF, or heregulin). NDF induces either mitogenesis or differentiation of mammary tumor cell lines but it does not bind to fibroblastic or ovarian cells that express *neu*/HER-2. This is due to the absence of another receptor tyrosine kinase of the EGF-receptor family, most likely the product of the *erb*B-4/HER-4 gene. The precursor of NDF is a transmembrane molecule containing several recognizable motifs, including an EGF-like domain, which functions as a receptor binding site. At least twelve different isoforms of proNDF exist, and their structural variation is confined to a stalk that connects the EGF-like domain with the transmembrane domain and undergoes proteolysis. Tissue-specific alternative splicing generates two groups of proNDF isoforms, that are enriched in either mesenchymal cells or in neuronal tissues, and probably perform distinct physiological functions. Certain splicing variants are expressed in human adenocarcinomas but their relevance to cancer development is still unclear. Our preliminary attempts to study this question will be discussed.

Polypeptide Receptors

Y 007 EXPRESSION OF THE C-*ERB*B-3 PROTEIN IN HUMAN TISSUES AND TUMOURS AND ITS INTERACTION WITH INTRACELLULAR SECOND MESSENGER SYSTEMS, William J. Gullick, Thangarajan Rajkumar and Sally A. Prigent, ICRF Oncology Unit, Hammersmith Hospital, Du Cane Road, London W12 0NN, U.K.

The c-*erb*B-3 protein is a member of the type 1 family of growth factor receptors. Much evidence has been presented that two of the other members of the family, EGF receptor and c-*erb*B-2, play a role in normal epithelial cell behaviour and in tumour development. We have therefore explored i) whether c-*erb*B-3 is also a growth regulatory receptor and ii) if there is any evidence for alterations in its structure or level of expression in human tumours. No ligand has yet been identified which binds to the c-*erb*B-3 protein. To facilitate ligand stimulation we therefore constructed a chimeric receptor which possesses an activatable kinase and promotes the growth of NIH 3T3 fibroblasts. Two of the proteins involved in c-*erb*B-3 signalling were identified. We have shown that upon stimulation the EGF receptor/c-*erb*B-3 chimeric protein physically associates with SHC and the p85 subunit of phosphatidylinositol 3-kinase, and that phosphatidylinositol 3-kinase activity is found in immune complexes from stimulated cells expressing the EGF receptor/c-*erb*B-3 chimera. p85 association was inhibited by two c-*erb*B-3 phosphopeptide sequences containing the motif Y(P)XXM. Whereas p85 is not phosphorylated to a significant extent, SHC appears to be a major substrate phosphorylation on tyrosine. In contrast to EGF receptor and c-*erb*B-2, we were unable to detect binding of activated c-*erb*B-3 to GRB2. We conclude the c-*erb*B-3 is likely to be a growth factor receptor and that the signalling pathways of c-*erb*B-3, EGF receptor and c-*erb*B-2 share some common features but differ in their interaction with GRB2. We have also explored the biological activity of the full length c-*erb*B-3 protein by raising a monoclonal antibody to its extracellular domain. This reacts specifically with live cells and can promote the growth of breast cancer cell lines expressing c-*erb*B-3, but does not affect the growth of cells lacking expression. In order to examine the expression of c-*erb*B-3 in normal tissues and tumours we have developed several polyclonal and monoclonal antibodies to synthetic peptides from the cytoplasmic domain of the protein. These react with c-*erb*B-3 in immunoprecipitations, Western blotting and in formalin-fixed paraffin-embedded tissues. We have surveyed the expression of the protein on normal fetal and adult tissues (Oncogene 1992, 7, 1273). Expression was found on several tissue types but interestingly was predominantly on differentiated non-dividing epithelial cells. The protein was apparently overexpressed on approximately 20% of breast, 30% of bladder and 35% of ovarian cancers. Overexpression was also found frequently on pancreatic and other G1 tract tumours. We are currently extending this study to cervical, prostate and lung cancers.

Y 008 ANTITUMOR EFFECTS OF ANALOGS OF LH-RH, SOMATOSTATIN AND BOMBESIN/GRP IN EXPERIMENTAL MODELS OF BREAST AND PROSTATE CANCER, Andrew V. Schally, Jacek Pinski, Karoly Szepeshazi, Tetsu Yano, Gabor Halmos, Yutaka Shirahige, Ana Maria Comaru-Schally, Ren-Zhi Cai, and Attila Nagy. VA Medical Center and Tulane University School of Medicine, New Orleans, LA 70146.

Attempts are being made to improve the methods for the treatment of sex-steroid dependent and independent breast cancer and prostate cancer by using various peptide analogs and their combinations to inhibit growth factors or their receptors. The growth of MXT estrogen-dependent mammary tumors in mice is inhibited by microcapsule formulations of the agonist D-Trp-6-LH-RH, and modern LH-RH antagonist SB-75, which produce sex-steroid deprivation and by somatostatin analog RC-160. Antagonist SB-75 caused the greatest inhibition. Combination of D-Trp-6-LH-RH with somatostatin analog RC-160 was more potent than single agents. In MXT estrogen-independent breast cancer, combination of D-Trp-6-LH-RH or SB-75 with RC-160 potentiated the reduction in tumor growth. The growth of MCF-7 MIII human breast cancer in nude mice was virtually arrested by administration of D-Trp-6-LH-RH, SB-75 or RC-160. Bombesin stimulates in vitro growth of MCF-7 MIII and MDA-MB 231 human breast cancer cell lines. Bombesin/GRP antagonist RC-3095 inhibits in vitro proliferation of these cells and in vivo growth of xenografts of these cancers in nude mice. Antagonist RC-3095 also powerfully inhibited the growth of MXT estrogen-independent breast cancers, possibly through the down-regulation of EGF receptors. Agonists and antagonists of LH-RH, containing cytotoxic radicals such as methotrexate and anthraquinone, bind to LH-RH receptors on breast cancers and are active in vivo as shown by growth inhibition of MXT estrogen-independent breast cancers in mice. LH-RH antagonist SB-75 causes an immediate and complete suppression of pituitary-gonadal axis in male rats and in men and appears to be superior to LH-RH agonists. Microcapsules of antagonist SB-75 powerfully inhibited the growth of Dunning R-3327H hormone-dependent prostate cancers in rats and PC-82 human prostate cancers in nude mice. Combination of LH-RH antagonist SB-75 or agonist D-Trp-6-LH-RH with analog RC-160 inhibits growth of these cancers more powerfully than either analog alone. The combination of bombesin/GRP antagonists with LH-RH agonists also had a greater inhibitory effect on tumor growth than single peptides. Somatostatin analog RC-160 and bombesin/GRP antagonist RC-3095 inhibited growth of androgen-independent Dunning R-3327-AT prostatic cancer in rats and DU-145 and PC-3 human prostate cancers in nude mice. Antagonist SB-75 also significantly prolonged the survival of rats bearing orthotopic implants of R-3327AT-1 tumors. Receptors for EGF were significantly down-regulated by treatment with the bombesin antagonist RC-3095 or somatostatin analog RC-160. Cytotoxic LH-RH analogs bind to LH-RH receptors on prostate cancers and inhibit growth of Dunning R-3327H androgen-dependent prostate cancers in rats, indicating that selective targeting is possible. Continued investigation of these analogs and their mechanisms of action and parallel clinical trials may lead to new therapeutic approaches.

Steroid Receptors

Y 009 **ABNORMAL ANDROGEN RECEPTOR EXPRESSION AND GENE STRUCTURE IN HUMAN PROSTATE CANCER.** **Evelyn R. Barrack, Department of Urology, The Johns Hopkins University School of Medicine, Baltimore, MD 21287-2101**

Reliable predictors of response for prostate cancer patients undergoing hormonal therapy are lacking. Since 80-90% of such patients do respond but the time interval between therapy and relapse (progression) is variable, our goal has been to predict the time to tumor progression following therapy rather than simply to predict whether a patient would respond. Based on androgen receptor (AR) immunohistochemistry, we found that the percentage of AR-positive malignant epithelial nuclei in stage D2 prostate cancer biopsies does not correlate with the time to tumor progression following androgen ablation therapy (Sadi et al., Cancer 67:3057, 1991). These and other data support the conclusion that the presence of AR is not a specific marker of androgen dependence. Consistent with this conclusion is the recent report that hormone refractory (i.e., androgen-independent) prostate cancers are predominantly and extensively AR-positive (van der Kwast et al., Int. J. Ca. 48:189, 1991). On the other hand, the intratumor AR concentration per cell is significantly more heterogeneous in patients with a shorter time to tumor progression after hormonal therapy than in patients with a longer time to progression, and the variance of the nuclear AR immunostaining intensity within a specimen appears to be a valuable predictor of the time to progression following therapy (Sadi and Barrack, Cancer 71:2574, 1993). This heterogeneity may be a manifestation of greater genetic instability in poor responders, and an indication that the tumor has progressed further towards androgen independence, thereby resulting in a shorter time to progression. Since the AR gene is located on the X chromosome, the heterogeneity of AR concentration may be due to X chromosome aneuploidy, which has recently been reported to occur in prostate cancer (Sesterhenn et al., J. Urol. 147:215A, 1992). Genetic instability, as reflected by the development of aneuploidy, which is a common feature of prostate cancer, may also result in AR gene mutations. AR gene mutations have indeed been documented in prostate cancer, first in the LNCaP human prostate cancer cell line (Veldscholte et al., BBRC 173:534, 1990). More recently, using the polymerase chain reaction and denaturing gradient gel electrophoresis to screen for the presence of mutations in the hormone binding domain of the AR gene in prostate cancer tissue, our laboratory identified a somatic mutation in a stage B prostate cancer specimen (the mutation was absent in peripheral lymphocytes of the same patient) (Newmark et al., PNAS 89:6319, 1992). This mutation, located in a region of the hormone binding domain that is highly conserved among members of the steroid receptor superfamily, alters the amino acid sequence. This raises the possibility that AR gene mutations may alter AR function and thereby affect the cancer cell phenotype. Much work remains to be done to fully elucidate the role and function of the AR in human prostate cancer. [Supported by NCI, CA16924 and CA58236.]

Y 010 AGONIST EFFECTS OF ANTAGONIST-OCCUPIED PROGESTERONE B-RECEPTORS: NEW MODELS OF HORMONE-RESISTANT BREAST CANCER, Kathryn B. Horwitz, Departments of Medicine and Pathology, and the Molecular Biology Program, University of Colorado School of Medicine, Denver, CO 80262

When antagonists have agonist-like effects, the clinical consequences are grave. I will discuss such effects with antiprogestins and normal progesterone receptors (PR). Human PR occur naturally as two isoforms -- 94 kDa A-receptors (hPR_A) and 120 kDa B-receptors (hPR_B) -- which form two homodimers (A:A and B:B) and one heterodimer (A:B). The dimers bind to DNA at progesterone response elements (PRE). B-receptors have a 164 aa N-terminal extension that we call the B-Upstream Segment (BUS). In transient expression systems, antagonist-occupied B-receptor homodimers stimulate transcription from a *tk*-CAT reporter in a PRE-independent manner, and from an MMTV-CAT reporter when cAMP levels are increased. By contrast, antagonist-occupied A-receptor homodimers are transcriptionally silent. In A:B heterodimers, A-receptors dominantly suppress the agonist-like effects of antagonist-occupied B-receptors. To show that these anomalous responses to antagonists persist when receptors are expressed at physiological levels, we developed new breast cancer cell lines that have only one receptor isoform. First, a monoclonal PR-negative T47D cell line (T47D-Y) was selected by flow cytometry. This cell line was then stably transfected with plasmids containing either hPR_A or hPR_B cDNAs, to produce cells expressing only A-receptors (YA) or B-receptors (YB). As in the transient systems, in these stable models, antagonists have inappropriate agonist-like transcriptional effects in YB cells but not YA cells. These cells also allow the study of long-term hormone effects on the mitotic cell cycle using flow cytometry. In YA and YB cells the agonist R5020 doubles the percent of cells in S-phase after 16 hours. Progesterone antagonists do not increase S-phase, and inhibit the increase produced by R5020. However, when cAMP levels are raised, the antagonist RU486 becomes an agonist -- it doubles the percent of cells in S-phase in YB cells but not in YA cells. To explain these unique properties of B-receptors we constructed expression vectors linking BUS to the DNA binding domain and nuclear localization signal of hPR (BUS-DBD-NLS). Transient transfection studies using a PRE_2-TATA-CAT promoter-reporter, show that BUS-DBD-NLS is a strong constitutive transactivator, stimulating CAT to levels comparable to those of full-length hPR_B. BUS-DBD-NLS stimulates transcription only when BUS is bound to DNA since no transactivation is obtained with BUS-NLS lacking the DBD, or with the DNA binding mutant, $BUS\text{-}DBD_{cys}$-NLS. The DBD alone has no intrinsic activation function, since a DBD-NLS fragment is transcriptionally inactive. The transcriptional activation function of BUS appears to be regulated by phosphorylation of *ser-pro* motifs located on this domain of B-receptors. CONCLUSIONS: The agonist-like effects of progesterone antagonists, as assessed by transcription or by growth analyses in new cell models, are restricted to the B-receptors. These inappropriate effects of B-receptors may be mediated by a third transactivation domain, TAF-3, that is activated by antagonists, and is missing in A-receptors. Inappropriate transcriptional activation by antagonists may account for "resistance" in breast cancer, or for their "agonist" actions in some normal tissues.

Y 011 GENE ACTIVATON BY ANTIESTROGENS VIA A COMPLEX OF ESTROGEN RECEPTOR AND AP-1, Paul Webb, Gabriela N. Lopez and Peter J. Kushner, Metabolic Research Unit, University of California, San Francisco, CA 94143.

Antiestrogens, such as tamoxifen and ICI 164,384 (ICI), are usually pure antagonists of estrogen action when tested on genes regulated by classical palindromic estrogen response elements (EREs). Nevertheless, antiestrogens sometimes exhibit potent, puzzling, and unexplained estrogen-like effects. Tamoxifen, for example, has potent estrogenic activity on growth and gene transcription in endometrial cells. These agonist effects of antiestrogens complicate their clinical use.

Here, we show that the human collagenase gene promoter exhibits an estrogen response in transfected mammalian cell lines mediated by the AP-1 site at -70 b.p. from the start of transcription. The AP-1 site binds the Jun/Fos proteins but does not bind ER. The estrogen response mediated by an AP-1 site can be as strong as that mediated by an ERE and can occur at physiological levels of ER found in endometrial cell lines This "indirect" estrogen response at AP-1 sites may, therefore, be a major pathway of estrogen action. Remarkably, antiestrogens are agonists at the collagenase AP-1 site, whereas they lack agonist activity when tested on a promoter in which the AP-1 site has been replaced by an ERE. The agonist activity of antiestrogens does not require the ER DNA binding domain but does require both the N-terminal and C-terminal domains. Transfection of F9 cells, which lack Jun/Fos, indicates that the indirect estrogen response requires Jun and Fos proteins as well as estrogen receptor (ER). The Jun-ER ratio, but not the Fos-ER ratio, is critical to the response. Moreover, either full length or C-terminal domain of the ER binds Jun, but not Fos, protein in solution. These observations suggest that the indirect response may occur when ER "piggybacks" on Jun/Fos, which are themselves bound to the AP-1 site. In support of this model we find that fusing an exogenous activation domain (Herpes virus VP16) to the ER creates a super-receptor that is more potent than wild type ER as an activator at AP-1 sites. We suggest that antiestrogen agonism through AP-1 sites may underlie the estrogen-like effects of these compounds that is sometimes observed in vivo.

Metastasis and Angiogenesis

Y 012 INVOLVEMENT OF STROMAL PROTEINASES IN TUMOR PROGRESSION, Paul Basset [1], Nicolas Rouyer [2], Catherine Wolf [1], Jean-Pierre Bellocq [2], Marie-Christine Rio [1], and Pierre Chambon [1]. [1] Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de l'INSERM, Institut de Chimie Biologique, Faculté de Médecine, and [2] Service d'Anatomie Pathologique Générale, Centre Hospitalier Universtaire, Strasbourg, France.

It has been long postulated that a number of secreted proteinases, particularly those of the matrix metalloproteinase family and those of the plasmin system, could be involved in the progression of human carcinomas (1). It is believed that these proteinases degrade the extracellular matrix components such that cancer cells can migrate through the basement membrane, and fill the interstitial spaces liberated by proteolysis. However, the recent observation that several of the proteinases implicated in tumor progression were not produced by cancer cells but by cells of the tumor stroma, suggests that extracellular proteinases may also contribute to stroma formation. In particular, interstitial type I collagenase, gelatinase A, urokinase and stromelysin-3, which are expressed in fibroblastic cells of tumor stroma, could be involved in the accumulation of these cells in breast and other human carcinomas (2-5). The production of mouse lineages in which the genes corresponding to these proteinases have been disrupted by homologous recombination, together with a better characterization of their enzymatic activities *in vivo*, will be helpful in further defining the role of extracellular proteinases during tumor progression. This, and a clarification of the relationships between stromal and cancer cells in human carcinomas, should assist in developing new classes of anti-cancer agents with which the non-cancerous stromal cells, rather than the cancer cells, would be targeted.

1. Liotta LA, Nageswara RC, Wewer UM. *Ann Rev Biochem* 1986 ; 55 : 1037-1057.
2. Basset P, Bellocq JP, Wolf C, et al. *Nature* 1990 ; 348: 699-704.
3. Pike C, Kristensen P, Ralfkiaer E, et al. *Am. J. Pathol.* 1991 ; 138: 1059-1067.
4. Poulsom R, Pignatelli M, Stetler-Stevenson WG, et al. *Am. J. Pathol.* 1992 ; 141: 389-396.
5. Wolf C, Rouyer N, Lutz Y, et al. *Proc. Natl. Acad. Sci. USA.* 1993 ; 90: 1843-1847.

Y 013 E-CADHERIN EXPRESSION IN PROSTATE CANCER. Jack A Schalken[1], Rayne Umbas[1], Laurence Giroldi[1], Pierre Paul Bringuier[1], Tilly W Aalders[1], Adri van Bokhoven[1], Frank Smit[1], William B Isaacs[2] and Marion JG Bussemakers[2], [1] University Hospital Nijmegen, NL-6500 HB Nijmegen, The Netherlands, [2] Johns Hopkins Hospital, Baltimore, MD 21205.

Prostate cancer is the most prevalent form of cancer among western males and its incidence is still rising. An adequate determination of treatment strategies is hampered by the fact that the biological potential of the prostatic lesions varies tremendously, ie some progress rapidly resulting in the death of the patient, while others remain dormant for many years. Clearly, a better understanding of the molecular cascade that is associated with acquisition of invasive and metastatic ability is necessary to improve diagnosis. In this respect the cadherins are of special interest. These homotypic-homophilic cell-cell adhesion molecules are known to play an important role in maintenance of tissue integrity. Moreover, it was shown that loss of expression of the epithelial cadherin (E-cadherin) was causally related to acquisition of invasiveness (Behrens *et al.*, JCB, 108, 2435; Vleminx *et al.*, Cell, 66, 107), and therefore, E-cadherin was considered an invasion suppressor gene. This and the fact that the chromosomal segment to which this gene is mapped (16q21) is frequently lost in prostate cancer development (Carter *et al.*, PNAS, 87, 8751;Bergerheim, Genes Chrom Cancer, 3, 215) prompted us to investigate the role of E-cadherin in prostate cancer progression. The fact that in a model system for prostate cancer progression E-cadherin expression was lost in all invasive tumour lines (Bussemakers *et al.*, Cancer Res, 52, 2916) further indicated an important role of E-cadherin in the progression of prostate cancer. In studies of human cancer we choose for an immunohistochemical approach, and found that decreased expression of E-cadherin (heterogeneous, cytoplasmic staining) correlated well with tumour grade (Umbas, *et al.*, Cancer Res, 52, 5104). Similar findings were reported for bladder, mammary and other carcinoma's. More recently we finished a study on the prognostic value of decreased E-cadherin expression. Even though the mean follow up time is short (4 years) the differences are striking even within the group of organ confined lesions that were removed radically. Of 12 patients that progressed, despite the radical surgery, eleven had an aberrant E-cadherin expression, while from the group of patients with a normal staining (27), only one progressed. We therefore strongly recommend a prospective trial to evaluate this observation in a large group of patients.

The mechanism that leads to the aberrant expression of E-cadherin is still not clear. In lesions showing LOH on 16q21 one might expect mutational inactivation of the remaining allele to be an important step, however this was found only in very few cases. Alternatively, allelic loss or transcriptional inactivation may lead to reduced dosage which in turn could lead to impaired E-cadherin mediated interaction. Finally, dysfunction of the attachment to the cytoskeleton through catenins, can occur as was found by Morton *et al.* (Cancer Res, 53, 3585). Even though the mechanism that leads to loss of E-cadherin mediated adhesiveness is not yet clear, these data strongly suggest that this is an important step in the aggressive behaviour of (prostate) cancer cells. Hence the usefulness as prognostic marker should be tested and the possibility as therapeutical target exploited.

Y 014 CLONING OF A HUMAN GENOMIC TIMP-1 REGULATORY ENHANCER ELEMENT FROM PROSTATIC PC-3 CLONES. Stearns, M.E. and Wang. Department of Pathology, Medical College of Pennsylvania, Philadelphia, PA 19129.

The tissue inhibitors of metalloproteinases (TIMPs 1/2) may be tumor suppressor genes which block invasion by malignant tumor cells. We have postulated that a trans-acting protein which normally regulates TIMP-1 expression might be inactivated in metastatic tumor cells. In testing this hypothesis we have cloned the TIMP-1 gene from human prostatic PC-3 cells and subcloned various 5'-flanking regions. Gel shift and footprinting assays have revealed that an ≈ 18 bp enhancer region (HTE-1) (≈ 1.1 Kb upstream of the 5' flanking region start site) binds a trans-acting nuclear protein(s) extracted from the nuclei of non-metastatic, non-invasive PC-3 subclones, but not from bone metastatic PC-3 ML clones (see Differentiation 48:115, 1991). In accordance, the relative levels of TIMP-1 were higher in the non-invasive clones than the PC-3 ML clones. Transfection experiments with pCAT-E (pCAT-promoter vector inserted with the HTE-1) constructs containing the TIMP-1 enhancer region have confirmed that only the non-invasive PC-3 lines (and not the PC-3 ML cells) expressed the trans-acting protein activity. Other experiments have revealed further that the transfection activity in non-invasive cells was upregulated at least 3.5-4-fold by 5-10% serum, and nanogram levels of IL6, IL10, TPA and TGF-β. In conclusion, we suggest that the enhancer dependent upregulation of TIMP-1 in benign hyperplasia prostate epithelial may prevent metalloproteinase lysis of the basement membrane and invasion of the surrounding tissue. Loss of expression of the trans-acting protein may result in reduced TIMP-1 expression and increased protease activity in malignant tissue. Supported by grant CA57180 to MES.

Cell Cycle/Paracrine Interactions

Y 015 PROSTATE CANCER - BONE STROMA INTERACTION LEADING TO ANDROGEN-INDEPENDENT PROGRESSION. Leland W. K. Chung[1], Ploutarchos Anezinis[1], George Thalmann[1], Shi-Ming Chang[1], Craig Hall[1], Haiyen E. Zhau[1], Nora Navone[2], Claudio Conti[2], and Sen Pathak[3], Urology Research Laboratory[1], Carcinogenesis Branch[2], Cell Biology[3], The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030.

Recent studies from our laboratory demonstrated that the growth of a human prostate cancer cell line, LNCaP, can be markedly accelerated by cell-cell interaction with prostate and bone fibroblasts (Gleave et al., Cancer Res. 51:3753, 1991). We have derived a number of LNCaP sublines from the primary and metastatic tumors maintained in intact and castrated mice and found that the sublines differed from their parental LNCaP cell line in their cytogenetic profiles, basal and androgen-induced growth and PSA expression, androgen receptor and extracellular matrix expression, and tumorigenicity (Wu et al., J. Urol. 149:425A, 1993). Using the cell-cell interaction model, we have begun to identify key and relevant protein factors that are responsible for stimulating PSA expression (Hsieh et al., Cancer Res. 53:2852, 1993) and tumor growth *in vivo* and tumor cell anchorage-independent growth *in vitro* (Chung et al., J. Cell Biochem. 16H:99, 1992). In this presentation, we will discuss our recent progress in furthering the development of this human prostate cancer model and in the characterization of the parental, tumorigenic, and metastatic LNCaP sublines.

Animal model: We have established metastatic human prostate cancer model is in which an androgen-independent LNCaP subline, C4-2, was found to acquire metastatic potential and disseminate to the lymph node and bone following tumor cell inoculation either subcutaneously or orthotopically. In this model, unexpectedly, we noted that osseous metastasis is more prevalent in castrated than intact male hosts.

LNCaP sublines: We have derived LNCaP sublines from tumors harvested from both primary and metastatic sites of intact and castrated hosts. All of the derived cell lines retained their parental cell-associated marker chromosomes. Additional chromosomal alterations, including specific translocations and deletions and p53 mutations, were found. The derived cell lines also expressed variable basal levels of PSA, growth factors, androgen receptor, and extracellular matrix and appeared to secrete autocrine growth factors to support anchorage-independent growth of the tumor cells.

Because of the consistent changes detected in LNCaP cells following cellular interaction with prostate and bone stromal cells, we propose that such interaction may be fundamental in leading to androgen-independence and subsequent acquisition of metastatic behaviors.

Y 016 CYCLIN GENES AND CELL CYCLE CONTROL IN BREAST CANCER. Robert L Sutherland, Colin KW Watts and Elizabeth A Musgrove, Cancer Biology Division, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, N.S.W. 2010, Australia.

The control of cellular proliferation in human breast cancer involves complex interactions between steroid hormones, peptide hormones, growth factors and cytokines. These regulatory molecules act via a diverse series of receptors and signal transduction pathways to control rates of cell cycle progression at defined points, predominantly in early to mid G_1 phase. The downstream target genes which control these cell cycle phase-specific events have not been well defined in breast cancer cells but emerging data from other experimental systems identify the cyclin family of genes as potential targets. Cyclins are the regulatory subunits of cell cycle specific kinases; sequential transcriptional activation of cyclin gene expression and the consequent activation of the kinase complex appears to regulate a series of control points within the cell cycle. Recent studies in this laboratory have focussed on the potential involvement of cyclin genes in the hormonal control of cell cycle progression in breast cancer cells.
Treatment of the hormone responsive breast cancer cell line T-47D, growth arrested in early G_1 phase by serum deprivation, with insulin or IGF-I leads to the sequential induction of mRNAs for cyclin D1 (early G_1 phase), D3, E, A (late G_1/early S phase) and B1 (G_2 phase). Interestingly, a similar sequential induction of cyclin genes has been reported by others following EGF stimulation of growth arrested normal mammary epithelial cells. Rapid induction of cyclin D1 gene expression was also observed following stimulation of T-47D cell cycle progression with the peptide mitogen bFGF and the female sex steroids, estrogen and progestin. In each case the level of induction of cyclin D1 was related to the proportion of cells which subsequently entered S phase implying that the level of cyclin D1 gene expression controls rates of cell cycle progression. This hypothesis was tested directly by constructing T-47D cell lines expressing human cyclin D1 under the control of a metal-inducible metallothionein promoter. Zinc induction of cyclin D1 resulted in a marked increase in the proportion of cells entering S phase due to a decrease in G_1 phase transit time, demonstrating that cyclin D1 was rate-limiting for progression through G_1 phase. Additional studies showed that induction of cyclin D1 in growth arrested cells allowed cells to complete the cell cycle without addition of exogenous growth factors. Since these data imply that dysregulated expression of cyclin D1 and other cyclin genes may contribute to the malignant phenotype the expression and amplification of several cyclin genes were studied in breast cancer cell lines. Seven of 20 cell lines (35%) showed increased expression of one or more cyclin genes (cyclins A, B1, D1 or E); 5 of these showed increased expression of cyclin D1. This gene was also overexpressed in a significant proportion of breast tumors. Conversely cyclin D2 was expressed at higher levels in normal breast epithelial cells than in breast cancer cells. Constitutive overexpression of cyclin D1 could potentially provide a growth advantage to cells by reducing or obviating their dependence on normal extracellular growth stimuli. This may also render cells insensitive to antiestrogen therapy since decreased expression of cyclin D1 appears to be a key determinant of reduced growth rates following antiestrogen treatment. These data implicate dysregulated expression of cyclin genes, particularly cyclin D1, as a potential factor in the development and progression of breast cancer; a hypothesis that is supported by recent data correlating amplification of 11q13 (the locus for cyclin D1) with poor prognosis in breast cancer patients.

Y 017 A ROLE FOR p53 IN PROSTATE CANCER METASTASIS IN THE MOUSE PROSTATE RECONSTITUTION MODEL SYSTEM. TC Thompson, TL Timme, SH Park, C Ren, PA Baley, LA Donehower and D Kadmon, Scott Department of Urology, Baylor College of Medicine, Houston, TX 77030.

We have developed a mouse model for prostate cancer metastasis using oncogenic recombinant retrovirus-infected mouse prostate reconstitutions (MPRs) in p53 "knockout" mice (Donehower *et al., Nature* **356**:215, 1992). The urogenital sinus (UGS) from day 17 mouse fetuses is isolated and alleotyped as to transgene status: -wild type (+/+), two normal copies of the p53 gene, heterozygous (+/-), one normal allele and one inactivated allele and; nullizygous (-/-), both alleles of p53 inactivated. Allelotyped UGS tissues are then infected with a helper-virus-free recombinant retrovirus, Zipras/myc 9, or a control non- oncogenic virus, BAGα. Control MPRs develop normal prostate morphology and do not form tumors whereas the Zipras/myc 9 -infected-MPRs develop carcinomas in a strain specific fashion. In the +/+ MPRs the predominant phenotype was benign hyperplasia and in one case a small focal carcinoma was observed. In the +/- and -/- MPRs carcinomas developed rapidly and metastatic deposits were found in lung, brain, mesentery, liver and bone.

p53 allelotype	Virus	n	weeks to harvest	wet weight (mg)+/SEM	Phenotype
+/+	BAGα	5	5	55 +/- 20	5/5 normal
+/+	Zipras/myc 9	21	5	87 +/-9	20/21 hyperplasis 1/21 focal cancer 0/21 metastatic
+/-	BAGα	8	5	62 +/-9	8/8 normal
+/-	Zipras/myc 9	14	3	1655 +/- 228	14/14 cancers 13/14 metastatic
-/-	BAGα	4	5	68 +/-19	4/4 normal
-/-	Zipras/myc 9	5	3	2210 +/- 303	5/5 metastatic

Analysis of the p53 status in cell lines derived from primary tumors and metastatic foci revealed that in +/-MPR's, loss of the wild type allele by gene conversion was a frequent occurrence. A 2 kb deletion of the wild type p53 allele was also observed in cell lines derived from the primary tumor and metastatic foci from the same animal. Retention of the wild type allele in a +/- MPR was only rarely observed and when present was not mutated in exons 5, 7 and 8 when screened by SSCP analysis. Southern blotting analysis of carcinoma cell lines derived from the primary tumor as well as metastatic deposits from the same animals indicated that clonal selection frequently occurred at the transition from primary tumor to metastasis suggesting that at least one genetic event in addition to *ras* + *myc* + loss of p53 is necessary for acquisition of the metastatic phenotype. Overall there studies demonstrate that in most cases complete loss of p53 function is necessary for the development of metastasis in this model. However, genetic alterations in addition to *ras* + *myc* + total loss of p53 may be required for acquisition of the metastatic phenotype.

Diagnosis, Treatment, and Prevention I

Y 018 TUMOUR ANGIOGENESIS IN BREAST CANCER - PROGNOSTIC FACTOR AND THERAPEUTIC TARGET, Adrian L Harris[1], Steve Fox[2],Roy Bicknell[1], Russell Leek[1], Kevin Gatter[2], [1]Molecular Oncology Laboratories, Institute of Molecular Medicine, John Radcliffe Hospital, Oxon, U.K. [2]Nuffield Dept of Pathology, John Radcliffe Hospital, Oxon, U.K.

In a study of 109 consecutive breast cancer patients with node negative tumours quantitative angiogenesis was assayed by using a Chalkley point eyepiece graticule. Using univariant and multivariant analysis survival including oestrogen receptor, EGF receptor, size and grade of tumour, vascular count was the major independent factor predicting relapse-free survival and overall survival (relative risks 3.3 and 6.9, P = .019 and .039 respectively). To evaluate the factors regulating tumour angiogenesis a double staining method was used to quantitate endothelial proliferation in the tumours after *in vivo* BUDR labelling. This showed that the labelling index for endothelium was 0.8 to 5.3 mean 2.2, much higher than labelling index in normal breast endothelium. However, there is no correlation of labelling index with vessel counts (Fox *et al*, Cancer Research 53: 4161, 1993). This suggests that vascular modelling and migration may be important as proliferation for breast tumour angiogenesis. To evaluate the growth factors concerned, RNA extracted from a series of 60 primary tumours was analysed by nuclease protection assays for VEGF, placenta growth factor, platelet derived endothelial cell growth factor, acidic and basic FGF, TGFβ1 and pleiotrophin. For the majority of growth factors there is approximately a 20 fold variation in expression between patients and VEGF and PDECGF were the most abundantly expressed. Approximately 50% of tumours expressed pleiotrophin but only 10% showed evidence of placenta growth factor expression. There was no correlation between the different vascular growth factors expressed. Immunochemistry confirmed that PDECGF was expressed within tumour cells. To evaluate PDECGF, MCF7 cells were transfected with human PDECGF and over-expressed in clones selected. Early passage of MCF7 cells required innoculation with 3T3 fibroblasts to produce tumours in nude mice with oestrogen supplementation. Cell lines transfected with PDECGF had a faster growth rate and were more vascular than controls. Similar studies were carried out with VEGF transfectants expressing the low molecular weight form of VEGF with 121 aminoacids. VEGF was shown to cause a significant increase in vascularisation and tumour growth. Thus these growth factors have been confirmed as having an angiogenic role in breast cancer models. This will be useful for analysing synergistic interactions between different vascular growth factors and assessment of anti-angiogenic agents. To further evaluate the role of endothelium in tumour progression, cell adhesion molecules that are regulated on endothelial cells by inflammatory cytokines was studied. ICAM1 was present in 69%, ICAM3 15%, VCAM 10%, E selectin 52% and P selectin 59% of the endothelium of tumour associated vessels. It was in arterioles, venules and capillaries. Expression of selectins was particularly related to the tumour periphery. Preferential location of the activation suggest that tumours may be able to use the properties of the cell adhesion molecules to promote tumour dissemination and metastastasis.

Y 019 INDICATORS OF INCREASED BREAST CANCER RISK, David L. Page, Roy A. Jensen, and William D. Dupont, Vanderbilt University Medical Center, Departments of Pathology and Preventive Medicine, Nashville, Tennessee 37232.

The evidence from epidemiologic and clinical studies identifies several predictors of breast cancer based on evidence from anatomic patterns in tissue. These predictors may be characterized by a magnitude of risk prediction as well as by the localization of the later appearing invasive cancer.

Specifically defined lesions termed atypical hyperplasia of ductal (ADH) or of lobular (ALH) types recognize a later risk of invasive disease in the magnitude of 4 to 5 times that of the general population matched for age and length of follow-up. The addition of a history of breast cancer in a first degree relative doubles this level of risk. Later cancers developing in these women are equally distributed between either breast. Three studies have verified this characterization of these atypical lesions since 1985. Other similar follow-up studies not using the same stringent, combined criteria have produced a lesser magnitude of separation between high- and low-risk groups identified by histology.

Lobular carcinoma *in situ* is closely associated with ALH but does recognize a higher level of risk, also in either breast. However, noncomedo carcinomas *in situ* of ductal pattern (DCIS) are unique in that the later developing carcinoma is in the same site of the same breast; indicating that this lesion may be identified as a true precursor lesion.

These atypical and CIS lesions lack features of polarity which are maintained in the hyperplasias without atypia. Other differentiation markers have been pursued, and most have shown a major difference between comedo and noncomedo DCIS. P53 and erbB-2 mutations and overexpression are virtually confined to comedo DCIS lesions as are high growth fractions demonstrated by various techniques. NM23, a protein related to non-metastatic capacity is more likely to be expressed in DCIS lesions without an invasive component.

Low replacement doses of conjugated estrogen after menopause do not further elevate risk beyond that identified by histology in our cohort of over 10,000 women who underwent benign breast biopsy in Nashville, TN. It would appear that these histologic lesions are not due to an estrogen effect, but are unrelated phenomena.

Diagnosis, Treatment, and Prevention II

Y 020 TARGETED THERAPY THROUGH THE EGF RECEPTOR IN BREAST CANCER, C. Kent Osborne[1], Ester B. Coronado-Heinsohn[1], Pat Bacha[2], Jill Shaw[2], C. Fred LeMaistre[1], [1]Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78284 and [2]Seragen, Inc., Hopkinton, MA.

EGF and TGFa are important growth factors for breast cancer cells. These ligands trigger their effects by binding to EGF receptor which is overexpressed in 40-50% of human breast cancer specimens raising the possibility for targeted therapy through this receptor pathway. Ligand neutralizing antibodies or receptor blocking antibodies, as well as inhibition of the EGF receptor tyrosine kinase activity, have been shown to inhibit breast cancer growth in both in vitro and in vivo models. Recently we have studied another targeted therapy in which a cytotoxic agent is delivered selectively to the tumor cell bearing high EGF receptor levels. $DAB_{389}EGF$ is a ligand fusion toxin produced by expression of a synthetic hybrid gene in which the genetic sequence encoding the receptor binding domain of diphtheria toxin has been replaced by those for EGF. The toxin binds with high affinity to EGFR, is internalized, and then inhibits protein synthesis leading to cell death. The potency of $DAB_{389}EGF$ in inhibiting growth of a panel of breast cancer cell lines parallels EGFR expression. Cells with low levels of EGFR on their surface (< 10,000 sites per cell) are affected only at high concentrations of the toxin whereas cell lines with high receptor levels are inhibited by very low levels of the toxin. CHO cells that do not express detectable EGFR are unaffected by the toxin. After an 18 hour incubation with cells growing in media-supplemented serum, $DAB_{389}EGF$ significantly inhibited protein synthesis in a dose-response fashion only in cells expressing intermediate or high EGFR content with IC50's ranging from 0.2 to 6 nM. Protein synthesis was not affected even at concentrations as high as 10 nM in cells with low levels of EGFR. Cell proliferation was even more sensitive to the toxin. Cells exposed to $DAB_{389}EGF$ for 7 days were again inhibited in a fashion that paralleled EGFR levels. IC50's for the intermediate or high expressing lines ranged from 3 pM to 50 pM, whereas IC50'S from the low expressing lines were greater than 1 nM. A brief exposure of cells for only 1 hour also resulted in some growth inhibition. The toxin also inhibited growth of a variety of human tumors in a colony forming assay at a concentration < 1 nM. Preliminary in vivo studies in mice, rats and monkeys demonstrate a short t 1/2 after a single bolus. Serum concentrations approaching 1 nM can be maintained throughout a 30 minute infusion. Daily treatment of nude rats with the infusion schedule resulted in significant tumor growth inhibition of an EGFR overexpressing cell line. Dose limiting toxicities at higher doses are hepatic and renal. These studies demonstrate that $DAB_{389}EGF$ can be used to selectively kill EGFR overexpressing cells in vitro at concentrations that are achievable in vivo with acceptable toxicity.

Y 021 NOVEL APPROACHES TO THE DIAGNOSIS, TREATMENT, AND PREVENTION OF PROSTATE AND BREAST CANCER, Kenneth J. Pienta, The Meyer L. Prentis Comprehensive Cancer Center, Wayne State University School of Medicine and the Michigan Cancer Foundation, Detroit, MI 48201.

The nuclear matrix is part of a dynamic skeleton of the cell, the tissue matrix system, which consists of an integrated three-dimensional skeletal network that organizes cellular structures and functions from the cell periphery through to the DNA. These skeletal networks or matrix systems consist of linkages and interactions of the nuclear matrix, the cytoskeleton, and the extracellular matrix. We have focused on the nuclear matrix as a diagnostic marker as well as a therapeutic target.

Intermediate biomarkers are needed to more effectively identify malignant progression as well as to develop the potential for more specific treatments and prevention strategies. The nuclear matrix is the RNA-protein network which forms the skeleton of the nucleus and participates in DNA organization as well as multiple cellular functions. Nuclear matrix proteins (NMPs) have been demonstrated to be tissue and cell type specific as well as to reflect the state of cell differentiation and/or transformation. We have demonstrated that nuclear matrices derived from normal human breast tissue and tumor tissue share common NMPs as well as demonstrate specific changes which appear to occur with the acquisition of the cancer phenotype. We have further demonstrated that NMPs are altered by chemopreventive and chemotherapeutic regimens and may provide intermediate endpoints for clinical trials.

We have designed a therapeutic regimen for hormone refractory prostate cancer based on utilizing the nuclear matrix as a therapeutic platform on which drugs interact. Estramustine phosphate (Emcyt) binds to the nuclear matrix and enhances the cytotoxicity of etoposide (VP-16), a topoisomerase II inhibitor which acts at the level of the nuclear matrix. In in vitro and in vivo models, these two agents demonstrated synergistic activity against prostate cancer cell lines. We have conducted a Phase II trial to evaluate the efficacy and toxicity of the combination of Emcyt and VP-16 in men with hormone refractory, metastatic prostate cancer. 50 patients, all who have failed primary hormonal therapy, but not previously treated with chemotherapy, have been enrolled in this trial which has demonstrated a 40-50% response rate.

The nuclear matrix appears to have potential as an intermediate biomarker as well as a chemotherapeutic target.

Late Abstract

BIOLOGIC EFFECTS OF HER-2/*NEU* OVEREXPRESSION AND THE HEREGULIN MOLECULES IN HUMAN BREAST CANCER. D. J. Slamon, M.D., Ph.D., Associate Professor of Medicine, Chief, Division Hematology-Oncology, UCLA School of Medicine, Los Angeles, California.

The HER-2/neu proto-oncogene encodes a growth factor receptor which is overexpressed in 20-30% of human breast cancers. This overexpression is associated with a decreased relapse free as well as overall survival in those patients whose tumors contain the alteration. The overexpression is most often due to amplification in a significant number of cases. This association between HER-2/neu amplification/overexpression and outcome suggests that the alteration may play some causal role in the pathogenesis. To test the potential role of HER-2/neu overexpression in altering the biologic activity of human breast normal and malignant epithelial cells, a number of in vitro studies were conducted in which single-copy, low expressing cell lines were converted to multiple copy, high expressing cell lines. The biologic effects of HER-2/neu overexpression were then measured including effects on DNA synthesis, cell growth, anchorage independent growth, and tumorigenicity. Overexpression of HER-2/neu resulted in an increase in those parameters in the malignant cell lines as well as the non-transformed immortalized breast cell lines. In the normal primary breast cells there was no evidence of these effects with HER-2/neu overexpression alone.

Monoclonal antibodies directed against the extracellular domain of the receptor can suppress all of the biologic effects induced by HER-2/neu overexpression both in vitro and in vivo. Preclinical studies indicate that these antibodies can be effective in completely suppressing growth of human tumor cells as well as malignant breast tissue xenografts when either are growing in vivo. The suppression is specific to cells and tissues overexpressing the HER-2/neu gene. Strategies using these antibodies in combination with other therapeutic modalities indicates that this cytostatic effect can be converted into a cytocidal effect. These observations have led to the development of new treatment strategies directed at this molecular alteration and these strategies are now in clinical testing. In addition, the recent identification, cloning and sequencing of a ligand for the HER-2/neu receptor has allowed for its recombinant expression. The availability of this ligand has led to further insights into the role of the HER-2/neu protein in the pathogenesis of human breast cancer.

Growth Factors I & II

Y 100 THE INTERACTION OF TRANSFORMING GROWTH FACTOR α AND C-MYC IN MOUSE MAMMARY GLAND TUMORIGENESIS **Laufey Thora Amundadottir[1], Michael D. Johnson[1], Gilbert H. Smith[2], Glenn Merlino[3] and Robert B. Dickson[1];** Lombardi Cancer Center, Georgetown University, Washington D.C. 20007[1], Laboratories of Tumor Immunology and Biology[2], and Molecular Biology[3], NCI, NIH, Bethesda, MD 20892. Transforming Growth Factor α (TGFα) binds to and activates the epidermal growth factor receptor (EGFR). Expression of TGFα is most predominantly found in transformed cell lines and tumors of epithelial origin, including breast tumors. The c-*myc* proto-oncogene is found amplified in about 30% of breast cancer. Both c-*myc* and TGFα are known to be induced by ovarian hormones in breast cancer. In various cell types *in vitro*, overexpression of c-*myc* results in increased responsiveness to the effects of mitogenic growth factors, including TGFα. In mouse and human breast cell lines Myc and TGFα have been shown to have additive or synergistic effects on anchorage independent growth in soft agar.

We are exploring the interaction of Myc and TGFα *in vivo* in mouse mammary gland tumorigenesis. We have mated a transgenic mouse strain heterozygous for TGFα (MT100) to a strain heterozygous for Myc (MMTV-c-*myc*) to yield double transgenic offspring for TGFα and Myc. 100% (15/15) TGFα/Myc animals have developed multiple mammary tumors at a mean age of 75 days. No single transgenic TGFα or Myc mice have developed tumors to this date, nor have animals negative for transgenes (age up to 10 months). Furthermore both female and male double transgenic animals develop mammary gland tumors with a similar latency time, suggesting the tumors could be estrogen independent. In contrast to TGFα and Myc single transgenic strains, where tumor formation is dependent upon multiple pregnancies, our double transgenic TGFα/Myc female animals develop tumors as virgins. All tumors are classified as adenocarcinomas type A and B that are locally invasive. No distant metastases have been found. Of other organs that co-express TGFα and Myc, salivary glands show ductule hyperplasia (sometimes with atypia), squamous metaplasia and/or adenoma. Salivary glands of single transgenic animals showed minimal ductule hyperplasia (TGFα mice) or no abnormalities (Myc mice and negative mice). In summary, TGFα and c-*myc* are powerful, synergistic-acting genes in breast carcinogenesis.

Y 101 A COMMON RESPONSE ELEMENT MEDIATES DIFFERENTIAL EFFECTS OF PHORBOL ESTERS AND FORSKOLIN ON TYPE-1 PLASMINOGEN ACTIVATOR INHIBITOR GENE EXPRESSION IN MCF-7 MAMMA CARCINOMA CELLS. Peter A. Andreasen, Helle Knudsen, Tina Olesen, Bente Madsen, Paola Ungaro and Andrea Riccio, Department of Molecular Biology, University of Aarhus, Denmark, and Department of Molecular and Cellular Biology and Pathology, University of Naples, Italy.

The urokinase-mediated pathway of plasminogen activation is implicated in invasion and metastasis in cancer, and urokinase and its type-1 inhibitor (PAI-1) are prognostic markers in breast cancer. In human MCF-7 mamma carcinoma cells, phorbol esters induce PAI-1, while the cAMP inducing agent forskolin suppresses the induction; estradiol enhances phorbol ester induction. We have mapped the 5'-flanking region of the PAI-1 gene for sequences mediating the effects of phorbol esters and forskolin. Transfection experiments with fusion genes showed that sequences mediating phorbol ester induction as well as its forskolin suppression were present between base pairs -100 and -30. Phorbol ester induction required both of two sequences with similarity to phorbol ester response elements (TREs). The proximal TRE-like sequence, the P-box (TGAGTTCA), bound a low-abundance, as yet unidentified protein in MCF-7 nuclear extracts (P-box binding protein), had a high affinity to purified c-jun homodimers, but a low affinity to c-jun/c-fos heterodimers in MCF-7 nuclear extracts. The distal TRE-like sequence, the D-box (TGAGTGG), had a low affinity to c-jun homodimers and to c-jun/c-fos heterodimers; binding of proteins to this sequence was facilitated by binding of proteins to the P-box. The P-box also had similarity to a cAMP-response element (CRE), but did not bind CRE-binding proteins in MCF-7 nuclear extracts. A mutation of the P-box abolishing the CRE-like symmetry abolished the forskolin suppression of the phorbol-ester response as well as the affinity to P-box binding protein. We conclude that the protein kinase C and the protein kinase A signal transduction pathways, with opposite effects on PAI-1 gene expression, converge in a common response element with two cooperatively acting TRE-like sequences, one of which also mediate the forskolin suppression.

Y 102 NEU DIFFERENTIATION FACTOR (NDF/HEREGULIN) INDUCES EXPRESSION OF INTERCELLULAR ADHESION MOLECULE 1 (ICAM- 1): IMPLICATIONS TO MAMMARY TUMORS. Sarah S. Bacus,* Andrei V. Gudkov,+ Carolyn R. Zelnick,* Randi K. Stern,* Dot M. Chin,* Ruth Lupu,^ Yosef Yarden.°
*BD-Cell Analysis Systems, Elmhurst, IL 60126, +University of Illinois, Chicago,IL 60612, ^Georgetown University, Washington, DC 20007, °Weizmann Institute of Science, Rehovot, Israel.

Neu differentiation factor (NDF, also called heregulin) is a 44 kilodalton glycoprotein that stimulates tyrosine phosphorylation of the Neu/HER-2 receptor and induces phenotypic differentiation of certain mammary cancer cell lines to growth arrested and milk-producing cells. To determine which molecules participate in the concomitant morphological alterations, we analyzed the expression of several cytoskeletal and surface molecules, and found that NDF elevated the expression of the intercellular adhesion molecule 1 (ICAM-1) in cultured AU-565 human adenocarcinoma cells. The levels of both the protein and the mRNA of ICAM-1 were elevated after 3-5 days of treatment with NDF. Elevated expression of ICAM-1 was induced also by interferon-gamma (IFN-γ) and by the tumor promoting phorbol ester (PMA), albeit with different kinetics. Down-regulation of protein kinase C, or its inhibition by calphostin C partially inhibited the effect of NDF, implying that the induction of ICAM-1 may be mediated by protein kinase C.

NDF transcripts were detectable in 30% of human mammary tumors, suggesting that the *in vitro* effect of the factor may be relevant to breast cancer. By selecting Neu-positive human mammary tumors, we found significant correlation between the expression of ICAM-1) and histological features of invasive ductal carcinoma with a prominent carcinoma *in situ* (CIS) component. When cultured *in vitro* the cells of these tumors grew in clusters and formed dome like structures reminiscent of comedo type CIS. In addition, the majority of patients with tumors that co-expressed ICAM-1 and Neu had no lymph node involvement, unlike most Neu-positive but ICAM-1-negative tumors, which metastasized to the lymphatic system. Taken together, our observations suggest that the induction of ICAM-1 by NDF may affect the morphology, differentiation state and metastasis of neu-expressing mammary tumor.

Y 103 GENETIC BASIS OF THE MULTIPLICITY OF NEU DIFFERENTIATION FACTORS (HEREGULINS). Noa Ben-Baruch and Yosef Yarden, Department of Chemical Immunology, Weizmann Institute of Science, Rehovot 76100, Israel.

NDF is a 44kDa glycoprotein that induces tyrosine phosphorylation of Neu in human mammary tumor cells. When tested on certain mammary tumor cell lines, the factor arrested cell growth and induced phenotypic cellular differentiation. NDF is a mosaic protein composed of an Immunoglobulin-like domain, a glycosilated spacer region, an EGF-like domain, a transmembrane domain and a long cytoplasmic tail. Multiple other factors with diverse biological activities and high homology to NDF were identified and studied. Detailed analysis of the c-DNA structure of the various ligands revealed alternative splicing at the EGF, juxtamembrane and cytoplasmic tail. Splice variants ate tissue specific; the neuronal forms lack the spacer region and contain a different N-terminal sequence, suggesting that they might be driven by a tissue specific promoter. The highest expression of mesenchymal NDF was found in ras-transformed fibroblasts, Rat1-EJ , while in Rat1 cells minimal expression was detected, suggesting that the mesenchymal promoter of NDF contains a ras-responsive element. To further define the promoter region and to elucidate the intron/exon structure of the alternatively splices isoforms, genomic cloning of NDF was undertaken. The characteristics of the NDF promoter region and initial intron/exon structure will be presented.

Y 104 Significance of erbB-2 receptor and gp30 ligand co-expression in breast cancer patients. M. Cardillo, C. Perez, C. Boulanger and R. Lupu. Vincent T. Lombardi Cancer Research Center, Georgetown University, Washington DC, 20007.

The *erbB*-2 receptor plays an important role in the prognosis of breast cancer and is expressed at very high levels in nearly 30% of human breast cancer patients. While evidence accumulates to support the relationship between *erbB*-2 overexpression and poor overall survival in human breast cancer, understanding of the biological consequence(s) of *erbB*-2 overexpression remains elusive. Our recent discovery of the *erbB-2* ligand (*gp30*) has allowed us to identify a number of related but distinct biological endpoints which appear responsive to signal transduction through the *erbB*-2 receptor. These endpoints of growth, invasiveness, and differentiation have clear implications for the emergence, maintenance and/or control of malignancy, and represent established endpoints in the assessment of malignant progression in human breast cancer. We have shown that low concentrations of *gp30* induces *in vitro* chemoinvasion of cells overexpressing *erbB-2* receptor. Preliminary data from our laboratory suggest that the estrogen and *erbB*-2 receptors represent alternative pathways of malignant growth and that activation of *erbB*-2 leads to a more invasive phenotype. To determine the relevance of *gp30* as a prognostic/diagnostic factor and the significance of *erbB-2/gp30* co-expression, we determined expression of erbB-2 and *gp30* in a variety of breast cancer tumors. The expression of gp30 was determined by RNAse protection, RT-PCR, Immunohistochemistry and In-situ Hybridization. Preliminary data indicate that 25 % of the breast cancer specimens expresses the *erbB-2* ligand (*gp30*) and only 6 % of those co-expresses *erbB-2* receptor. Additional studies are on the way the define the role of the *erbB-2* ligand in breast cancer tumor progression.

Y 105 EXPRESSION OF IGFs, THEIR RECEPTORS, AND THEIR BINDING PROTEINS IN ANDROGEN INDEPENDENT HUMAN PROSTATE CANCER CELLS. **José A. Figueroa, James G. Jackson, Timothy W. Kozelsky, and Douglas Yee. University of Texas Health Science Center at San Antonio, San Antonio TX 78286.**

The use of drugs which block growth factor action (i.e. suramin) in patients with prostate cancer represent a novel therapeutic strategy for this disease. Although the IGFs are known mitogens for several neoplastic cells, little is known about their role in the pathophysiology of prostate cancer. Thus, in this study we investigated the expression of IGF ligands, receptors ($IGFR_1$, $IGFR_2$), and binding proteins (IGFBPs) in the androgen-independent cell line DU145, and examined the mitogenicity of both IGF-I and IGF-II on these cells. Using RNase protection assays, we detected mRNA transcripts for IGF-II, $IGFR_1$, $IGFR_2$ and IGFBPs-2 through 6. Both wild type and alternately spliced $IGFR_1$ mRNA transcripts were detected. IGFBPs-3, and -6 were the most abundant species while IGFBPs-2, -4, and -5 were expressed at a lower level. Expression of IGF-I or IGFBP-1 mRNA was not found. Ligand blotting confirmed secretion of distinct IGFBPs into cell's conditioned media. Compared to controls, treatment of DU145 with increasing concentrations (5-20nM) of either IGF-I or IGF-II resulted in a dose-dependent increase in cell number and DNA synthesis as well as autophosphorylation of $IGFR_1$. IGF-I and IGF-II growth effects were inhibited by 10μg/ml αIR-3, an antibody against $IGFR_1$, suggesting that activation of $IGFR_1$ tyrosine kinase is the crucial step leading to cell proliferation. Our results suggest that the IGF family of proteins plays a role in the regulation of hormone-independent prostate cancer cell growth.

Y 106 THE ROLE OF GROWTH FACTORS IN BRANCHING MORPHOGENESIS IN THE DEVELOPING RAT ANTERIOR PROSTATE, Barbara A. Foster and Gerald R. Cunha, Department of Anatomy, University of California, San Francisco, CA 94143.

A serum-free culture system was developed to study the effect of growth factors and neutralizing antibodies to growth factors on branching morphogenesis in the developing rat anterior prostate. Anterior prostates from 0 day Fisher-344 rats were isolated and cultured in serum-free medium for six days. The glands in culture exhibited androgen-dependent branching morphogenesis. Without androgens the glands survived, but the epithelium did not branch. Acidic FGF (aFGF) enhanced branching morphogenesis in the presence of androgens, where as TGF-β_1 and TGF-β_2 inhibited it. A neutralizing antibody to TGF-α also inhibited branching morphogenesis. This effect was not due to toxicity as the glands resumed branching when transferred to antibody-free medium. Glands grown with TGF-α plus testosterone had a distorted branching pattern. Glands grown with TGF-α but without testosterone did not branch. These data suggest that TGF-α may play a role in mediating the effects of androgens, but cannot fully replace androgens in the developing anterior prostate. HGF, KGF, and bFGF had no effect on the parameters used to quantitate branching morphogenesis. In this system TGF-α, TGF-β_1, TGF-β_2 and aFGF were found to modulate branching morphogenesis and may be important mediators of androgens in the morphogenesis of the rat anterior prostate.

Y 107 AUTOCRINE/PARACRINE ROLES OF INSULIN-LIKE GROWTH FACTORS IN PROSTATE CANCER CELL GROWTH, Fujita-Yamaguchi, Y., Kasuya, J., Giannini, S., Kawachi, M.H. and Kimura, G., Beckman Research Institute of the City of Hope, and City of Hope Medical Center, Duarte, CA 91010

Potential autocrine/paracrine roles of insulin-like growth factors (IGFs) were evaluated in cell cultures using three prostate cancer cell lines, LNCaP, DU145 and PC-3. By employing reverse-transcriptase polymerase chain reaction, mRNAs for IGF-II and IGF-I receptor were detected in all three cell lines whereas IGF-I mRNA was not detectable in DU145 and PC-3 cells. LNCaP cells, however, showed a trace amount of IGF-I mRNA. Radioimmunoassay indicated that IGF-II but not IGF-I was released into conditioned media of three cell lines, ^{125}I-IGF-I binding assays showed that functional IGF-I receptor was available in the membrane fractions of the three cell lines. These results strongly suggested that an autocrine/paracrine loop of IGF-II and IGF-I receptor plays an important role in prostate cancer cell growth. Based on this observation, we have examined whether or not prostate cancer cell growth can be inhibited by the disruption of the autocrine/paracrine loop. The disruption can be achieved by either blocking the IGF-I receptor or suppressing the expression of IGF-II mRNA. Basal (autocrine growth) and IGF-II-stimulated (paracrine growth) DNA synthesis of prostate cancer cells were inhibited in the presence of our new monoclonal antibody against human IGF-I receptor (1H7) which has been shown to inhibit IGF-I or -II-stimulated DNA synthesis of NIH3T3 cells expressing a large number of human IGF-I receptors more effectively then monoclonal antibody αIR-3 (Li *et al.* in press, Biochem. Biophys. Res. Comm.). To suppress IGF-II mRNA expression, a phosphorothioate antisense oligonucleotide specific to IGF-II mRNA was included in cell cultures. Preliminary studies indicated that the antisense oligonucleotide inhibited prostate cancer cell growth. In summary, inhibition of prostate cancer cell growth by the IGF-I receptor specific antibody or the IGF-II specific antisense oligonucleotide is consistent with the notion that the autocrine/paracrine loop of IGF-II and IGF-I receptor is responsible for prostate cancer cell growth. These agents may be useful for prostate cancer therapy.

Y 108 TGFα EXPRESSION IS A RELATIVELY LATE EVENT IN THE PROGRESSION OF PROSTATIC ADENOCARCINOMA. William E. Grizzle, Russell B. Myers, Denise Oelschlager, Sudhir Srivastava. Department of Pathology, University of Alabama at Birmingham, Birmingham, AL 35294 and National Cancer Institute, Bethesda, MD 20892.

Previously we reported that transforming growth factor alpha (TGFα) is expressed selectively by the malignant cells of prostatic adenocarcinomas. In the present study we examined the expression of TGFα in prostatic intraepithelial neoplasia (PIN), localized prostatic adenocarcinoma as well as matching primary tumors and lymph node metastasis from patients with stage D adenocarcinomas. Immunohistochemical analysis of TGFα expression was conducted using a monoclonal antibody directed against TGFα. Immunoreactivity was graded as none, low, moderate or strong based upon the intensity and extent of staining. Significant expression of TGFα was not detected in the benign epithelium (normal by histology) located within the adenocarcinoma specimens. Expression of TGFα was infrequently detected among the PIN lesions of the 20 cases examined. When TGFα was detected in PIN lesions, expression was typically low. TGFα expression was detected in all the 30 localized (Stage B or C) adenocarcinomas examined but the intensity of expression was typically low or moderate with the exception of one adenocarcinoma in which strong expression was detected. In contrast, strong expression of TGFα was detected in 4 of 17 primary lesions and 5 of 18 nodal metastasis from patients with stage D adenocarcinoma. The increased expression of TGFα in the stage D adenocarcinomas as compared to localized lesions and PIN suggests that the expression of TGFα occurs relatively late in the development and progression of prostatic adenocarcinomas. Supported in part by NCI contract #N01-CN-15340-02.

Y 109 MAMMARY DERIVED GROWTH INHIBITOR (MDGI) IS A NEW DIFFERENTIATION FACTOR FOR THE MAMMARY GLAND

Richard Grosse**, Yangmin Yan*, Eva Spitzer*, Wolfgang Zschiesche*, Gilbert H. Smith+and David Salomon+
* Max Delbrück Center for Molecular Medicine, 13122 Berlin, Germany.
+ National Cancer Institute, NIH, Laboratory of Tumor Immunology and Biology, Bethesda, MD 20892.
** University of Bergen, Haukeland Hospital, The Gade Institute, N-5021, Bergen, Norway.

MDGI is a member of the Fatty Acid Binding Protein (FABP) family assumed to control intracellular levels of eicosanoids, retinoids, and long chain fatty acids. Recombinant and wild-type forms of MDGI and Heart-FABP, but not FABPs from liver and intestine, selectively inhibit growth of normal mouse mammary epithelial cells (MEC) in primary culture. In whole mammary gland organ culture, inhibition of DNA synthesis in MEC affects ductular elongation and branching. In parallel functional differentiation was markedly stimulated by MDGI as evaluated by following the levels of milk proteins ß-casein and WAP. In addition, a strong autostimulation of MDGI expression in MEC was found indicating an autocrine loop. These regulatory properties can be fully mimiced by an eleven amino acid sequence, represented in the C-terminus of MDGI and related FABPs. This peptide does not bind fatty acids. The differentiation promoting MDGI activities can be antagonized by EGF. Thus, physiologically a balance between MDGI and EGF/TGF-alpha dependent pathways may regulate normal ductal growth and alveolar differentiation.

Y 110 **THE ROLE OF RELAXIN IN PROMOTING PROSTATE CANCER CELLULAR MOTILITY AND WOUND HEALING.** Deborah J. Hansell and Edward P. Gelmann, Lombardi Cancer Research Center, Georgetown University, Washington, DC 20007.

We have shown that relaxin is expressed in benign prostate hypertrophy, in prostate cancer specimens and in the prostate cancer cell line LNCaP. No expression was detected in DU-145 and PC-3 prostate cancer cell lines. We also detected relaxin secreted into the conditioned medium from cultured LNCaP cells. Relaxin, a member of the insulin-like growth factor family of peptides, has been shown to cause an increase in collagen turnover by dermal fibroblasts and connective tissue remodeling during pregnancy. Relaxin is not mitogenic for any of the the prostate cancer cell lines. In modified Boyden chamber chemotactic assays human recombinant relaxin at a concentration ranging from 10^{-8} - 10^{-10} M promoted prostate stromal cell chemotaxis from between 4-7 fold over the control conditions. No effect was observed on LNCaP cells. At a concentration of 10^{-8} M relaxin stimulated the faster migration of DU-145 cells across a scored wound at 8 hours when compared to control media of IMEM plus 5% dialysed FBS. LNCaP cells were not stimulated by relaxin in the wound healing assay. This suggests that relaxin, expressed by prostate epithelial cells, may serve a role in cellular motility in the prostate and perhaps in prostate cancer.

Y 111 EGF IS A DIFFERENTIATION FACTOR RESPONSIBLE FOR THE ACQUISITION OF OVARIAN HORMONE RESPONSIVENESS IN NORMAL MAMMARY GLAND. Sandra Z. Haslam and April St. John, Department of Physiology, Michigan State University, East Lansing, MI 48824

The ovarian hormones, estrogen (E) and progesterone (P) are required for mammary epithelial cell proliferation during the ovarian cycle and pregnancy in rodents and humans. Growth factors like EGF are also believed to play an important role in the growth and development of the mammary gland and may interact with ovarian hormones. During postnatal development in the mouse, there is sequential development of hormone responsiveness such that E-induced progesterone receptors (PR) and P-induced mammary cell proliferation are not acquired until 7 weeks of age. The purpose of the present study was to investigate the roles of E, P and EGF in the ontogeny of P responsiveness. The induction of E-dependent PR was investigated in the mammary glands of immature mice treated with E, P, or EGF, both in vivo and in vitro in organ culture. In vivo injection of E alone to ovariectomized, immature mice for a minimum of 10 days, resulted in the precocious acquisition of E-dependent PR; P had no effect. To investigate the contribution of indirect systemic effects of E, PR inducibility was investigated in organ culture. The minimal hormone combination required for the acquisition of E-dependent PR was E+EGF. Interestingly, maximal induction also required 9-10 days in culture. However, in vivo implantation of EGF alone into the mammary glands of ovariectomized, immature mice resulted in the acquisition of E-dependent PR as soon as 2 days after implantation. This effect of EGF was independent of its growth promoting effects. From these studies we conclude that EGF may act as a differentiation factor and can cause the acquisition of E- and P-dependent growth control in normal mammary gland. Furthermore, it is likely that one of the roles of E during mammary gland development is to increase systemic and local concentrations of EGF. Understanding how ovarian hormones and growth factors modulate hormonal responsiveness in the normal mammary gland may provide new insights into the loss of hormone responsiveness in breast cancer and may lead to the development of new therapeutic strategies. This work was supported by NIH Grant 1 RO1 CA40104.

Y 112 Expression of epidermal growth factor (EGF) family growth factors in the aged mouse mammary gland.
Herrington, G., Ram, T., Mills, R. and Hosick, H. Departments of Zoology and Genetics/Cell Biology, Washington State University, Pullman, WA.

Since members of the EGF family have been implicated in mouse mammary tumor development, we have screened glands of mice of different ages and parity to determine whether changes in EGF, transforming growth factor alpha (TGFα), amphiregulin, and cripto-1 (CR-1) production correlate with increased age and its associated increased risk of tumor development. First, TGFα, amphiregulin and CR-1 were immunolocalized in histological sections of glands from 2-4 month-old nulliparous, 2-4 month-old (12-16 day) midpregnant, and 18-26 month-old nulliparous and multiparous BALB/c mammary glands. To date, the 18-26 month-old aged gland has shown positive immunostaining for TGF-α in 34% (11 of 30) of glands screened. Positive CR-1 immunostaining was observed in all of the aged glands from both multiparous and nulliparous sections as well as a spontaneous fibrosarcoma from a 25 month-old multiparous #4 gland. To date, no conclusive staining has been observed in any other age group. In a second approach to study stromal-epithelial paracrine interactions, we isolated mammary fibroblast cell populations from the three age groups. Preliminary results show positive amphiregulin and CR-1 expression in both epithelial and stromal populations. Conditioned medium harvested from these mammary fibroblast cell cultures consistently stimulated characteristic branching outgrowth of PMME cells in a collagen matrix. In a third paradigm, we used the Polymerase Chain Reaction (PCR) to look at TGFα and EGF mRNA expression in the isolated fibroblast and PMME cells. Interestingly, TGFα and EGF transcripts were detected in all fibroblast isolates, compared with only EGF mRNA being detected in the PMME cells. These observations suggest that the EGF family of growth factors could contribute to tumor development in the older mammary gland, and that it may be produced in the old gland by stromal cells.

Y 113 STROMAL EXPRESSION OF GROWTH FACTORS IN BREAST CANCER. Ann Hornby, Christian Singer, Audrey Rassmussen, and Kevin Cullen, Vincent T. Lombardi Research Center, Georgetown University, Washington, DC 20007

Breast cancers are almost exclusively epithelial cancers, however, clinically significant breast cancers, are a complex and heterogeneous mix of epithelium, stromal cells, matrix proteins and vascular elements. Our group has begun to examine the role of interaction between tumor epithelium and stroma in the genesis and proliferation of breast cancers. Previously, we have examined expression of IGF-I and IGF-II in cell lines derived from primary tissue and shown that IGF-I expression was seen in the majority of lines derived from benign lesions, whereas, IGF-II expression was seen in the majority of lines derived from malignant lesions. Currently we are examining the interaction between erbB2 and its ligand, gp30. We found that the pattern of expression of gp30 mRNA was very similar to what we previously reported for IGF-II. The majority of fibroblasts derived from breast cancers expressed gp30 (8 out of 15), while none of the fibroblasts derived from surrounding normal breast tissue or from benign lesions (0 out of 14) expressed the gp30 ligand. We also found a low level of erbB2 expression in both peripheral and tumor fibroblasts. While we are postulating a paracrine role for gp30 in breast cancers, an autocrine role cannot be ruled out at the present time due to the presence of erbB2 expression by the fibroblasts themselves. Further studies aimed at understanding the role of gp30 in breast cancers are underway.

Y 114 DETECTION OF A PROLACTIN-LIKE FACTOR IN NEOPLASTIC MOUSE MAMMARY EPITHELIAL CELLS, Gloria D. Jahnke, Carol S. Trempus, Frank Kari and Richard P. DiAugustine, Hormones and Cancer Workgroup, N.I.E.H.S., Research Triangle Park, NC 27709

Prolactin, a member of the growth hormone family, has been identified in extra-pituitary tissues in both rodents and humans. Forms of prolactin have been identified in uterine myometrium, placenta, lymphocytes and recently in the rat lactating mammary gland. Since ectopic production of growth factors can occur with neoplasia, we investigated whether a prolactin-like factor is ectopically produced by neoplastic mouse mammary epithelial cells and stimulates local growth of the gland. Cell lines derived from normal and neoplastic mouse mammary tissue were screened for the presence of prolactin transcripts by RT-PCR. Our results suggest that there are at least two forms of prolactin transcript present in some neoplastic cell lines tested. One form is the same size as pituitary prolactin transcript and the other form includes intron 4. Northern blots of poly A+ RNA from these cell lines and hybridization to a rat prolactin specific probe revealed a 1kb transcript coincident with the size obtained for pituitary RNA and consistent with the size observed from RT-PCR for both forms. Evidence of prolactin transcripts in normal mouse mammary tissue was only found during lactation; the alternately spliced form was present. The Nb2 cell proliferation assay for prolactin bioactivity confirmed the presence of a prolactin-like factor in conditioned media from NOG8 cells and the levels detected are within physiological range. Studies are in progress to determine the molecular weight of this protein and to assess whether an autocrine pathway is present in these cells.

Y 115 MODULATION OF TGFß-EXPRESSION BY DIFFERENT MEMBERS OF THE STEROID HORMONE FAMILY AND THEIR ANTAGONISTS, Cornelius Knabbe, Andreas Kopp, Walter Jonat and Gerhard Zugmaier, Departments of Clinical Chemistry and Gynecology, University Hospital Eppendorf, D-20246 Hamburg and Dpt. of Hematology and Oncology, University of Marburg Medical Center, D-35043 Marburg, Germany.

Transforming growth factor ß proteins (TGFß) are multifunctional polypeptides with a broad spectrum of activities. We have previously shown that the growth inhibitory antiestrogen tamoxifen induces the expression of TGFß in human breast cancer cells in vitro, suggesting a possible role of TGFß as autoinhibitory mediator of antiestrogen action. We now addressed the question if expression of TGFß can also be modulated by other members of the steroid hormone family and their antagonists in different in vitro systems and obtained the following results: 1) Growth inhibitory concentrations of glucocorticoids, type-1 and type-2 antiestrogens induce the expression of TGFß-2 in estrogen receptor positive MCF-7 and T 47-D human breast cancer cells, growth stimulatory estradiol loweres TGFß-2 expression. No modulation of TGFß-2 is seen in estrogen receptor negative human breast cancer cell lines under estrogen/antiestrogen treatment. 2) Growth inhibitory concentrations of retinoic acid induce the expression of TGFß in pancreatic carcinoma cell lines. These data suggest that TGFß is a marker of (anti)hormonally regulated growth inhibition. This hypothesis was tested in a clinical setting. Using a specific immuno assay, we determined the blood level of TGFß-2 in 13 patients with metastatic breast cancer before and under treatment with the antiestrogen tamoxifen. Blood samples were taken every two weeks, clinical staging was performed after 12 and 24 weeks. The main results are: 1) All patients who were in remission showed a significant increase in TGFß-2 at the time of 4 weeks after begin of therapy. 2) 80 % of those patients who showed a relapse had unchanged or diminuished TGFß-2 values after begin of therapy followed by a secondary increase preceding the clinical manifestation of the progress. These data point to a bifunctional role of TGFß in vivo: In hormone responsive tumors, TGFß can act as autoinhibitory mediator of steroid hormones and their antagonists. In anti(hormone) resistant cases the (auto)inhibitory function appears to be lost, tumor promoting effects of TGFß like induction of angiogenesis and immunosuppression might become more important and allow the diagnostic use of TGFß as marker of tumor progression.

Y 116 REGULATION OF THE GROWTH OF HUMAN TUMOR CELL LINES BY HEREGULIN, Gail D. Lewis and Mark X. Sliwkowski, Departments of Biological Chemistry and Protein Chemistry, Genentech, Inc., South San Francisco, CA 94080

A number of ligands for p185^{HER2} have recently been characterized. These include the heregulins (HRG-α, β1, β2, and β3); *neu* differentiation factor (NDF), the rodent homolog of HRG; a family of related glial growth factors (GGF), and acetylcholine receptor-inducing activity (ARIA). Because overexpression of HER2 is correlated with poor prognosis in certain malignancies, we have studied the effects of recombinant HRG on the growth of human tumor cell lines expressing a range of levels of p185^{HER2}. Initial studies in monolayer culture showed that the response to HRG of a cell line with low p185^{HER2} levels (MCF7) differed from that of the p185^{HER2}-overexpressing line, SK-BR-3. These growth responses were differentially influenced by serum concentration and extent of treatment. Subsequent examination of the growth of other breast tumor lines, as well as a gastric and ovarian line, in both monolayer and soft agar, has shown that: 1) not all cell lines expressing p185^{HER2} respond to HRG, 2) the effects of HRG vary with assay conditions and cannot be predicted by level of p185^{HER2} expression alone, and 3) the responses to HRG can be distinguished from epidermal growth factor and from the growth inhibitory anti-p185^{HER2} monoclonal antibody,4D5.

Y 117 KERATINOCYTE GROWTH FACTOR AND RECEPTOR EXPRESSION IN BENIGN AND MALIGNANT PROSTATE. McGarvey, T. and Stearns, M. E. Department of Pathology, Medical College of Pennsylvania, Philadelphia, PA 19129.

Keratinocyte growth factor (KGF) and its receptor may play a role in the growth of adenocarcinomas. Northern and slot blots showed that three different immortalized cell lines expressed KGF, including non-invasive and bone metastatic human prostatic PC-3 ML subclones, the MCF-10 breast cells, and W1-38 fibroblasts. Only the MCF-10 cells expressed KGF receptor, however. In situ hybridization studies show that the KGF and KGF receptor genes were expressed in the stromal and epithelial cells, respectively, in human benign (BPH) prostate tissue. In low and high grade carcinoma both the KGF gene and receptor were expressed in the glandular epithelial cells. The labeling was significantly increased with Gleason scoring and in metastatic nodules. Interestingly, stromal expression of KGF was not detectable in carcinomas or in metastatic lesions. We interpret the data to mean that a paracrine growth factor loop in BPH may be replaced by an autocrine loop in adenocarcinomas. Supported by CA 57180 to MES.

Y 118 GENE EXPRESSION IN MULTIPLE STAGE MAMMARY TUMORIGENESIS, Daniel Medina, LiXin Zhang, Thenaa Said, Joseph Jerry and Janet S. Butel, Departments of Cell Biology and Molecular Virology, Baylor College of Medicine, Houston, TX 77030

Mouse mammary tumorigenesis proceeds through well-defined intermediate stages. The best documented intermediate stage is the hyperplastic alveolar nodule which is characterized by the biological properties of immortalization, morphological hyperplasia and increased risk for tumor development. Recent experiments have demonstrated that these three properties are independent and assortable properties, each of which is represented in different in vivo outgrowth lines. The molecular changes which are responsible for each of these properties are not clearly understood. We examined the activities of several genes often altered in tumorigenesis. The expression of the tumor suppressor gene, p53, was frequently altered in mammary hyperplasias. The alteration of p53 expression was detected as overexpression of both mutant and wild-type protein and correlated with the property of morphological hyperplasia but not with immortalization or enhanced tumorigenic potential. The expression of mammary specific wnt genes-2,4,5b was examined by Northern blots and the results indicated that wnt5b was constituitively expressed only in hyperplasias with enhanced tumorigenic potential. A similar result was obtained with the status of cdc2 activity. Non-tumorigenic hyperplasias exhibited primarily inactive cdc2 in contrast to tumorigenic hyperplasias with active cdc2, as measured by tyrosine phosphorylation and kinase assays. Finally, differential display RNA-PCR demonstrated the presence of 2 mRNAs preferentially expressed in tumorigenic hyperplasias. The cDNAs for these genes are currently being sequenced. These data indicate that specific genes are preferentially activated at different stages of mammary tumorigenesis and are correlated with specific biological properties acquired during the evolution of mammary tumors.

Y 119 PROLACTIN REGULATION OF RAT AND HUMAN PROSTATE, Marja T. Nevalainen, Paula Martikainen, Eeva M. Valve, Wu Ping, Martti Nurmi and Pirkko L. Harkonen, MediCity Research Laboratory, Department of Anatomy and Department of Surgery, University of Turku, Finland.

Besides androgens, prolactin and estrogen are thought to be involved in the regulation of normal and malignant growth of the prostate. Our previous results show that the morphology, DNA contents, DNA labeling with (3H)thymidine and expression of tissue specific genes M-40 and RWB encoding secretory proteins (probasin and SVII) are regulated by prolactin in both dorsal and lateral prostate of rat in organ culture, although the hormone responsiveness of dorsal and lateral lobe differ (Nevalainen MT et al.,Endocrinology 129:2, 1991). Also in human hyperplastic prostatic tissue (n=17 patients undergoing cystoprostatectomy for bladder carcinoma) cultured for seven days in medium containing 5% dextran charcoal treated fetal calf serum, insulin and dexamethasone, prolactin had androgen independent effects on morphology and DNA synthesis.

The receptor for prolactin belongs to the cytokine/GH/Prl receptor family. Three forms of transmembrane receptor proteins have been identified in rat tissues and one form in human tissues.

We have studied the expression of prolactin receptor mRNA and the binding of prolactin to its receptor in rat dorsal and lateral prostate both ***in vivo*** and ***in vitro*** and in human prostate ***in vitro***. Rats were castrated and injected with testosterone (1mg/day) or estrogen (50ug/day). In the ***in vitro*** experiments explants of rat dorsal and lateral prostate were cultivated in the chemically defined medium supplied with insulin and corticosterone with or without testosterone or estrogen. Poly(A+)RNA was prepared and analyzed by Northern blotting and hybridized with probes recognizing the long and short forms of the prolactin receptor. Only the long receptor form was expressed in rat dorsolateral prostate. The mRNA:s for prolactin receptor were differentially regulated by steroid hormones both in human and rat prostatic tissues.

Human and rat prostate thus provide a useful model for the studies of prolactin receptor gene expression and prolactin-triggered/stimulated signal transduction pathway which is regulated by steroid hormones at least at the level of the membrane receptor.

Y 120 STRUCTURE-FUNCTION ANALYSIS OF EGF-RECEPTOR INTERACTION BY SITE-DIRECTED MUTAGENESIS. Salil K. Niyogi, Douglas K. Tadaki, and Stephen R. Campion. Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-8077.

In view of the implication in breast cancer of EGF-like peptides and their cognate receptor (cERB2) which is closely related to the EGF receptor (EGFR), studies of EGF-receptor interaction have assumed increased importance. Protein engineering was employed to examine structure/function relationships of human EGF (hEGF). Interaction of hEGF with EGFR requires residues scattered throughout the growth factor molecule, including the specific hydrophobic residues Tyr13, Leu15, Ile23, and Leu26 in the N-terminal domain, and Leu47 in the C-terminal domain. In general, ionic residues in hEGF are not critical for the formation of a stable hEGF-receptor complex. However, the guanidinium group of the highly conserved Arg41 residue was found to be of critical importance for the functional activity of hEGF. Quantitative evaluation of double-site mutant hEGF analogues indicates cumulative effects of simultaneous mutations on relative receptor affinity and suggests that each of the individual sites interacts with the receptor essentially independently. The decreased binding observed with mutants of critical hydrophobic residues also translates into a decreased ability to activate the EGFR tyrosine kinase activity. Specific EGF analogues are currently being utilized as affinity probes to map the ligand-binding pocket of EGFR. [Research supported by USDOE contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc., USPHS grant CA 50735, and NCI postdoctoral training grant CA09336.]

Y 121 MURINE ENDOGLIN IS A TGF-β RECEPTOR COMPONENT OF MOUSE FIBROBLASTS, Nadia Pece, Sonia Vera, Urszula Cymerman, and Michelle Letarte, Division of Immunology and Cancer Research, Hospital for Sick Children, and Department of Immunology, University of Toronto, Toronto, Canada, M5G 1X8

Human endoglin is a 180 kDa homodimeric membrane glycoprotein expressed primarily on human vascular endothelium. Human endoglin binds TGF-β1 and TGF-β3 with high affinity and is an essential component of the transforming growth factor-beta (TGF-β) receptor system on human endothelial cells. Murine endoglin has recently been cloned and sequenced and is 72% identical to human endoglin. By *in situ* hybridization and immunofluorescence, murine endoglin was shown to be expressed not only on endothelial cells but also on fibroblast-like stromal cells of connective tissue in several organs. This suggested that certain fibroblast lines might be suitable for the biochemical and functional characterization of murine endoglin. We first tested by PCR analysis the presence of endoglin messenger RNA. Mouse NCTC 2071 fibroblasts were found to express high levels of endoglin mRNA whereas the NIH 3T3 line expressed approximately 1% of that level. Affinity labelling of NCTC 2071 cells with ^{125}I-TGF-β1 followed by immunoprecipitation revealed that murine endoglin binds TGF-β1 and is also a homodimer of approximately 170 kDa. We have used the low expressing NIH 3T3 fibroblasts for transfection of human endoglin. A series of transfectants were generated by electroporation using the pcEXV-LENDO plasmid containing a full length cDNA of human endoglin. Several lines espressing very high levels of human endoglin were identified by flow cytometry. We are currently using these transfectants to test the role of endoglin in adhesion to various substrates and in migration assays. We will also test the response of the transfectants to TGF-β. These experiments should allow us to determine if endoglin plays an essential role in the response of fibroblasts to TGF-β.

Y 122 **CLONING OF THE cDNA OF A GLYCOPROTEIN OF BREAST EPITHELIAL CELLS WITH EGF-LIKE AND CELL ADHESION SEQUENCES THAT IS A TARGET FOR RADIOIMMUNOTHERAPY,** Jerry A. Peterson, Simon Godwin, Joseph R. Couto, and Roberto L. Ceriani, Cancer Research Fund of Contra Costa, Walnut Creek, CA 94596.

The entire cDNA sequence of a 46 kDa glycoprotein (BA46) that is present in the human milk fat globule membrane and expressed by breast carcinoma cells has been determined. The encoded protein contains an N-terminal putative signal sequence followed by a cell adhesion sequence (RGD), an EGF-like sequence, and a C-terminal sequence with homology to C1/C2 region of human coagulation factors V and VIII. The presence of the RGD sequence suggests that BA46 may be involved in cell interaction, possibly by interacting with integrins which are also present on the breast cell surface and which have specific receptors for the RGD peptide sequence that is present on fibronectins. Increased expression of BA46 in breast carcinoma cells could thus reduce the breast cell's interaction with the basement membrane by interfering with integrins, and thus play a role in metastasis. The EGF-like sequence suggests a possible growth factor. BA46 is also a target for radioimmunotherapy, where two monoclonal antibodies (Mc3 and Mc8) that bound to different functional domains had different effectiveness. Mc3 was far superior to Mc8, in many cases completely eradicating the transplanted tumors. This demonstrates that some moieties of the same antigen are better targets for radioimmunotherapy than others. Also, Mc3 may act on the tumor by not only carrying the radiotoxicity but by also interfering with a cell growth factor. Supported in part by NIH grants HD30444, CA39932, and CA61258.

Y 123 **PENTOSAN POLYSULFATE PRODUCES A SYNERGISTIC INCREASE IN THE CYTOTOXIC EFFECTS OF ACIDIC FIBROBLAST GROWTH FACTOR-PSEUDOMONAS EXOTOXIN FUSION PROTEINS.** **Rae,J.M., Gottardis,M.M., Siegall,C.B., Pastan,I. and Lippman,M.E. Lombardi Cancer Center, Washington D.C. 20007 (J.M.R., M.M.G., M.E.L.), Bristol-Myers Squibb, Seattle, WA 98121 (C.B.S.) and N.C.I., N.I.H., Bethesda, MD 20205 (I.P.).**

The fibroblast growth factors (FGF) are a family of heparin binding growth factors (HBGFs). Chimeric fusion proteins consisting of acidic fibroblast growth factor fused to various truncated forms of Pseudomonas exotoxin (aFGF-PE) were found to be FGF receptor specific and cytotoxic to many tumor cell lines. The heparinoid, pentosan polysulfate (PPS), has been shown to inhibit tumor cell growth by blocking the action of HBGFs. We treated tumor cells with PPS in combination with 2 chimeric proteins to look for cooperation between these two agents. PPS produced a synergistic increase in the effects of the aFGF-PE toxins. Cytotoxicity was determined using an anchorage dependent growth assay and an anchorage independent soft agar cloning assay. MDA MB 231 tumor cells are unaffected by PPS at concentrations as high as 100ug/ml and are resistant to the exotoxins at levels up to 100ng/ml. In combination these concentrations prove 100% lethal to MDA MB 231 cells. Dose response curves were generated with fixed amounts of one drug and varying amounts of the other and vise versa. The results showed that PPS at 1ug/ml could give aFGF-PE an EC50 50ng/ml. The shift in dose response of aFGF-PE by PPS was seen in several other tumor cell lines. Similar potentiation was seen in the soft agar cloning assays. FGFs released by the tumor cells compete with aFGF-PE for binding of receptor. PPS binding to FGFs could prevent them from acting as an competitive antagonist towards aFGF-PE. aFGF-PE is a promising new compound that may have a utility as an anticancer agent. PPS is currently in clinical trials. The PPS potentiation could increase specificity and decrease the side effects of aFGF-PE. *In vivo* experiments are now under way to determine if these effects can be achieved in animals.

Y 124 ROLE OF HEPATOCYTE GROWTH FACTOR (HGF) IN BREAST CANCER: A NOVEL MITOGENIC FACTOR SECRETED BY ADIPOCYTES N. Rahimi, R. Lall, R. Saulnier and B.E. Elliott. Cancer Res. Lab., Queen's Univ., Kingston, Ontario, CANADA, K7L 3N6

Stromal cells can dramatically affect the growth and metastatic capability of breast carcinoma cells. Growth factors are considered to be important mediators of this process, acting as either mitogenic or mitoinhibitory regulators. We have developed an *in vitro* co-culture system to examine the influence of adipocytes, a dominant mammary stromal cell type, on the growth of a murine mammary carcinoma, SP1. We have shown that substrata or conditioned medium (CM) from 3T3-L1 adipocytes can promote *in vitro* growth of SP1 cells. The stimulatory activity of CM was inhibited by: heparin, an inhibitor of certain heparin binding growth factors; erbstatin, a tyrosine kinase inhibitor; and suramin, an inhibitor of many growth factors. These results indicate that growth stimulatory substances of 3T3-L1 CM activate receptor tyrosine kinase activity in SP1 cells and are within the heparin binding growth factor superfamily. Antibody against HGF at 15 μg/ml completely reduced CM stimulatory activity; antibodies against IGF-I, bFGF, PDGF, and EGF had no inhibitory effect. HGF was mitogenic for SP1 cells at concentrations between 10 and 20 ng/ml. A synergistic effect was observed on the proliferation of SP1 cells in response to both HGF and bFGF together. These data demonstrate that 3T3-L1 CM contains a mitogenic (HGF-like) component which regulates SP1 cell growth. Acid-treated CM (pH 3, 30 min) showed no stimulatory activity and suppressed growth of SP1 cells, suggesting that CM contains biologically inactive inhibitory molecules which are activated by mild acidification. Together, our present data demonstrate that 3T3-L1 CM contains both mitogenic (HGF-like) and mitoinhibitory growth substances which regulate SP1 growth. These results highlight the importance and the complexity of stromal-tumor cell interactions, and show that stromal cells, in particular adipocytes may not only provide growth substances for tumor cells, but growth inhibitory molecules as well. (Supported by grants from NCI(C) and MRC).

Y 125 MULTISTEP MAMMARY CARCINOGENESIS: IDENTIFICATION OF COOPERATING PROTO-ONCOGENES (*Wnt-1* and AIGF/*Fgf-8*) BY RETROVIRAL INSERTION IN TRANSGENIC MICE.

Gregory M. Shackleford, Craig A. MacArthur and Deepa B. Shankar, Division of Hematology-Oncology, Departments of Pediatrics and Microbiology, Childrens Hospital Los Angeles and University of Southern California School of Medicine, Los Angeles, CA 90027

We are using retroviral insertional mutagenesis as a means of activating and molecularly tagging proto-oncogenes that participate in mammary tumorigenesis in mice. We have previously found that the kinetics of mammary tumorigenesis in *Wnt-1* transgenic mice is accelerated by infection with mouse mammary tumor virus (MMTV), and that resulting tumor DNAs contain proviruses integrated near the transcriptionally activated proto-oncogenes *int-2/Fgf-3* or *hst/Fgf-4* (ref. 1). By examination of the tumors that lack activation of these genes (and other genes that are usual targets of MMTV insertions), we have detected a new common integration site for MMTV, which contains the recently identified AIGF/*Fgf-8* gene (androgen-induced growth factor; ref. 2). Eight of 80 tumors examined from infected *Wnt-1* transgenics contained insertions in this locus, and 7 of the 8 showed transcriptional activation of *Fgf-8* (by northern analysis of total cellular RNA). *Fgf-8* RNA was absent in other tumors and in normal mammary tissue. These findings strongly suggest that *Fgf-8* cooperates with *Wnt-1* in mammary tumorigenesis. Further characterization of the *Fgf-8* gene showed that normal expression was detectable only in testis and ovary (by poly(A)+ RNA northern analysis of 13 tissues). Sequencing of genomic and cDNA clones revealed that *Fgf-8* has 5 coding exons, in contrast to the 3 exons found in other *Fgf* genes. Alternative splicing between two splice donors and two splice acceptors in exons 2 and 3, respectively, was detected in 3 cDNA clones; each clone encoded a different isoform of the protein. The above results identify a third *Fgf* gene as a collaborator with *Wnt-1* in mammary tumorigenesis, and suggest that genes of the *Wnt* and *Fgf* families cooperate effectively in this process.

1. Shackleford GM, MacArthur CA, Kwan HC, and Varmus HE. *Proc. Natl. Acad. Sci.*, USA **90**:740-744 (1993).
2. Tanaka A, Miyamoto K, Minamino N, Takeda M, Sato B, Matsuo H and Matsumoto K. *Proc. Natl. Acad. Sci.*, USA **89**:8928-8932 (1992).

Y 126 IN VITRO MODEL FOR STROMAL/EPITHELIAL CELL INTERACTIONS IN HUMAN PROSTATE.

S. C. Strom, M.T. Thompson, T.A. Howard, K.C. Song and M.J. Becich. Cell and Molecular Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA 15261.

Cultures of replicating epithelial or stromal cells were obtained from human radical prostatectomy specimens by sequential collagenase digestion. Fresh isolates contain both epithelial and stromal components, however, selective stimulation of the growth of specific cell types results in cultures which are predominantly (>90%) stromal or epithelial. Stromal cell growth is stimulated in media containing 5% serum, while epithelial cell growth is stimulated in serum-free media containing Epidermal Growth Factor (EGF) or Hepatocyte Growth Factor (HGF). Stromal cells in culture express muscle actin, vimentin and are PSA negative. Epithelial cells express cytokeratin 903, and PSA. Ultrastructurally, the epithelial cells contain junctional complexes, have well developed endomembranes and contain numerous vesicles, presumably containing secretory products. Isolated cells have a finite lifespan and continue to proliferate for 6-12 weeks. There is evidence for a paracrine pathway of interactions between stromal and epithelial cells components in the prostate. Stromal cells, but not epithelial cells, express mRNA for the growth factor, HGF. Epithelial cells, but not stromal cells, express c-met, the plasma membrane receptor for HGF. The role of the HGF/c-met pathways in neoplasia was investigated by immunohistochemical techniques on parafin sections of pathology specimens. While most tumors express low or normal levels of c-met, as compared to normal prostate, a higher level of expression of c-met was observed in locally invasive carcinomas. These results suggest that production of HGF by stromal cells may contribute, in a paracrine manner, to the growth and/or differentiation of epithelial cells in the prostate and that alterations in the HGF/c-met pathway may contribute to neoplasia.

Y 127 α_1 ANTITRIPSIN INHIBITS TGFα RELEASE FROM MCF-7 CELLS, Jonathan Yavelow, Anna Tuccillo, Patricia McClister, Department of Biology, Rider College, Lawrenceville, NJ 08648 and Thomas Finlay, NYU Medical Center, New York, NY 10016.

Human breast cancer cells synthesize and release a variety of growth modulating substances in response to estrogen stimulation and it is generally accepted that the growth promoting effects of estrogens are due, at least in part, to this autocrine/paracrine mechanism. Several of these growth modulating substances, including TGFα, the insulin-like growth factors and TNFα and its receptor, have been shown to require pericellular proteolysis for activation or release and it is our working hypothesis that this pericellular proteolysis is regulated by endogenously synthesized protease inhibitors. In earlier studies we demonstrated that MCF-7 human breast cancer cells were able to synthesize the protease inhibitors α_1-antitrypsin (α_1-AT) and α_1-antichymotrypsin (α_1-ACHY) and that the synthesis of these two proteins varied amongst different MCF-7 sublines. In one subline, α_1-AT synthesis which normally was very low, could be stimulated more than 10-fold by the cytokine IL-1. Using this subline we showed a negative correlation between anchorage-independent growth of MCF-7 cells in soft agar (a well-established measure of tumorigenesis) and endogenous synthesis of α_1-AT i.e. that agents which stimulated synthesis of α_1-AT also blocked colony formation. Consistent with the above, we have identified a membrane-anchored pericellular protease on MCF-7 cells that is neutralized by α_1-AT.

In this communication we show, using a sensitive ELISA assay, that MCF-7 cells synthesize and release TGFα from its membrane-bound precursor (a process known to require proteolytic cleavage) and that release of TGFα is stimulated by estradiol and blocked by α_1-AT. Similar results were obtained by immunoprecipitation of soluble TGFα from conditioned media of MCF-7 cells metabolically labeled with ^{35}S-cysteine. Since TGFα is known to stimulate colony formation by MCF-7 cells growing in soft agar, these results add support to our hypothesis that the tumorigenic/metastatic potential of a cancer cell is a function of the amount or ratio of amounts of the protease inhibitors and proteases it produces.

Y 128 ROLE OF THE *c-erb* B2/*neu* ONCOGENE IN HUMAN PROSTATIC CANCER PROGRESSION. Haiyen E. Zhau and Jianxin Zhou, Department of Urology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030.
c-erb B2/*neu* (or *neu*) has been demonstrated to be a transforming oncogene in both rodent (Sikes and Chung, Cancer Res. 52:3174, 1992) and human (Zhou et al., J. Urol. 149:374A, 1993) prostatic epithelial cells. Previously (Zhau et al., Mol. Carcinog. 5:320, 1992), we identified that *c-erb* B2/*neu* or HER-2, was overexpressed but not amplified in human prostate cancer. To understand the potential role of *neu* in human prostatic cancer progression, we used a gene transfer procedure to determine whether *neu* amplification/overexpression leads to increased tumor growth and metastasis. We chose the poorly tumorigenic, androgen-independent PC-3 cell line as a target for gene transfer. PC-3 cells were transfected with (1) pSV*neu*-T (a point-mutated rat *neu* oncogene construct) and pSV2*neo*, and (2) pSV2*neo* as control. Fifty single-cell clones were selected for further characterization. Southern blot analysis demonstrated that four cell clones had different sites and copy numbers of pSV*neu*-T integration. These clones were selected for further molecular and biological analysis. Clone 35 was found to have increased *neu* amplification and overexpression of *neu* mRNA and protein. Although this clone showed a rate of *in vitro* growth comparable with that of the controls, it acquired the ability to grow in soft agarose and exhibited marked tumorigenic and metastatic potential. Subcutaneous inoculation of clone 35 resulted in an enhanced rate of tumor growth in athymic mice, but orthotopic injection of the same clone to the dorsal prostate of athymic mice resulted in widespread dissemination of PC-3 cells to various sites, notably the lymph nodes, kidney, spleen, interlobular space of the pancreas, bone and the soft tissues surrounding the bone, and body wall musculature. Although clones 17, 21, and 22 were all found to overexpress *neu* mRNA and protein, only clones 21 and 22 were tumorigenic when injected subcutaneously into mice. Reverse transcription and PCR of total cellular RNA of the transfected cells revealed that the transfected *neu* oncogene was dominantly expressed in the cell clones. Further characterization of these cell clones is in progress. In conclusion, although the point-mutated form of the *neu* oncogene has not been identified in human malignancies, *neu* amplification and/or overexpression has been detected in human prostate cancer. The present model of *neu*-induced human prostatic cancer growth and metastasis further suggests that the *neu* oncogene plays a role in human prostatic cancer progression.

Polypeptide Receptors; Steroid Receptors

Y 200 TIME-DEPENDENT INHIBITION OF RAT 5α-REDUCTASE ISOZYMES 1 AND 2 BY 4-aza- AND 6-aza-STEROIDS, Ken W. Batchelor*, J. Darren Stuart*, H. Neal Bramson *, and Stephen V. Frye+, Departments of Cellular Biochemistry* and Medicinal Chemistry+, Glaxo Research Institute, Research Triangle Park, NC 27709
Steroid 5α-reductase (EC 1.3.99.5) is responsible for conversion of testosterone to dihydrotestosterone (DHT) in target tissues, such as the prostate gland. Inhibition of 5α-reductase blocks the formation of DHT and reduces prostate size in rats and in man as a treatment for benign prostatic hypertrophy. Recent studies have demonstrated the finasteride is a time-dependent inhibitor of both human prostatic 5α-reductase (predominantly isozyme 2) (Faller, B., et al (1993)) and of human 5α-reductase 1 (Tian, G., et al submitted). Therefore, we have investigated time-dependent inhibition of rat 5α reductase isozymes 1 and 2 by 4-aza- and 6-aza-steroids with a view to understanding better species differences as they relate to in vivo activity and potential clinical efficacy.

Initial studies have indicated that the 4-aza-1,2-enes are time-dependent and probably irreversible inhibitors of the rat 5α-reductase 2. However, in contrast to the human isozyme, none of these compounds exhibit time-dependent inhibition of the rat 5α-reductase 1. The significance of these observations for in vivo effects on DHT levels and prostate weight are discussed.

Y 201 ANTAGONISM OF ESTROGEN RECEPTOR BINDING TO DNA AND ESTROGEN RESPONSE ELEMENT-DEPENDENT TRANSCRIPTION BY A NOVEL SUBSTITUTED INDOLE IS UNRELATED TO BINDING OF ESTRADIOL TO ITS RECEPTOR. Alan J. Bitonti, Jennifer A. Dumont, Francesco G. Salituro, Ian A. McDonald, Paul S. Wright, Russell J. Baumann, Marion Merrell Dow Research Institute, Cincinnati, OH 45215.
A novel substituted indole (8-{2-[1-(4-chloro-benzoyl)-5-hydroxy-2-methyl-1*H*-indol-3-yl]-acetylamino}-octanoic acid butyl-methyl-amide, MDL 101,906) was synthesized with the aim of finding a new pure antiestrogen. MDL 101,906 was synthesized by addition of the 7α alkyl side chain from the pure antiestrogen ICI 164,384 onto the 3-position of a substituted indole nucleus. MDL 101,906 (5-20 μM) inhibited estrogen receptor (ER) binding to an estrogen response element (ERE) in DNA mobility shift assays done with nuclear extracts from MCF-7 human breast cancer cells treated with the drug. Mobility shift assays with cytosol extract were not as significantly inhibited. Addition of the drug directly to mobility shift assays was not inhibitory. Mobility shift assays conducted with nuclear extracts and response elements for SP1, CTF/NF1 and glucocorticoid receptor were unaffected by drug treatment. MDL 101,906 did not compete for estradiol (E2) binding to MCF-7 ER. In transfection experiments with an estrogen-dependent luciferase reporter plasmid in MCF-7 cells, MDL 101,906 inhibited ER-dependent transcription (IC_{50}=2-5 μM). The effects of the drug on E2/ER-dependent processes was apparently due to depletion of ER from MCF-7 nuclei . Measurement of ER in MCF-7 nuclear extracts using an ER-enzyme immunoassay (Abbott) showed that MDL 101,906 caused significant depletion of nuclear ER (60% depletion with 20 μM). MDL 101,906 inhibited the growth of MCF7 and tamoxifen-resistant LY-2 cells with IC_{50}'s of 3.8 and 4.7 μM, respectively. Experiments to determine antitumor efficacy of MDL 101,906 are in progress.

Y 202 SERUM, PHORBOL ESTER AND TESTOSTERONE REGULATE EXPRESSION OF A FIBROBLAST GROWTH FACTOR 1 (FGF-1) TRANSCRIPT. Ing-Ming Chiu[1], Robert A. Payson[1], and Stephen E. Harris[2], Department of Internal Medicine[1], The Ohio State University, Columbus, OH 43210 and Department of Medicine[2], University of Texas, San Antonio, TX 78249

The human fibroblast growth factor 1 (FGF-1) is a potent mitogen for mesoderm-derived cells such as fibroblasts, smooth muscle cells, and endothelial cells. It also stimulates the growth of cells of ectodermal origin such as prostate epithelial cells. This implicates FGF-1 as a growth factor in the pathogenesis of atherosclerosis as well as in prostate cell growth and malignancy. There now appears to be at least four 5' non-coding exons for the human FGF-1 gene. These exons and their associated promoters confer tissue-specific expression. As such, FGF-1.A transcript is expressed in kidney; 1.B transcript is expressed in brain; while 1.C and 1.D transcripts are expressed in a variety of tissue cultured cells. Here we show that expression of the FGF-1.C transcript is upregulated by testosterone in the androgen-dependent prostate carcinoma cell line LNCaP. In contrast, FGF-1.C is expressed in the absence of testosterone in PC-3 cells, an androgen-independent prostate carcinoma cell line. No detectable levels of FGF-1.C transcript are present in brain or kidney as ascertained by RNase protection analysis. We further show that FGF-1 promoter 1.C is activated in PC-3 cells treated with either serum or phorbol ester, PMA, following serum starvation. These data suggest that: 1) FGF-1 is at least one growth factor that abrogates the requirement for testosterone in androgen-independent prostate cancer; and 2) serum mitogens may function at least in part through protein kinase C to induce FGF-1 expression in PC-3 cells. We have observed that the AP-1 binding site, known to be involved in the transcriptional response of cells to phorbol ester, is present twice 5' of the 1.C transcription initiation site. Also located 5' is a putative androgen response element (ARE). We are currently investigating the functions of these putative *cis*-acting sequences.

Y 203 EXPRESSION OF PROLACTIN RECEPTOR IN HUMAN BREAST CARCINOMA, Charles V. Clevenger, Theresa L.M. Pasha and John E. Tomaszewski, Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center, Philadelphia, PA 19104

The neuroendocrine hormone prolactin (PRL) is a growth factor required for the proliferation and terminal differentiation of the epithelial components within the human breast. These effects are mediated by the PRL receptor (PRLr), a member of the growth factor receptor family. Three PRLr isoforms (long, intermediate, and short) have been identified in the rat, which differ in the length of their intracytoplasmic domains. In humans, however, only the long PRLr isoform had been identified previously. We present here, for the first time, that expression of all three PRLr isoforms occurs in human breast carcinoma when assayed by immunoblot analysis and RT/PCR. PRLr expression in breast carcinoma is heterogenous; this heterogeneity ranges from abundant expression of each PRLr isoform to virtually undetectable expression of any isoform. This contrasts to the normal breast where moderate expression of the intermediate isoform is observed. Immunohistochemical analysis of breast tissues using an anti-PRLr antibody confirms these analyses, and demonstrates specific labelling of the cell surface and cytoplasm of epithelial cells and activated fibroblasts. A statistically significant correlation between expression of the PRLr and the estrogen receptor was observed. These data suggest that quantitation of the PRLr may serve as a prognostic marker and indicate a role for the PRLr in the pathogenesis of human breast carcinoma.

Y 204 17ß-ESTRADIOL STIMULATES MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION IN ZR-75-1 BREAST CANCER CELLS. Anthony A. Colletta, Fiona V. Howell and Christopher J. Marshall, Hartwell Laboratory and Chester Beatty Laboratories, Institute of Cancer Research, Fulham Road, London SW3 6JJ, UK.

The mechanism by which steroid hormones promote mitogenesis in breast cancer cells is not known although data from several laboratories suggest some crosstalk between steroid hormone action and signal transduction pathways. We have examined the activation of the mitogen activated protein (MAP) kinase pathway by estradiol (E2) in ZR-75-1 breast cancer cells. Treatment with 10nM E2 stimulates MAP kinase activity 4-5 fold, assessed by immunoprecipitation of MAP kinase and phosphorylation of myelin basic protein as the kinase substrate. This kinase activity is maximal within 30 minutes and returns to basal levels within 120 minutes. The activation of kinase function is accompanied by the 42 to 44 kDa shift seen with activation of this kinase due to phosphorylation on tyrosine and threonine residues.

MAP kinase activation by E2 is not reversed by co-treatment of the cells with 1µM tamoxifen or ICI 164,384 suggesting an E2 receptor (E2R) independent effect. Overexpression of E2R in ZR-75-1 cells followed by E2 treatment does not augment the induction of MAP kinase seen in the wild-type untransfected cells, again suggesting an E2R independent mode of action of E2. Transient expression of E2R in Cos-1 cells does not confer MAP kinase induction following 10nM E2 treatment and surprisingly leads to a ~40% reduction in kinase activity compared to E2R-transfected control Cos-1 cells.

These data suggest that E2 may promote mitogenesis in breast cancer cells by a complex interplay of transcriptional events mediated by E2R and by interaction with as yet unidentified components of cellular signal transduction pathways.

Y 205 THE EXPRESSION OF THE EXON 5-DELETION VARIANT OF ER mRNA IN TAMOXIFEN RESISTANT BREAST TUMOURS AS DETECTED BY RT/PCR, Angela A.I. Daffada, S.R.D. Johnston, N.King and M. Dowsett, Academic Department of Biochemistry, Institute of Cancer Research, Royal Marsden Hospital, Fulham Road, LONDON SW3 6JJ, UK.

The growth of the majority of breast cancers is dependent on oestrogen, which binds to and stimulates the trans-activator activity of oestrogen receptor (ER) protein. Anti-oestrogens such as Tamoxifen inhibit the proliferation of cancer cells by binding to ER and altering its transcriptional activity. However, many breast cancers are 'de novo' resistant or develop 'acquired' resistance to tamoxifen. In addition, some ER negative (ER-) tumours express progesterone receptor (PgR) and PS2 proteins whose expression is normally dependent on oestrogenic stimulation through ER. These findings may be due a variant ER such as the exon 5-deletion splice variant (Fuqua *et al.*, 1991) which still binds to DNA and is constitutively transcriptionally active, independent of both ligand and Tamoxifen.

We have used RT/PCR to investigate the incidence of this ER mRNA species in 79 human breast cancers, including primary untreated tumours and Tamoxifen resistant tumours. Exon 5-deletion variant mRNA (V) was present in addition to wild type ER mRNA (WT) in 90% of tumours. We have confirmed a high relative expression of V to WT in tumours in the ER-,PgR/PS2+ phenotype (median V/WT ratio 46.5%). Overall the V/WT ratio was greater in this phenotype compared either with ER+,PgR/PS2+ tumours (9.5%, $p = 0.0002$), or ER-,PgR/PS2- tumours (25.3 %, $p = 0.03$). Untreated ER+ primary breast cancers expressing PgR and/or PS2, which would be expected to be sensitive to Tamoxifen had a low V/WT ratio (median 6%, range 0 - 15%). Tumours which were 'de novo' resistant to Tamoxifen had a significantly higher V/WT ratio (26%, $p = 0.02$), whereas tumours which acquired resistance following an initial response had a median V/WT ratio of 14% (NS). Tumours resistant to adjuvant tamoxifen when the initial hormone sensitivity was unknown had a significantly higher expression of V/WT mRNA compared with controls (35%, $p = 0.02$).

These results confirm a high relative expression of this exon 5-deletion splice variant mRNA species is associated with the presence of PgR and PS2 in ER- tumours, and suggest that a role for the variant may exist in Tamoxifen resistant breast cancers.

Y 206 MODULATION OF TYROSINE KINASE SIGNALING PATHWAYS IN HUMAN BREAST CANCER CELLS. Roger J. Daly, Michele D. Binder, Peter W. Janes and Robert L. Sutherland. Cancer Biology Division, Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney, NSW 2010, Australia.

A number of intracellular targets for receptor tyrosine kinases (RTKs) have recently been identified. These include the src homology (SH)-2 domain-containing proteins phospholipase Cγ-1, the GTPase activating protein for ras and the adaptor protein Grb2. Grb2 serves to link tyrosine kinases to ras signaling by complex formation with the GTP-GDP exchange protein Son of Sevenless (Sos), and overexpression of Grb2 leads to increased activation of ras and the mitogen activated protein (MAP) kinase cascade in response to epidermal growth factor and insulin.

Since upregulation of Grb2 could modulate the growth factor sensitivity of tumor cells by amplification of the ras signaling pathway, the expression of the Grb2 gene was investigated in a series of 19 human breast cancer cell lines and compared to normal breast epithelial cells. The majority of cell lines expressed similar Grb2 mRNA levels to the normal controls. However, 7 cell lines exhibited overexpression of Grb2 mRNA and protein and in some cell lines, including MDA-MB-361 and -453, this was accompanied by amplification of the Grb2 gene locus.

In order to determine whether ras signaling was activated in breast cancer cells expressing high levels of RTKs, two breast cancer cell lines that overexpress c-erbB2 (SK-BR-3 and BT-474) were used as models. Western blotting of erbB2 immunoprecipitates from serum starved cells revealed both receptor tyrosine phosphorylation and co-precipitation with Grb2, suggesting stimulation of the ras pathway. However, MAP kinase activity could only be detected in SK-BR-3 cells and not in BT-474 cells or in cell lines expressing normal levels of c-erbB2 (MCF-7 and T-47D). After preincubation of the cells with the protein tyrosine phosphatase (PTPase) inhibitor sodium orthovanadate, MAP kinase activity was markedly increased in BT-474 but not in MCF-7 or T-47D cells. This suggests either down regulation of pathways leading to MAP kinase activation or dephosphorylation of MAP kinase itself by PTPase activity in some c-erbB2 overexpressing lines. Thus activation of MAP kinase is not an inevitable consequence of c-erbB2 overexpression.

These results identify both positive (Grb2) and negative (PTPase) modulators of the ras signaling pathway in human breast cancer cells and indicate that growth factor receptor expression *per se* may not be an accurate indicator of mitogenic signaling potential.

Y 207 VITAMIN D RECEPTORS AND ACTIONS IN CULTURED HUMAN PROSTATE CANCER CELLS, David Feldman, Roman Skowronski, Donna Peehl and Scott Cramer, Departments of Medicine and Urology, Stanford University School of Medicine, Stanford, CA 94305

It has been suggested that vitamin D deficiency may promote prostate cancer, although the mechanism is not understood. In this study, we examined three human prostate carcinoma cell lines (LNCaP, DU-145 and PC-3) as well as primary cultures of epithelial and fibroblastic cells established from normal, BPH and malignant tissues. The cells were studied both for the presence of specific $1,25(OH)_2$-vitamin D receptors (VDR) and also employed to assess the effects of hormone on cell proliferation. Ligand binding experiments demonstrated classical VDR in all cells with binding capacity ranging between 20-80 fmol/mg protein. The presence of VDR was confirmed by immunocytochemistry. In addition, a 4.6 kb mRNA transcript hybridizing with a specific human VDR cDNA probe was identified in all cells. $1,25(OH)_2D_3$ treatment stimulated induction of 24-hydroxylase mRNA, employed as a marker of $1,25(OH)_2D_3$ action. $1,25(OH)_2D_3$ also caused a dose-dependent stimulation of prostate specific antigen (PSA) secretion by LNCaP cells. Physiological concentrations of $1,25(OH)_2D_3$ dramatically inhibited proliferation of the LNCaP, PC-3 and primary cells but not DU-145 cells. Both epithelial cells and fibroblasts were growth-inhibited but morphology and keratin expression were not appreciably altered. A series of vitamin D analogues, with reduced hypercalcemic activity *in vivo*, were examined for their ability to bind to VDR and inhibit growth of LNCaP cells. Some analogues were more potent than $1,25(OH)_2D_3$ in binding and growth inhibition, raising the possibility that antiproliferative activity might be achieved in patients without inducing hypercalcemia. In conclusion, these results demonstrate that benign and malignant human prostate carcinoma cells possess VDR and that $1,25(OH)_2D_3$ treatment can elicit an antiproliferative action in these cells. The findings lend support to the hypothesis that vitamin D might exert beneficial actions on prostate cancer.

Y 208 RECOMBINANT SINGLE CHAIN IMMUNOTOXINS SPECIFIC FOR EGF & ERBB-2 RECEPTORS INHIBIT IN VIVO & IN VITRO TUMOR CELL GROWTH , N.E. Hynes, B.M. Marte, P. Hellmann, R. Beerli, B. Groner* & W. Wels*, Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel, Switzerland, *Tumor Biology Center, P.O. Box 1120, D-79011 Freiburg, Germany.

cDNA coding for heavy and light chain variable domains of Mab225, which binds the extracellular domain of the EGFR, was derived by reverse transcription and PCR amplification of hybridoma cell mRNA. The variable domains were connected with a synthetic linker sequence and fused to a truncated *Pseudomonas* exotoxin A (ETA) gene. Recombinant scFv(225)-ETA was expressed in E.coli and purified by affinity chromotography. scFv(225)-ETA behaves as the parental Mab in that it competes with EGF for binding to EGFR and inhibits ligand activation of the receptor. scFv(225)-ETA inhibits, in a dose dependent manner, the *in vitro* growth of both the EGFR overexpressing tumor cell lines A431 and MDA-MB468. scFv(FRP5)-ETA, a recombinant single chain immunotoxin specific for the erbB-2 receptor (Wels et al., 1992, Cancer Res. 52:6310-6317) inhibited the growth of erbB-2 overexpressing SKBR3 cells but had no effect on MDA-MB468 cells which do not express erbB-2. Suprisingly, the growth of A431 cells, which express low numbers of erbB-2 receptors, was strongly inhibited by scFv(FRP5)-ETA. Combination treatment with scFv(FRP5)-ETA + scFv(225)-ETA led to synergistic inhibitory effects on the *in vitro* growth of A431 cells. The data suggest that in addition to the level of receptor expression other cell type specific factors such as the rate of receptor turnover or EGF/erbB-2 receptor cross-talk contribute to the efficacy of immunotoxin treatment. In a nude mouse tumor model of A431 xenografts, treatment with scFv(FRP5)-ETA and the combination of scFv(FRP5)-ETA + scFv(225)-ETA led to a growth arrest of established tumors during the course of treatment. scFv(225)-ETA treatment alone was the most effective leading to tumor shrinkage, whereas treatment with Mab225 only led to retarded tumor growth. This shows that the growth inhibitory effect of Mab225 can be potentiated by recombination of the antigen binding site with a toxic effector function.

Y 209 INFLUENCE OF DIFFERENT HORMONAL AGENTS ON COLONY FORMATION AFTER DIFFERENT DOSES OF RADIATION TREATMENT OF DIFFERENT CANCER CELLS.

Lundgren S, Paulsen GHU, Marthinsen ABL, Strickert T. Department of Oncology, University of Trondheim, Norway.

Treatment of breast cancer is often a combination of different treatment modalities (i.e. surgery, radiation, hormonal and cytotoxic treatment). An increased number of women are offered conservative surgery and radiotherapy. Some of those women will also receive hormonal and/or cytotoxic therapies.

This study focuses on the possible influence of different hormonal treatment (estradiol, antiestrogens and progestins) on colony formation of different breast cancer cell lines in soft agar. Both estradiol reseptor positive and negative (MCF-7 and MDA-MB-231) cell lines have been studied. The MCF-7/TAM^R (AL-1) cell line has also been studied. Radiotherapy as 6 MV photons is given both as different single dose (0-10 Gy) and as multiple doses to obtain survival curves. Other parameters as steroid receptor (ER and PgR), DNA ploidity, reduced and oxidised gluthathione content will also be presented.

Y 210 DIFFERENTIATION THERAPY WITH THE PROGESTERONE ANTAGONIST ONAPRISTONE IN EXPERIMENTAL BREAST AND PROSTATE CANCER

Horst Michna, Martin Schneider, Svante Gehring, Karsten Parczyk, Research Laboratories of Schering AG, Berlin, F.R.Germany

The presence of progesterone receptors in a high percentage of mammary carcinomas provides the rationale for the testing of the growth inhibitory potential of progesterone antagonists in experimental breast cancer models. Progesterone antagonists proved to possess mammary carcinoma inhibitory potential in a panel of both chemically induced and transplantable rodent tumors as well as xenografts of human tumors in nude mice. Interestingly, their growth inhibiton is accompanied by differentiation. Our panel of indicators of differentiation include a decrease in the volume fraction of undifferentiated epithelial cells, in tumor grading, in the amount of lectin binding sites containing α-L-fucose and in immunolocalized tenascin expression as well as an increase in the volume fraction of dysplastic ducts, casein filled vacuoles, epithelial TGF β1 expression, of cells in G_0G_1 and the proportion of apoptotic epithelial nuclei. Finally, in the R 3327 H experimental prostate carcinoma model Onapristone induces signs of differentiation and a reduction of tumor growth identical to the efficacy of castration if the amount of progesterone receptors is stimulated by a concomitant administration of the estrogen diethylstilbestrol.

Y 211 SLOW, TIGHT BINDING INHIBITORS OF TYPE II HUMAN STEROID 5-ALPHA REDUCTASE,

Moss, M.L., Stuart, J. D., Kuzmic, P.,‡ Bramson, H.N., Tian, G., McGeehan, J., Batchelor, K.W., Frye, S.V., and Wiseman, J.S., Departments of Biochemistry, Cellular Biochemistry, and Medicinal Chemistry, Glaxo Research Institute, 5 Moore Dr., Research Triangle Park, NC 27709 and ‡School of Pharmacy, Department of Medicinal Chemistry, University of Wisconsin, Madison, 53706

MK-906 is a drug used clinically to alleviate symptoms of benign prostatic hyperplasia, a disease driven primarily by the androgen dihydrotestosterone. The drug's capacity to inhibit 5-α-reductase, an enzyme that converts testosterone to dihydrotestosterone, also makes it potentially efficacious in the treatment of prostate cancer. Recently, a novel class of 6-aza steroid inhibitors has been reported that shows promising activity against both the type I and II human steroid 5-α reductases (Frye et al., Submitted to J. Med. Chem.). We have compared the mechanism of inhibition of the type II enzyme by the Δ-1,4-aza steroid, MK-906, and by the 6-aza steroid, 17β-N,N-diethylcarbamoyl-6-aza-androst-4-en-3-one. Both steroids inhibit the type II 5-alpha reductase in a time dependent fashion at pH 6 and 37°C. The slow onset of inhibition observed with MK-906 is at least partly due to its ability to covalently modify the type II 5-α-reductase. Labelling studies performed with ^{14}C-MK-906 demonstrate that the enzyme is modified stoichiometrically in a 1:1 ratio. The 6-aza steroid, 17β-N,N-diethylcarbamoyl-6-aza-androst-4-en-3-one, in contrast to MK-906, is a reversible competitive inhibitor. Inhibition of the type II enzyme by 17β-N,N-diethylcarbamoyl-6-aza-androst-4-en-3-one proceeds in a stepwise fashion. The inhibitor and enzyme form an initial complex with a K_i of approximately 20 nM. A subsequent step follows to generate EI* with an overall inhibition constant estimated to be 0.2nM. The data presented here demonstrate that both classes of inhibitors are potent against type II 5-alpha reductase, although their mechanisms of inhibition are different.

Y 212 TUMOR NECROSIS FACTOR AND INTERFERON ARE SELECTIVELY CYTOSTATIC FOR HORMONE-DEPENDENT AND HORMONE-INDEPENDENT HUMAN BREAST CANCER CELLS, Heinz Mueller, Nathalie, Flury, Rong Liu, Sibylle Scheidegger and Urs Eppenberger, Department of Research and Gynecology, University Medical School, CH-4031 Basel, Switzerland

Tumor necrosis factor α (TNF-α) inhibited the growth of nonstimulated, epidermal growth factor-, insulin-like growth factor 1-, and estrogen-stimulated hormone-dependent human breast cancer cells with a half-maximal inhibition at 0.25nM. In contrast, the growth behavior of hormone-independent cells was not affected by TNF-α alone. However, in the presence of 500-1000 U/ml interferon (INF) α or γ, high concentrations of TNF-α (1nM) were able to inhibit the growth of hormone-independent cells. TNF-α increased the c-myc message in hormone-dependent cells, whereas in hormone-independent cells only in the presence of INF-γ a significant increase in c-myc message upon TNF-α stimulation could be demonstrated. No significant changes of either the p75 or the p55 TNF receptors were observed upon INF-γ treatment of hormone-independent cells. This indicates that the increased responsiveness of these cells for TNF-α is not due to a change in available TNF receptors. The affinity of the receptors for TNF-α are one order of magnitude different for the two cell types (2-5nM and 20-50nM for the hormone-dependent and the hormone-independent cells, respectively). After INF-γ treatment, the EC_{50} of hormone-independent cells shifted towards the EC_{50} of hormone-dependent cells. Thus, treatment of TNF-unresponsive human breast cancer cells with INF interconverted the low-affinity TNF receptors to high- affinity. Taken together, our data imply that the most benefical treatment for breast cancer patients with an advanced disease is a combination therapy of TNF-α and other cytokines such as INF.

Y 213 INTRON 1 ELEMENTS ARE INVOLVED IN EGFR GENE REGULATION IN BREAST CANCER CELLS

Jane M. Murphey and Susan A. Chrysogelos, Department of Biochemistry and Molecular Biology and Lombardi Cancer Center, Georgetown University, Washington, DC 20007

The estrogen independent state of human breast cancer is characterized by the loss of estrogen receptor (ER) and the acquisition of high levels of epidermal growth factor receptor (EGFR). EGFR has been shown to be a better predictor for failure with endocrine therapy than ER status, strongly supporting the hypothesis that overexpression of EGFR is a critical step in the progression to estrogen independence. DNase I hypersensitivity assays were performed in several human breast cancer cell lines with a wide range of EGFR levels to localize regions of the EGFR gene that are functionally implicated in its expression. In ER negative breast cancer cell lines with high levels of EGFR a group of hypersensitive sites appear within the first intron indicating that this region of the gene is involved in regulation of expression. Several CAT constructs containing the SV40 promoter and portions of the first intron have been created and utilized in transient transfection assays in selected breast cancer cell lines. In cell lines that express low levels of EGFR there is a 2-3 fold increase in transcription when the intron region is utilized as an enhancer. However, in high expressors of EGFR a 15 fold increase in transcription is observed. The sequence of the first 2.5 kb of the first intron was also analyzed and this analysis revealed several consensus regulatory elements. Additionally, in response to the inaccuracy of the B-gal method of normalization, we have devised a method of measuring transfection efficiency involving Southerns to measure a ratio between the transfected DNA and an endogenous histone H4 gene. This ratio gives us a more accurate representation of transfection efficiency and eliminates the possibility of artifact due to promoter cross-competition. Experiments are in progress to delineate regulatory elements within the intron region of EGFR through CAT assays, gel shift assays and DNase I footprinting.

Y 214 COMPARATIVE IMMUNOHISTOCHEMICAL ANALYSES OF C-ERB B2, P53 AND BCL2 EXPRESSION IN ARCHIVAL PROSTATE CANCER TISSUES, K. Shankar Narayan, John A. Arcadi, and Lawrence W. Jones. Huntington Medical Research Institutes, Pasadena, CA.

This communication summarizes the results of our analyses for the expression of bcl-2 and c-erb-b2 oncoproteins and p53 mutant protein in 87 archival prostate cancer specimens obtained from routine fixed and paraffin embedded TUR tissues over a 11 yr. (1980-91) period. Expression of these proteins in the selected specimens was detected by immunohistochemical techniques utilizing commercially available monoclonal antibodies: p53 DO_7 (Novacastra labs, U.K.); c-erb-B2 CB-11 (Biogenex Labs, CA and Novacastra, U.K.) and bcl-2- 124 (Dako Corporation, CA) and the Vectastain Elite ABC kit from Vector Labs, CA. Markedly improved detection of the various antigens was obtained by the use of a microwave heating procedure (10 minutes heating in 10mM citrate buffer, pH 6.0, Shi et al J Histochem. Cytochem. 39: 74, 1991). Significant to extensive expression of the c-erbB2 oncoprotein was detected in 87% (65/87) of the specimens with expression in tumor cells spanning a wide range of differentiation. Marked expression of bcl-2 oncoprotein was detectable in a large number of tumor cells present in 44% of archival specimens with a tendency for preferential expression in cells of well to moderately differentiated glandular tumors. In contrast, significant p53 mutant protein expression was found in only 29% (26/86) of the tumor specimens studied. Staining patterns for p53 mutant protein was difficult to categorize but often appeared to be in moderate to poorly differentiated tumor cells.

Y 215 EVIDENCE OF A HIGH AFFINITY NEUROTROPHIN RECEPTOR IN HUMAN PROSTATE EPITHELIA AND STIMULATION OF TYROSINE PHOSPHORYLATION OF THE RECEPTOR BY HUMAN PROSTATE STROMAL PROTEIN. Beth R. Pflug, Craig Dionne, John H. Lynch and Daniel Djakiew, Department of Cell Biology and Department of Surgery, Division of Urology, Georgetown University Medical Center, Washington DC 20007 and Cephalon, West Chester, PA 19380

A nerve growth factor (NGF)-like protein secreted by human prostate stromal cells has been shown in our laboratory to have a mitogenic effect on the TSU-pr1 prostate epithelial tumor cell line. We have previously localized the low affinity neurotrophin receptor (p75) to human normal prostate epithelium while expression of p75 was absent from the prostate epithelial cell lines, TSU-pr1, DU-145, PC-3 and LNCaP. The low affinity receptor (p75) and the high affinity neurotrophin receptors (*trk*) have been shown to bind neurotrophins. However, only the *trk* receptors mediate neurotrophin signaling while the role of p75 is still not clear. Our current studies reveal a high affinity neurotrophin receptor localized primarily to the epithelia of prostate tissue by immunocytochemistry using a pan-*trk* antibody. Immunoblot analysis showed a 140 kD immunoreactive band present in TSU-pr1 cell lysates immunoprecipitated with a pan-*trk* antibody. In TSU-pr1 cells stimulated for 5 minutes with a neurotrophin present in human prostate stromal cell secretory protein (hPS), a 140 kD band was evident by immunoblot analysis using an anti-phosphotyrosine antibody, while phosphorylation was not observed in untreated cells. Displacement of ^{125}I-NGF from TSU-pr1 cells and primary prostate epithelia with NGF and hPS was observed while no displacement was evident with EGF nor PDGF. Scatchard analysis revealed only high affinity binding in TSU-pr1 cells (K_d = 10 pM) and both high (K_d = 10 pM) and low (K_d = 1 nM) affinity binding was observed in primary prostate epithelial cells. This study suggests that functional high affinity neurotrophin receptors are present on prostate epithelia and are responsive to a NGF-like protein secreted by prostate stromal cells.

Y 216 SIGNIFICANCE OF IMMUNOHISTOCHEMICAL C-ERB B-2 LOCALIZATION FOR PROGNOSIS OF PRIMARY BREAST CANCER, Eva Spitzer[1], Wolfgang Zschiesche[1], Ines Schönborn[2] and Richard Grosse, [1] Max-Delbrück-Center for Molecular Medicine, 13122 Berlin, [2] Department of Gynecological Pathology, University Hospital "Rudolf Virchow", Freie Universität Berlin

Recently, we provided evidence that overexpression of c-erbB-2 as analysed by immunostaining of membranous p185 is an independent prognostic factor especially for breast cancer patients at high risk. In all studies of c-erbB-2 overexpression, cytoplasmatic reactivity has been neglected or even interpreted as artifactual. Recent studies have revealed, however, that binding of potential ligands of p185 in tumor cells leads to a transient shift from membranous to cytoplasmic p185 immunostaining, functional differentiation and decreased proliferation of the cells. Therefore, we investigated histopathological and immunochemical characteristics of 463 primary breast cancers concerning a possible correlation of the immunochemical localization pattern of p185 with clinical parameters and its relevance to prognosis. The percentages of p185 membrane reactive (m+), cytoplasma (c+) and membrane/cytoplasma reactive carcinomas (m/c+) amounted to 21%, 26% and 20%, respectively, whereas the negative (-) tumor portion was 33%. Immunocytochemistry revealed an highly significant relationship between estrogen receptor and progesterone receptor status and c-erbB-2 overexpression. An inverse correlation was found for c-erbB-2 (-) and m+ tumors, whereas both c+ and m/c+ immunostaining were directly correlated. In analogy, c-erbB-2 (-) and m+ carcinomas correlated directly, both c+ and m/c+ tumors correlated inversely with the grading. There was no significant relation between c-erbB-2 localization pattern and histological tumor type, lymph node status and tumor size. Concerning the overall survival after 9 years there were no differences between those carcinomas with (-) reaction (72%), c+ (69%) and m/c+ (76%) reaction. For patients with c-erbB-2 m+ carcinomas the overall survival was 30%.

Y 217 ANDROGEN RECEPTOR MUTATIONS IN HUMAN HORMONE-INDEPENDENT PROSTATE CANCER, Taplin, ME, Bubley,G, Franz,ME, Balk, SP, Division of Hematology/Oncology, University of Massachusetts Medical Center, Worcester, MA 01655 and Beth Israel Hospital,Boston, MA 02215.

Prostate cancer cells were obtained from bone marrow metastases of patients with hormone independent disease. Androgen receptor (AR) message (exons B-H) was amplified and quantitated using RT-PCR. Significant levels of AR transcripts were detected in all samples. PCR products from three patients were cloned into pBluescript and multiple independent isolates were completely sequenced. Several point/mutations in AR were found in multiple plasmids from two patients. The AR germline DNA was normal.

Pt.	Domain	Codon	Substitution	Amino Acid	Frequency
1	hormone-binding	877[2]	C→G	Thre→Ser	Majority
1	hormone-binding	877[3]	T→C	Thre→Thre	Minority
1	hormone-binding	899[3]	C→T	Ile→Ile	Minority
1	hormone-binding	792[2]	A→G	Gln→Arg	Minority
2	DNA-binding	595[2]	G→A	Cys→Tyr	Majority
2	DNA-binding	605[2]	A→G	Lys→Arg	Majority
2	hormone-binding	880[1]	A→G	Thr→Ala	Minority
2	hormone-binding	902[2]	A→G	Gln→Arg	Minority

These data indicate that the AR is expressed in high levels in the hormone independent state and that in a subset of patients mutations in the AR are heterogenous and may be involved in the evolution to hormone independence.

Y 218 DIFFERENTIAL EXPRESSION OF ESTROGEN RECEPTORS AND EPIDERMAL GROWTH FACTOR RECEPTORS IN HUMAN BREAST CANCER USING DUAL-STAINING IMMUNOHISTOCHEMISTRY.

Ton van Agthoven[1], Mieke Timmermans[2], John A. Foekens[3], Sonja C. Henzen-Logmans[2] and Lambert C.J. Dorssers[1]. Departments of Molecular Biology[1], Pathology[2] and Endocrine Oncology[3], Dr. Daniel den Hoed Cancer Center, P.O. Box 5201, 3008 AE Rotterdam, the Netherlands.

Expression of EGFR is inversely associated with ER expression in primary breast tumors. Although expression of ER and EGFR is inversely related, about 50% of ER positive tumors contain EGFR, using biochemical assays. However these assays do not allow for the identification of the cellular origin of these receptors. We have established a double immunohistochemical method to determine simultaneously ER and EGFR expression patterns in biopsies of breast tumors at the single cell level. Using a panel of transfected human breast cancer cell lines the procedure was optimized and verified for specificity. In this study ER and EGFR were assayed on 28 selected ER positive breast tumors and 10 non-malignant breast tissues. ER positive tumor cells were detected in 26 cases (92.9%). EGFR positive tumor cells were detected in 7 cases (25%). In 5 of these tumors both ER and EGFR were detected in tumor cells, but localized in distinct cells. Only in 1 case of DCIS coexpression was observed in a subset of tumor cells. ER and EGFR expression was observed in normal cells present in most biopsies. Furthermore, coexpression of ER and EGFR on individual luminal epithelial cells was frequently observed in normal or benign breast tissues. This study shows that ER and EGFR in breast tumor cells are inversely related at the single cell level. However, demonstration of ER and EGFR in normal luminal cells shows that expression is not mutually exclusive. In addition to biochemical assays, immuno-histochemical analysis of ER and EGFR expression may help to give better understanding and more accurate predictions concerning prognosis and response to hormonal therapy. Current studies focus on the interplay between these signal transduction pathways.

Y 219 STUDIES ON GROWTH OF THE HUMAN PROSTATIC CANCER CELL LINE LNCaP

J. Veldscholte[1], M.H.A. Oomen[2], J.P.T.M. van Leeuwen[3], E. Mulder[1], and G.J. van Steenbrugge[2], Depts. of Endocrinology & Reproduction[1], Urology[2], and Internal Medicine III[3], Erasmus University Rotterdam, P.O.B. 1738, 3000 DR Rotterdam, The Netherlands

LNCaP cells contain androgen receptors which show an altered steroid specificity, due to a mutation in the steroid binding domain (Thr 868 replaced by Ala). Despite the absence of receptors for progesterone and for estradiol, the growth rate of the parental, androgen dependent LNCaP-FGC (FGC) cells increased when cultured in the presence of either estrogens or progestagens. In addition, most antiandrogens were growth stimulators as well. Only the antiandrogen ICI 176 334 could block transcription and cell growth through the mutant receptor. This was probably the result of the capability of ICI 176 334 to block dissociation of heat-shock proteins (hsp56, hsp70, and hsp90) from the androgen receptor and to prevent tight nuclear binding of the androgen receptor.

VD_3 (1,25 dihydroxyvitamin D_3, which did not bind to the androgen receptor in LNCaP cells) inhibited androgen-induced proliferation of the androgen-dependent FGC line at concentrations between 1 and 100 nM, but did not change the rate of proliferation when given alone. Two androgen-independent sublines of LNCaP, LNO and R, respectively, both containing androgen receptors, showed a reduction of their growth rate above 1 nM of either the androgen R1881 or VD_3. The combination of R1881 and VD_3 was most potent in reducing the growth rate of these sublines.

In conclusion: induction of growth of FGC cells by estrogens, progestagens, and most antiandrogens is caused by a mutation in the steroid binding domain of the androgen receptor which prevents a blockade of receptor function by most antiandrogens, but not by ICI 176 334. This might be due to a difference in the mechanism by which these compounds block receptor function. VD_3 inhibits both androgen-induced and androgen-independent growth of LNCaP cells by a mechanism that does not involve androgen receptor binding.

Study supported by the Dutch Cancer Society (Grant IKR 90-13).

Y 220 PROTEIN KINASE C IN ANDROGEN-SENSITIVE AND INSENSITIVE PROSTATIC TUMOUR CELLS: EFFECTS ON CELL GROWTH AND GENE EXPRESSION, Pirkko Vihko and Pirkko Henttu, Biocenter and Department of Clinical Chemistry, University of Oulu, FIN-90220 Oulu, Finland

Unstimulated DU-145 and PC-3 prostatic cells, which are androgen-insensitive, contained 4-5.5-fold more protein kinase C activity that was sensitive to Ca-ions and phospholipid than did the androgen-sensitive LNCaP cells. TPA-activation increased the amount of PKC activity about 2.2-fold in LNCaP cells within 3 hours, but slightly decreased the activity in DU-145 and PC-3 cells, to 60 and 81% of the control, respectively. TPA-treatment did not affect the growth of PC-3 cells, but slightly decreased the growth of DU-145 cells during the first three days of treatment. In contrast, treatment of LNCaP cells with TPA resulted in the death of approximately 50% of cells within 2 days. TPA had only minor effects on the expression level of the immediate early genes c-Fos and C-Jun in PC-3 cells, whereas in DU-145 and LNCaP cells the amounts of these mRNAs were up-regulated within 3 hours after stimulation. Interestingly, the amount of c-Myc mRNA was up-regulated by TPA in DU-145 and PC-3 cells but down-regulated in LNCaP cells. Activation of PKC in LNCaP cells resulted in the down-regulation of the steady-state levels of mRNAs coding for androgen receptor and two prostatic marker proteins: prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA). Furthermore, in LNCaP cells that contained TPA-activated PKC androgen-regulation of these mRNAs was abrogated. However, the androgenic up-regulation of PSA and down-regulation of PAP mRNA did not require the presence of active PKC as was demonstrated by LNCaP cells that were depleted of PKC with a 2-day treatment of TPA. In contrast, the effect of androgens on the human androgen receptor mRNA was inversely correlated to the activity of PKC. These results suggest that commonly used prostatic cell lines LNCaP, DU-145 and PC-3 differ in respect to the activity and effects of PKC. Lack of prostate-specific gene expression may partly result from the high basal level of PKC activity found in DU-145 and PC-3 cells. Furthermore, PKC activation interferes with androgen-regulated gene expression.

Y 221 RHAMM IS REQUIRED FOR THE MOTILITY AND INVASION OF HUMAN BREAST CANCER CELLS *IN VITRO*.

Chao Wang and Eva A. Turley. Manitoba Institute of Cell Biology,and Department of Pediatrics and Physiology,University of Manitoba,100 Olivia Street, Winnipeg, Manitoba, Canada R3E 0V9

RHAMM (Receptor for Hyaluronan-Mediated Motility) regulates the locomotion of smooth muscle cells, ras-transformed fibroblasts, TGF-β stimulated fibroblasts, maligant human B cells, activated human thymocytes, and macrophage chemotaxis *in vitro*. Here we investigated the role of RHAMM in five human breast cancer cell lines (HBCCL). MDA-MB-231, and MDA-MB-468 are esotrogen receptor negative and hormone independent, while MCF7, ZR-75-1 and T47D are esotrogen receptor positive and hormone dependent in their growth characteristics. Only MDA-MB-231 forms invasive colonies in a Matrigel invasion assay, is invasive and metastatic in NCr nude mice and is more invasive in Boyden chamber chemoinvasion assays. Immunofluorescence of cell monolayers revealed homogenous staining for RHAMM in all HBCCL. Western blot analysis of RHAMM in cell lysates showed that one RHAMM isoform (48KDa) was elevated in MDA-MB-231. Correspondingly a 5.8 Kb RHAMM mRNA was elevated as detected by Northern blot analysis. Quantitation of the rate of cell locomotion by image analysis indicated that MDA-MB-231 moved 1.8 - 4.0 times faster than other cell lines. The addition of anti-RHAMM antisera, specific to a sequence ($aa^{269-288}$) encoded in RHAMM cDNA, or of a peptide mimicking one of RHAMM's HA binding domains significantly reduced MDA-MB-231 cell random locomotion by 60%. Invasion of collagen gels was also significantly blocked by anti-RHAMM antisera (anti-peptide $aa^{269-288}$ antibody and anti-RHAMM fusion protein antibody). These results indicate that RHAMM contributes to the motility and invasion of metastatic human breast cancer cells *in vitro*.

Y 222 HUMAN GROWTH HORMONE TRANSGENIC MICE DEVELOPS MALIGNANT MAMMARY TUMORS; POSSIBLE INVOLVEMENT OF THE PROLACTIN RECEPTOR.

Håkan Wennbo and Jan Törnell, Department of Physiology, University of Göteborg, Medicinareg 11, S-413 90 Göteborg, Sweden.

Growth hormone (GH) is an important regulator of somatic growth but it's possible role in pathological growth (eg tumor formation) is less clear. A high incidence of malignant tumors in the female mammary gland have been reported in patients with acromegaly and GH's possible role in mammary tumors is also indicated by that forty percent of breast cancer patients have elevated GH levels.

We have previously reported a high incidence of mammary adenocarcinomas in female mice from two different strains of human-GH (hGH) transgenic mice.

HGH can bind both to somatogenic GH-receptors and lactogenic prolactin (PRL) receptors.

In this study have we tried to clarify the importance of PRL-receptors in the tumor development.

Method: We used metallothionein promoter bovine-GH (bGH) transgenic mice. Bovine-GH binds only to GH-receptor and not to PRL-receptor. Transgene integration was identified by PCR. The transgenic mice were examined for mammary tumors by palpation and histologically.

Results: Nine female bGH transgenic mice from the same founder-animal were included in this study. BGH levels in serum was 1440 ± 204 ng/ml in female mice from this fonder and the weight at 80 days of age were 38,1 ± 1,5g in female transgenic animals and 24,3 ± 2,1g in the control group. Eight of the nine female transgenic mice were 52-73 weeks of age when sacrificed. There were no palpable tumor in any of them. Histological examinations were carried out in seven of the nine mice. The mammary gland was normal in all histologically examined mice.

Conclusion: BGH transgenic mice do not develop mammary cancer in contrast to hGH transgenic mice. This indicates the importance of the PRL-receptor in the tumor induction of hGH. HGH induces malignant tumors in the mammary gland in transgenic mice by the PRL-receptor alone or in combination with the GH-receptor.

Metastases and Angiogenesis; Cell Cycle/Paracrine Interactions

Y 300 **NEW CD44 SPLICE VARIANTS ASSOCIATED WITH HUMAN BREAST CANCERS**, Lilly Y. W. Bourguignon and Naoko Iida, Department of Cell Biology and Anatomy, School of Medicine, University of Miami, Miami, FL 33101

Recent studies indicate that the expression of CD44 on the cell surface changes profoundly during tumor metastasis, particularly during the progression of various carcinomas. In this study we have acquired 20 human breast carcinoma samples that were collected following radical mastectomy. Our data, using RT-PCR techniques, indicate that multiple species of CD44v are detected in all 20 of the breast carcinomas tested, but not in normal tissues or cells. Most importantly, we have found that larger CD44v species (>700bp) are present in only the metastatic but not the non-metastatic breast carcinomas. Furthermore, the PCR products of CD44 variant isoforms were cloned and sequenced. Nucleotide sequence analysis has revealed at least two different CD44 variants displaying unique splice choices of variant exons. The first CD44 variant analyzed [designated as CD44v-I] contains the insertion of exons 10,11,12,13 and 14 within the membrane proximal variable region (between exon 5 and exon 15). This differs from the epithelial form (CD44E) and standard form (CD44s) known to contain only exons, 12,13 and 14 or no insertion between exon 5 and exon 15, respectively. The expression of CD44 variants with additional exons 10 and 11 has been suggested previously to be responsible for tumor progression and metastasis. Therefore, it is quite possible that the expression of CD44v-I is directly associated with tumor metastasis. The second CD44 variant [designated as CD44v-II] shows unique splice choices of variable exons in which exon 12 and 13 are inserted, but exon 14 is spliced out. Preliminary data indicate that exon 14 displays extensive O-and N-linked glycosylation sites which may be important for a number of biological functions (e.g. adhesion, migration and metastasis). To our knowledge, the expression of CD44v-II has not been described in any other tumors. The preferential expression of CD44v-II in various metastatic breast cancer tissues suggests that CD44v-II may be unique for human breast cancer and could potentially be a very useful marker for establishing the progression of breast cancer development.

Y 301 THE ROLE OF ANDROGEN IN STROMAL-EPITHELIAL INTERACTIONS IN THE PROSTATE, Anne. Collins and David. E. Neal, Department of Surgery, The Medical School, University of Newcastle, Newcastle upon Tyne, U.K. NE2 4HH

Stromal proliferation plays a key role in the development of benign prostatic hyperplasia in humans, yet little is known about the effect of sex steroids on human prostatic stromal growth. Stromal and epithelial cells derived from collagenase-digested, benign hyperplastic adult prostates were characterised as to their androgen receptor status and stromal cells to their growth response to mibolerone, dihydrotestestone (DHT), hydrocortisone, oestradiol and the anti-androgen cyproterone acetate.

Steroid hormone binding was quantified in intact cells using [^{3}H] Mibolerone. Specific high affinity binding sites were detected in both stromal and epithelial cells (K_d = 3.8 ± 0.3 x 10^{-9}M and 4.6± 0.16 x 10^{-9}M respectively) with each containing 10^4 receptors per cell. Mibolerone (10^{-10}M) had a significant stimulatory effect on DNA synthesis (only in serum-free media). This effect was steroid-specifc as neither hydrocortisone, oestrodiol and DHT had any effect on DNA synthesis. This mibolerone-mediated stimulation could be effectively antagonized in culture by the specific androgen receptor antagonist cyproteone acetate suggesting that the mitogenic action of mibolerone on prostatic stromal cells may be mediated by an androgen receptor pathway.

Y 302 THE USE OF A NOVEL MOUSE MODEL FOR PROSTATE CANCER METASTASIS TO DETERMINE ALTERATIONS IN GENE EXPRESSION IN THE METASTATIC PHENOTYPE. **J.A. Eastham, S. H. Park, C. Ren, T.L. Timme, P.A. Baley, D. Kadmon, L..A. Donehower, P.T. Scardino, T.C. Thompson, Houston, Texas.**

Although metastatic disease is responsible for the majority of prostate cancer deaths, there is little information regarding the natural history of metastasis and/or the phenotype of metastatic prostate cancer at the molecular and cellular level. This profound lack of information derives in part from the lack of adequate *in vivo* models for prostate cancer metastasis. We have recently modified the mouse prostate reconstitution (MPR) model system by using p53 "knockout" mouse tissue as a target for transduction of initiating genetic alterations. The *ras* and *myc* oncogenes are transduced using Zipras/myc 9 into total urogenital sinus (UGS) derived from wild-type (+/+) 129/Sv mice; heterozygous (+/-) p53 "knockout" mice; and homozygous (-/-) p53 "knockout" mice. Subsequently MPRs are introduced under the renal capsule of normal wild-type (129/Sv) adult male mice and evaluated 3-5 weeks later for phenotypic progression. Zipras/myc 9 produced predominantly hyperplastic tissue with only one case of focal prostate cancer in +/+ MPRS (n=21). Interestingly, Zipras/myc 9 produced malignant carcinomas in all cases and resulted in metastatic deposits in both +/- (n=14) and -/- (n=5) MPRs. The pattern of metastasis was similar to that seen in humans including the studding of the mesentery with small metastatic deposits as well as extensive skeletal and lung metastases. RNAs isolated from early passage cell strains derived from the primary tumors and clonal cell lines derived from metastatic deposits removed from the same animal (n=7 animals) were examined for levels of TGF-β1 mRNA. In 3 of 4 +/- and 3 of 3 -/- animals there was significant reduction in TGF-β1 mRNA levels in metastatic cells compared to primary carcinoma cells (26%-69% reduction in normalized mRNA levels). All of the metastatic clones were either originally p53 nullizigous or had lost expression of p53 in the primary tumor and metastatic cell lines. Interestingly, in the one +/- MPR which retained expression of p53, TGF-β1 mRNA levels were increased 33% in the metastatic versus the primary cancer cells. Therefore down-regulation of TGF-β1 mRNA levels is associated with the acquisition of the metastatic phenotype and may be related to the complete loss of p53 function.

Y 303 EXPRESSION OF THROMBOSPONDIN (TSP-1) AND ITS RECEPTORS (CD36, CD51) DURING DEVELOPMENT OF THE HUMAN MAMMARY GLAND AND IN NORMAL, HYPERPLASTIC AND NEOPLASTIC HUMAN BREAST

L. Frappart, P. Clezardin, C. Péchoux, C.M. Serre, JL. Vauzelle. Laboratoire d'Anatomie Pathologique, Bât 10 and INSERM U 234, Pavillon F, Hôpital Edouard Herriot, F 69437 Lyon

The role of extracellular matrix proteins during development of the human mammary gland is largely unknown. Thrombospondin (TSP) is a trimeric extracellular matrix glycoprotein involved in embryogenesis and organogenesis. Five genes encoding for four distinct TSPs (TSP1, TSP2, TSP3, TSP4), and cartilage oligomeric matrix protein (COMP) have recently been described. In this study, the patterns of expression of TSP1 and of two known cell surface receptors: CD36 and CD51 have been determined during development of the human fetal mammary gland and in normal, hyperplastic and neoplastic human breast. Using both immunohistochemistry and immunoelectron microscopy, TSP1 was found in the dense mesenchyme immediately adjacent to the mammary bud, and at the membrane of budding epithelial cells invading the surrounding mesenchyme. As formation of the ductal tree system occurs, TSP1 becomes expressed at the myoepithelial-stromal junction of mammary ducts. Such an immunolocalization of TSP1 in buds and ducts of the fetal mammary gland has been confirmed at the mRNA level by *in situ* hybridization using an antisense probe and also by polymerase chain reaction assay. Comparison of TSP1 immunolocalization with that of CD36 and CD51, revealed no colocalization of TSP1 with these receptors during mammary gland development. As opposed to TSP1, CD36 was strongly expressed at the membrane of preadipocytes present in the fat pad tissue, but absent from epithelial cells of the mammary bud. CD51 was only weakly expressed by budding mammary epithelial cells and did not colocalize with TSP1. By contrast, in budding mammary epithelial cells, TSP1 was colocalized with CDw49b, a collagen receptor which has also been reported as being a putative TSP receptor. In nonlactating ducts of normal and hyperplastic breast, TSP1 and CD51 are expressed in the basement membrane and in the basal surface of myoepithelial cells, respectively. In lactating adenomas, both TSP1 and CD51 disappear from the myoepithelial-stromal junction of the ducts. However, TSP1 becomes selectively expressed at the apices of secretory epithelial cells of lactating ducts together with CD36, suggesting that the distribution of TSP1 and the appearance of its receptors are dependent on the secretory activity of human mammary ducts. In neoplastic human breast, a strong immunoreactivity for TSP1 is observed in the basement membrane surrounding *in situ* carcinomas and excessive TSP1 deposits are observed in desmoplasia of invasive ductal carcinomas and in fibroblast present in desmoplastic areas. On the other hand, few invasive ductal carcinoma cells express TSP1, while CD51 is moderately expressed by some neoplastic clusters, and no immunoreactivity is observed for CD36. By contrast, TSP1 is codistributed with CD51 in most of the invasive lobular carcinoma cells and with CD36 in a subpopulation. Taken together, these findings suggest that TSP1 plays an important role during development and differentiation of the human mammary gland and in carcinomas of the breast.

Y 304 THE PATTERN OF DEPOSITION OF HYALURONIC ACID AND HYALURONIDASE IN THE DEVELOPING PROSTATE IS RECAPITULATED IN PROSTATE CANCER

P. Gakunga, S. Shuster, G. Cunha, R. Stern., University of California, San Francisco

Hyaluronic acid (HA) is associated with rapid tissue growth observed during embryological development. In parenchymal glands it is associated with mesenchymal-epithelial interactions that result in branching morphogenesis. HA enhances this process by creating hydrated pathways, as well as by direct interaction with cells through specific HA-cell surface receptors. Prostate cancer is associated with changes in the stroma that facilitate tumor progression. These changes are an apparent recapitulation of the embryologic development pattern.

Histolocalization of HA in 0-60 day-old mouse prostate gland sections was determined using a biotinylated HA-binding peptide from the tryptic digestion of cartilage-derived proteoglycans. HA deposition was localized to the stromal matrix, more prominently around the proliferating ductal tips. There was reduced deposition in association with the developmentally quiescent glands. Hyaluronidase, the principal enzyme that degrades HA, was immunolocalized to the cytoplasm of epithelial cells. The pattern of HA and hyaluronidase deposition seen in the developmentally active early stages was recapitulated in human prostate cancer tissue, but not in normal nor benign tumor prostatic tissue. Evidently, epithelial-mesenchymal interactions during embryonic glandular development are comandeered to facilitate prostate cancer invasion. Whether such patterns are prognostic indicators for an aggressive course is currently under investigation.

(Supported by NIH grant HD 25505, CA44768)

Y 305 A NOVEL APPROACH FOR DETECTING p53 MUTATIONS IN HETEROGENEOUS PROSTATE TISSUE SAMPLES USING TA-CLONING-PCR-SSCP. Angelo E. Gousse, Kevin Slawin, Thomas Wheeler, Peter T. Scardino, and Timothy C. Thompson, Houston, TX.

Mutations in the p53 gene have been detected in a significant proportion of high-grade, late-stage prostate cancers. However, studies performed using conventional PCR–SSCP have rarely detected p53 mutations in early clinical prostate cancers. It is possible that p53 mutations are present, but escape detection due to the technical limits of the assay. When screening for mutations using PCR–SSCP or dot-blot oligonucleotide hybridization, the researcher often makes subjective decisions by comparing the analyzed sample to (+) and (-) controls. These controls are usually either 100% wild-type or 100% mutant, yet test samples can lie anywhere between these two extremes depending on the heterogeneity within the sample itself. We have developed a novel approach for screening tissues for gene mutations in a more objective and rigorous manner than is commonly performed. The steps involved include: 1) Tissue microdissection and DNA preparation 2) Exon specific PCR amplification 3) TA-Cloning (Invitrogen, Inc.) of the amplified gene product 4) PCR/SSCP screening of pooled clones 5) repeat screening of selected unpooled clones with identification of individual TA-mutants 6) Direct solid-phase DNA sequencing of identified mutant clones. After a typical tissue analysis, a profile is obtained consisting of the number of wild-type fragments identified, as well as the number and sequence of any mutant species detected. The sensitivity of this technique is limited only by the number of clones one is willing to screen, and by the spontaneous error rate of *Taq* polymerase. This error rate is low, and the errors are relatively specific in nature. We have used this approach to screen *ras*-initiated mouse prostate reconstitution tissue specimens as well as hyperplastic human prostate samples for mutations in the p53 gene. While conventional PCR–SSCP failed to detect any mutations, our modified approach has identified missense base substitutions within exon 5 of the p53 gene occurring in approximately 5% of all clones analyzed, after correcting for presumed polymerase-generated errors. We are currently screening a panel of human prostate cancer specimens for p53 mutations using this technique. We propose this novel approach will reveal subsets of cells harboring p53 mutations which would not be identifiable using conventional methods. The identification of p53 mutations within small populations of tumor cells may have clinical significance in prostate carcinogenesis.

Y 306 PROSTATE AND BREAST ADENOCARCINOMA DEVELOPMENT IN TRANSGENIC MICE, Maroulakou, I.[1], Garrett, L.[1], Anver, M.[2], Papas, T.[3], Green, J.[1], [1]LMO, NCI; [2]PRI/DynCorp, Frederick, MD; [3]CMSB, HCC, Charleston, SC.

Few useful animal models exist for the study of prostate carcinoma. We have developed a transgenic mouse system in which the progression of mild hyperplasia to overt adenocarcinoma may be studied. The rat C3(1) hormone responsive promoter directs the expression of SV40 T_{AG} to prostate tissue in males, whereas female animals express T_{AG} in mammary tissue and develop adenocarcinoma of the breast. This construct also directs expression of T_{AG} to other tissues with the development of osteosarcoma and follicular thyroid carcinoma. Interestingly, these animals develop abnormalities in cartilage growth, especially of the epiphyseal plates and trachea. These transgenic animals provide a useful model for studying and treating the progressive development of cancer in the prostate and breast, as well as studying hormone-responsive elements in vivo.

Y 307 REGULATION OF THE INVASIVENESS OF THE HUMAN BREAST CANCER CELL LINE MCF-7 BY AGENTS THAT ACT THROUGH PROTEIN KINASE-C. M.D. Johnson, J. Torri, M.E. Lippman and R.B. Dickson, Lombardi Cancer Center, Georgetown University, Washington, DC 20007. Successful metastasis depends on several tumor cell properties: the motility of the cells, the ability to bind to other cells and substrata such as the basement membrane, and the ability to proteolytically degrade such substrata. There is evidence that all of these processes are regulated at some level by Protein Kinase-C (PKC) mediated pathways. We have measured the effects of agents that modulate PKC activity on the motility, invasiveness and attachment properties of the human breast cancer cell line MCF-7. Motility was assessed by the Boyden chamber assay in which the ability of the cells to move towards a chemoattractant is measured. Invasiveness was determined in a similar assay except the cells were required to pass through an artificial basement membrane. Matrigel adhesion was measured in an assay that determines the ability of the cells to adhere to a substratum. Even at low concentrations (10^{-10}M), TPA produced a 14 fold increase in the motility of MCF-7 cells. Higher concentrations were required to stimulate the invasiveness of the cells (maximal at 10^{-8}M) but again the increase was significant (18 fold). TPA increased the ability of the cells to adhere to fibronectin, laminin, and to the extra-cellular matrix extract matrigel. The macrocyclic lactone Bryostatin-1 which has been shown to compete with TPA for binding to PKC was able to inhibit the effects of TPA; equimolar concentrations producing approximately 50% inhibition. The ability of cells to degrade the extracellular matrix is believed to depend on their ability to elaborate matrix metallo-proteinases (MMPs). We have therefore studied the effects of TPA and Bryostatin-1 on the synthesis and secretion of MMP-9 by these cells. TPA produced an at least 100 fold induction of MMP levels in conditioned media (Maximal at 10^{-8}M). This induction was inhibited by Bryostatin-1. Further studies are underway to determine the mechanisms underlying these effects and to continue the work *in vivo* in the nude mouse to allow the potential of Bryostatin-1 as an antimetastatic agent to be assessed.

Y 308 SERUM METALLOPROTEINASES IN PROSTATIC CANCER, Jonathan Waxman, Simon Tickle*, Harpreet Wasan and Terry Baker*, Department of Clinical Oncology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London and Celltech*, Slough, United Kingdom.

Metastases and local progression may result from the inappropriate activity of metalloproteinases or of their regulatory peptides. We have developed assays for interstitial collagenase, stromelysin 1 and tissue inhibitors for metalloproteinase 1 and 2 which we have used to study sera from 40 patients with prostatic cancer, 21 patients with active rheumatoid arthritis and 56 age matched controls. Patients with prostatic cancer had higher levels of TIMP-1 and collagenase ($p=0.0001$) and lower levels of TIMP-2 ($p=0.003$) than controls. Patients with metastatic cancer had higher levels of collagenase than those without metastases ($p=0.02$). Patients with rheumatoid arthritis had higher levels of stromelysin than controls ($p=0.002$) or patients with cancer ($p=0.008$). Serum TIMP-1 in combination with collagenase levels was as sensitive as prostate specific antigen as a marker of metastatic disease.

Y 309 REGULATION OF T-47D BREAST CANCER CELL CYCLE PROGRESSION BY CYCLIN D1. Elizabeth A Musgrove, Christine SL Lee, Michael F Buckley and Robert L Sutherland. Cancer Biology Division, Garvan Institute of Medical Research, St Vincent's Hospital, Sydney, NSW 2010, Australia.

The sequential transcriptional activation of cyclins, the regulatory subunits of cell cycle specific kinases, is thought to regulate progress through the cell cycle. Cyclin D1 is most abundant during G_1 phase and is rapidly induced upon treatment of breast cancer cells with steroid and growth factor mitogens. Amplification and overexpression of the cyclin D1 gene are observed in a significant proportion of breast tumors. Therefore, the consequences of increased cyclin D1 expression were investigated using T-47D breast cancer cells expressing cyclin D1 under the control of a metal-inducible metallothionein promoter.

Zinc induction of cyclin D1 led to an increase in cyclin D1 protein levels within 3 h (to a maximum of 7-fold at 9-12 h). This was followed by a marked increase in the proportion of cells in S phase, commencing approximately 9 h after treatment. The entry of cells into S phase was preceded by induction of cyclin D3 mRNA and phosphorylation of the retinoblastoma gene product (pRB), to which D-type cyclins bind, suggesting that these late G_1 events depend, at least in part, on the action of cyclin D1. Increased expression of cyclin D1 accelerated cells through G_1, demonstrating that cyclin D1 is rate-limiting for progress through G_1 phase. In cells treated with a range of zinc concentrations, the increase in the fraction of cells entering S phase was proportional to the relative level of cyclin D1 protein. Thus, the initiation and rate of progress through G_1 both depend on the level of cyclin D1. Furthermore, induction of cyclin D1 was sufficient to cause serum-deprived, growth-arrested cells to progress through G_1 phase into S phase, a process which required the addition of growth factors (e.g. IGF-I) in control cells containing the expression vector alone.

These data demonstrate a critical role for cyclin D1 in human breast cancer cell cycle control and suggest that deregulated expression of cyclin D1 is likely to reduce dependence on normal physiological growth stimuli, potentially providing a growth advantage to tumour cells.

Y 310 BIOLOGY OF MICROMETASTATIC TUMOR CELLS IN BONE MARROW IN BREAST AND PROSTATE CANCER,

K. Pantel, G. Schlimok, S. Braun, D. Kutter, Ch. Klein, R. Oberneder, R. Riesenberg and G. Riethmüller, Institute of Immunology & Dept. of Urology, University of Munich, Munich; Medical Clinic II, Central Hospital, Augsburg, Germany

We have recently demonstrated the prognostic relevance of an immunocytochemical assay with monoclonal antibodies against cytokeratin component no. 18 (CK18) that allows the specific detection of individual carcinoma cells in bone marrow aspirates (Schlimok et al., PNAS 84:8672, 1987; Lindemann et al., Lancet 340:685, 1992; Pantel et al., Cancer Res. 53:1027, 1993). To gain insights into the biology of these cells, we performed immunocytochemical double marker analysis and in vitro cell culture of marrow specimens from patients with breast and prostate cancer.

Mononuclear cells in bone marrow aspirates were isolated by Ficoll-Hypaque density centrifugation. Epithelial tumor cells on cytological preparations were identified with mAb CK2 to CK18 and double stained with mAbs to the respective target proteins.

Coexpression of prostate-specific antigen on CK18+ cells in bone marrow of patients with prostate cancer demonstrated the prostatic origin of these cells. Typing of structures relevant to the immune response against tumor cells revealed a deficient expression of MHC class I antigens on micrometastatic breast cancer cells in 65.4 % of the specimems analyzed (n=26). While proliferation-associated nuclear proteins such as Ki-67 or p120 on micrometastatic cells were only detected in 2 out of 23 (8.7%) breast cancer patients, overexpression of the tyrosine kinase erbB2 was found in 48 out of 71 marrow specimens (67.6%). The incidence of $erbB2^+/CK18^+$ cells was positively correlated to the clinical stage of tumor progression. The proliferative potential of CK+ micrometastatic cells was further underlined by their in vitro response to EGF and bFGF, inducing a more than 100-fold increase in tumor cell numbers in 12 of 36 (33.3%) or 12 of 23 (52.2%) cultures from patients with breast or prostate cancer, respectively.

In conclusion, although the majority of micrometastatic tumor cells appears to be in a dormant state, they inherit a certain growth potential and develop effective mechanisms to escape the immune surveillance. The presented assays might therefore provide clinically relevant information regarding the oncogenic potential of individual carcinoma cells in bone marrow.

Y 311 CHARACTERIZATION OF THE METASTASIS SUPPRESSOR ACTIVITY ON HUMAN CHROMOSOME 11p11.2-13: DENTIFICATION OF A PUTATIVE METASTASIS SUPPRESSOR GENE FOR PROSTATIC CANCER.

Carrie W. Rinker-Schaeffer[1], Jin Tang Dong[2], Patty Lamb[2], J. Carl Barrett[2], and John T. Isaacs[1], [1]The Johns Hopkins University School of Medicine, Baltimore MD, and [2]The National Institute of Environmental Health Sciences, Chapel Hill, NC.

Metastatic progression of prostatic cancer (PC) involves multiple genetic changes. We have demonstrated that acquisition of metastatic ability requires both the loss of metastasis suppressor function(s) and the activation of oncogenes (Cancer Res. 52; 3486, 1992). These studies demonstrated that there is an activity(s) on human chromosome 11p11.2-13, which when introduced into rodent PC cells by microcell-mediated chromosomal transfer, is capable of suppressing metastatic ability without affecting tumorigenicity or growth rate *in vivo*. In contrast, introduction of this region has no effect on the *in vivo* growth rate or metastatic ability of rodent mammary cancer cells. This indicates that the metastasis-suppressor activity encoded by human chromosome 11p11.2-13 is PC-specific. We are presently using two approaches to identify potential metastasis-suppressor genes encoded by this region. The first approach addresses the biochemical mechanism of chromosome 11p11.2-13-mediated metastasis suppression. Initial studies characterized a number of biochemical properties of the metastasis-suppressed and control cell lines. These studies demonstrated that cellular motility is the parameter best correlated with the metastatic potential of the cancer cells. Additional studies examined factors which contribute to cellular motility, including collagenase and NM23 expression and ATP/GTP-binding protein profiles. Thus far, none of these individual parameters have shown a correlation with metastatic potential in our system. To identify other metastasis-suppressor activities, we have introduced the beta-galactosidase reporter gene into our cell lines by defective retrovirus transduction. This enzyme allows for sensitive detection of cancer cells *in vivo* and should enable the identification of the point of 11p11.2-13-mediated metastasis suppression and thus identify likely factors that contribute to this activity. These cell lines will also be used to establish an experimental bone metastasis system to allow further dissection of the metastatic pathway. Our second approach consists of positional cloning of genes which are differentially expressed in the metastatic and metastasis-suppressed cell lines. Specifically, Alu PCR was used to amplify sequences encoded by the human 11p11.2-13 region in the metastasis-suppressed rodent PC cells. Partial sequences of these PCR products were used to generate oligonucleotide probes for screening cDNA libraries. This method identified cDNA clones which were specifically expressed in the metastasis-suppressed cells. One such clone, JTD161, encodes a 267 amino acid protein which is highly homologous to the ME491 family of melanoma membrane antigens. JTD161 is strongly expressed in metastasis-suppressed PC cells and normal prostatic tissues. The expression of this gene is down-regulated in metastatic cancer cell lines examined thus far. JTD161 does not suppress the metastatic ability of rodent mammary cancer cells, suggesting that it may have PC specific metastasis-suppressor activity. (Supported by NCI Grant CA58236)

Y 312 PROSTATE STROMA-SPECIFIC GENE EXPRESSION,

David R. Rowley, Michael J. Gerdes, Truong D. Dang, and Lauren McBride, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

Prostatic epithelial cell differentiation is induced and directed by adjacent stroma through unknown mechanisms. In development, fetal urogenital sinus mesenchyme is androgen regulated to induce the differentiation program of epithelium. In the adult prostate gland, stromal-epithelial interactions are necessary for maintenance of androgen regulation and growth/differentiation control. It is likely that androgen regulated and stroma-specific genes are key regulators in prostate biology, specifically in regulation of growth control and may be critical to initiation and/or progression of prostatic carcinoma. Prostate stromal cell lines were developed to identify androgen regulated prostate stroma- specific genes. The PS-1 (adult rat ventral prostate) and U4F cell lines (fetal urogenital sinus) were characterized, and adapted to continuous culture in chemically-defined media. Proliferation of U4F and PS-1 cells was significantly stimulated by physiological concentrations of androgens (5-20nM testosterone, dihydrotestosterone) in comparison to other steroid hormones (glucocorticoids, progesterone, and estradiol). To identify androgen regulated transcripts, cDNA was prepared from these cell lines in +/- androgen conditions and analyzed for differential transcript expression with differential display reverse transcription polymerase chain reaction (DDRT-PCR) procedures. Multiple analyses of separate preparations of RNA/cDNA to identify consistent androgen regulated transcripts have identified 14 candidate transcripts from the PS-1 cell line. Each of these have been isolated, re-amplified, and cloned into the pCR II TA cloning vector for sequence analysis and probe development. Confirmation of specific expression of cloned transcripts is being assessed by Northern analysis utilizing in vivo tissue RNA obtained from intact and castrated rats, as well as human prostate tissues from normal, BPH, and prostatic carcinoma samples. Additional efforts are being directed at identification of corresponding full length cDNA sequences through the screening of prostate cDNA libraries.

Supported by NIH grants CA58093 and DK45909.

Y 313 DEPOSITION OF HYALURONIDASE IS AN INDICATOR FOR FAVORABLE OUTCOME IN BREAST CANCER.

Svetlana Shuster, Ann D. Thor, Helene S. Smith, Dan Moore, and Robert Stern. Brush Cancer Res. Inst., Cal. Pac. Med. Center, SF, CA, Lawrence Livermore Natl. Labs., Livermore CA, and Depts. of Path., UC San Francisco and Mass. Gen. Hosp., Harvard Med. School, Boston, MA.

Breast cancer is associated with stromal changes involved in local tumor progression and invasion. Enhanced deposition of hyaluronan (HA) is among such compositional changes. However, hyaluronidase, the enzyme that degrades HA, was found predominantly in tumor epithelial cells. Immunohistochemical localization was accomplished using rabbit polyclonal antisera to purified liver hyaluronidase. Such staining was performed on routine formalin-fixed, paraffin-embedded sections. At a dilution of the antisera of 1/1200, no staining was evident in normal breast tissue (reduction mammoplasty), nor in benign breast tumors. However, most breast cancers stained positively at this dilution of the probe. 211 archival cases with follow-up data available were examined. Staining intensity was scored using an arbitrary scale of 0 to +4. Increased intensity of staining correlated with a favorable outcome. Data were analyzed using Kaplan-Meyer survival curves. Of patients whose tumors stained > +2, overall survival was 70 % after 15 years. Of those with tumors that stained +2 or less, overall survival was 45% after ten years and 29% after 15 years, Staining for hyaluronidase may be an important prognostic indicator for breast cancer .

(Supported by DHHS NIH PO grant CA-44768)

Y 314 EFFECT OF 1,25-DIHYDROXYVITAMIN D_3 ON PROLIFERATION AND APOPTOSIS IN MCF-7 BREAST CANCER CELLS. Maura Simboli-Campbell, JoEllen Welsh and Martin P.R. Tenniswood, Department of Biochemistry, University of Ottawa, Ottawa, ONT K1H 8M5

The active form of vitamin D, $1,25(OH)_2D_3$, is a potent inhibitor of breast cancer cell growth, both *in vivo* and *in vitro*. Although several groups have investigated the anti-proliferative effects of $1,25(OH)_2D_3$, the link between $1,25(OH)_2D_3$ and apoptosis or active cell death (ACD) has not yet been investigated in breast cancer cells. Since cell number (and therefore tumor mass) represents a balance between the rates of proliferation and ACD, measurement of cell number alone will not identify which process is affected by $1,25(OH)_2D_3$. Treatment of subconfluent MCF-7 cells with $1,25(OH)_2D_3$ caused a 25% decrease in cell number by 48 hours and a 50% decrease by 96 hours. However cell proliferation, determined by ^{3}H-thymidine incorporation into DNA, did not decline until 72-96 hours following $1,25(OH)_2D_3$ treatment. Flow cytometric analysis of MCF-7 cells following 48 hours of $1,25(OH)_2D_3$ treatment showed an accumulation of cells in the G_0/G_1 phase of the cell cycle with minimal reduction in S phase cells. In these cells, we observed distinct nuclear condensation of NuMA, a 240 kD nuclear matrix protein, within 48 hours and for up to 96 hours of $1,25(OH)_2D_3$ treatment. Morphological alterations of this kind in the nuclear matrix are believed to precede the release of nuclear matrix proteins which accompanies ACD. These changes in NuMA occurred in the absence of changes in the expression of TRPM-2, a gene expressed during MCF-7 tumor regression *in vivo*, and that is associated with changes of the cell membrane and membrane remodelling during the fragmentation of the dying cell into apoptotic bodies. In summary, the initial 25% decrease in MCF-7 cells treated for 48 hours with $1,25(OH)_2D_3$ appears to result from induction of ACD, while the 50% decrease in cell number occuring following 96 hours of $1,25(OH)_2D_3$ treatment appears to be due to a combination of inhibition of proliferation and induction of ACD. Supported by the American Institute for Cancer Research.

Y 315 FENRETINIDE SELECTS FOR ALTERNATIVE PATHWAYS OF CARCINOGENESIS IN THE MOUSE PROSTATE RECONSTITUTION (MPR) MODEL SYSTEM. Kevin Slawin[1], Dov Kadmon[1], Sang Hee Park[1], Peter T. Scardino[1], Mario Anzano[2], Michael B. Sporn[2] and Timothy C. Thompson[1], [1]Scott Department of Urology, Baylor College of Medicine, Houston, TX 77030 and [2]National Cancer Institute, Bethesda, MD 20892.

Human prostate cancer is a highly heterogeneous disease in which selected clones of cancer cells infrequently progress to clinical significance. Cancer progression is thought to result from the sequential accumulation of genetic alterations within subpopulations of tumor cells. In human prostate cancers, inactivation of the p53 gene by mutation has been identified as one such genetic alteration associated with late events of tumor progression. However, wild-type p53 protein may also be inactivated through binding by *mdm-2*, a cellular protein which has been shown to be amplified in a number of human malignancies. The relative potentials of different p53-related abnormalities to facilitate tumor progression is largely unknown. We recently reported that dietary fenretinide produced a 49% reduction in the tumor incidence and a 52% reduction in the tumor mass of *ras*+*myc*-induced cancers in the MPR model system. We investigated whether these effects could be due to differences in the frequencies of p53 mutation in tumors from the two groups. Initially we used PCR/SSCP to screen 6 tumors from fenretinide-fed hosts and 12 tumors from control animals for mutations in exons 5, 7 and 8 of the p53 gene, but none were detected. However, when we probed Southern blots of tumor DNAs with *mdm-2*, we found that 2/6 tumors from the fenretinide-treated group but 0/8 tumors from the control group demonstrated genomic amplification of the *mdm-2* locus. Our experiments suggest that fenretinide may block the progression of oncogene-induced prostate cancer by inhibiting an aggressive molecular pathway which does not involve mdm-2 amplification, allowing a more indolent pathway involving *mdm-2* amplification to progress in a subset of these cancers.

Y 316 MECHANISM OF LINOMIDE'S ANTIANGIOGENIC AND CHEMOPREVENTIVE ABILITIES. Jasminka Vukanovic and John T.Isaacs. The John Hopkins Oncology Center, Baltimore, MD 21231

Linomide a quinoline 3-carboxamide has a growth inhibitory effect against a series of androgen dependent and independent rat and human primary prostatic cancers (the later being grown in scid mice) and their metastases. This therapeutic ability is due to linomide's immunomodulatory and antiangiogenic activities. Due to its antiangiogenic ability linomide decreases tumor microvessel density and coresponding tumor blood flow for about 40% (Vukanovic et al. Cancer Res. 53: 1833, 1993). Linomide has chemopreventive abilities as well. It is demonstrated by ~ 2/3 reduction in the percentage of the animals developing cancer of sex accessory tissues in Lobund-Wistar rats induced by methylnitrosourea i.v. and promotion with testosterone proprionate within one year. Since tumor angiogenesis is not only required for the growth of the primary cancer, but also for its ability to metastasize, the effect of linomide on metastasis was directly tested using a quantitation metastasis assay. These in vivo experiments demonstrated that daily linomide treatment decrease by ~3 fold the extent of dissemination of cancer cells to the lungs. Treatment with the antibody (aaGM1) against natural killer (NK) cells resulted in slightly increased number of lung metastases. Combination of aaGM1 and linomide, however, substantially reduced the number of lung metastases even further. Since there is a large body of evidence demonstrating the role of immune cells in angiogensis, prostate tumor tissues were obtained and stained with a series of different antibodies against macrophages, T, B and NK cells. These studies indicated that linomide treatment decreased the number of macrophages from ~32-70%. No change in the number of T lymphocytes or their subsets has been noticed so far. Since the critical role has been suggested for TNF-α, a major secretory product of activated macrophages, in the process of angiogenesis, the amount of TNF-β in those tumors, as well as TNF-α serum concentrations are presently being assayed.

Y 317 SELECTIVE EXPRESSION OF THE CELLULAR ADHESION MOLECULE CD44 IN HUMAN PROSTATE CARCINOMA CELL LINES. Catherine F. Welsh and Lilly Y.W. Bourguignon, Department of Cell Biology and Anatomy, University of Miami, Miami, FL 33101

CD44 is a transmembrane cellular adhesion molecule originally identified as an ~85 kDa lymphocyte homing receptor (also known as CD44 standard form) important in cell-cell and cell-extracellular matrix interactions. Expression of alternatively-spliced high molecular weight (MW) forms (CD44 variant isoforms) have since been detected in various solid tumors and may correlate with metastatic behavior. We have characterized CD44 on several cell lines derived from human prostate cancer. Reverse transcriptase polymerase chain reaction demonstrated that CD44 standard form RNA was present in Alva 31, DU145, and PPC-1, but not in LNCaP, a relatively well-differentiated cell line with a less malignant phenotype. Likewise, immunofluorescence with fluorescein-conjugated human anti-CD44 confirmed a uniform labelling of the cell surface in all cell lines except LNCaP. Using a double labelling technique, ankyrin was shown to accumulate beneath CD44 capped structures on the cell surface, suggesting an interaction between the intracellular domain of CD44 and the cytoskeleton. Immunoblot analysis indicated that Alva 31 CD44 migrated as a higher MW form (~100-120 kDa) than the standard form from lymphoma cells and contained wheat germ agglutinin binding sites. Further, cell adhesion assays demonstrated that PPC-1 and Alva 31, but not LNCaP adhere strongly to hyaluronic acid (HA) and type I collagen, ~50% less to keratin sulfate, and least well to chondroitin sulfate, paralleling the ability of CD44 to bind extracellular matrix proteins. Selective expression of CD44, differential glycosylation, and specific adhesion properties may influence the tumorigenicity of these cell lines.

Y 318 GENE EXPRESSION AND AMPLIFICATION OF mdm-2 IN MOUSE PROSTATE CARCINOMA CELLS. K. Yoshida, P.A. Baley, L.D. Truong, D.L. George, T.C. Thompson, Houston, Texas.

It is well established that the loss of normal p53 function contributes to tumorigenesis in multiple organ systems. Inactivation of p53 can occur via missense mutations in specific conserved domains. In addition, inactivation of the wild-type protein can occur via binding to either viral or cellular proteins such as adenovirus E1b and mdm-2, respectively. Overexpression and amplification and mdm-2 has been demonstrated in human sarcomas where it appears to underlie progression of the disease. We previously demonstrated that the *ras* and *myc* oncogenes can induce poorly differentiated carcinomas in the mouse prostate reconstitution (MPR) model system. Cell lines derived from *ras+myc* carcinomas were subsequently shown to contain stabilized wild-type p53 protein. This phenotype was in contrast to that demonstrated for cell lines derived from *ras*-induced hyperplasia which demonstrated specific p53 mutations. Therefore, the introduction of the myc oncogene in these studies appeared to participate in the induction of stabilized wild-type p53 protein in a fashion similar to that induced by DNA damage and possibly similar to that seen in human lung carcinoma cell lines and/or human soft-tissue sarcomas. To study potential molecular mechanisms for this stabilized wild-type p53 phenotype, we analyzed the mdm-2 gene for overexpression and amplification in *ras+myc*-induced prostate carcinoma cell lines. We show here overexpression of mdm-2 mRNA (4-5 fold) in two out of seven cell lines derived from these carcinomas. In one case where overexpression was observed, amplification of mdm-2 (4-fold) was also demonstrated both *in vitro* and *in vivo* following subcutaneous injection by southern blotting. These data to our knowledge indicate for the first time that overexpression and amplification of mdm-2 can occur in prostate carcinoma cells. Additional northern blotting analysis demonstrated that transforming growth factor-β1mRNA levels were significantly lower (approximately 40%) in the two cell lines with elevated mdm-2 mRNAs compared with the other five. Further studies unexpectantly demonstrated an inverse correlation between mdm-2 mRNA levels and growth potential *in vivo* using four carcinoma cell lines. Overall our data document amplification of mdm-2 in experimental prostate cancer and further demonstrate an inverse relationship between mdm-2 mRNA levels and growth potential *in vivo*. mdm-2 overexpression may not modulate a highly aggressive phenotype.

Molecular Genetics

Y 400 COMPARATIVE GENOMIC HYBRIDIZATION AND ALLELIC IMBALANCE OF CHROMOSOME 8 IN PROSTATE CANCER, Michael L. Cher, Peter R. Carroll, and Ronald H. Jensen, Departments of Urology and Laboratory Medicine, University of California School of Medicine, San Francisco, CA and Donal MacGrogan and Rob Bookstein, Canji Corporation, San Diego, CA.

We compared physical changes in sequence copy number as detected by comparative genomic hybridization (CGH) with loss of heterozygosity on chromosome 8 as measured by polymerase chain reaction (PCR-based allelic imbalance) in 18 prostate cancers. The PCR assay was performed at 21 loci on 8p and 5 loci on 8q. The results showed that CGH and PCR correlated extremely well. CGH detected a loss on all p arms when PCR showed allelic imbalance and showed no changes in all cases where PCR showed retention. CGH also delineated some partial p arm losses: of 10 cases, CGH generally correlated with PCR in 7. On the q arm, CGH was better than PCR at detecting changes because PCR was performed at a few dispersed loci and CGH "paints" the entire arm. CGH also showed that q arm allelic imbalance was due to a gain rather than a loss in copy number. In 5 cases, CGH showed simultaneous loss of the whole p arm and gain of the whole q arm suggesting isochromosome formation. CGH has the additional advantage of examining the entire genome: the cases without 8p loss showed 1-2 other changes (mean 1.25), while the cases with 8p loss showed 2-9 total changes (mean 5.3). Changes that were frequently associated with 8p loss included gains of 8q, 13q, and losses of 17p and q, 16p and q, and y. We conclude that on chromosome 8 in prostate cancer: (1) CGH and PCR-based allelic imbalance are valid techniques that correlate highly with each other; (2) CGH can detect some partial p arm losses; (3) allelic imbalance occurs by physical deletion on the p arm and copy number gain on the q arm; (4) simultaneous p whole-arm deletion and q whole-arm gain is seen frequently suggesting isochromosome formation; (5) PCR is more precise at mapping deletions if densely mapped loci are tested but CGH is a more powerful screening technique; and (6) other gains and deletions throughout the genome are associated with 8p deletion.

Y 401 Generation and characterization of neutralizing antibodies which recognize the *erb*B-2 ligand, *gp30*. C.Cho, J. Payne, M.Cardillo and R. Lupu. Vincent T. Lombardi Cancer Research Center, Georgetown University, Washington DC, 20007.

Polyclonal antibodies have been generated which recognize and neutralize the effects of *gp30*, a member of the Heregulin or *neu* differentiation factor (NDF) family of proteins. These proteins serve as the cognate ligands to the *erb*B-2 (also called neu or *HER-2*) oncogene product, a 185-kD phosphoprotein which has been found to be amplified and overexpressed in some types of adenocarcinomas and correlates with poor prognosis. The presence of ligand may be essential to induce tyrosine kinase phosphorylation for receptor activation. Therefore, we have generated and affinity purified polyclonal antibodies against synthetic peptides corresponding to the EGF-like domain of *gp30* which confers its receptor binding ability. Immunoblot experiments show that these antibodies are able to recognize purified, denatured *gp30* as well as specifically recognize protein secreted from various breast adenocarcinomas and fetal fibroblast cell lines. A combination of these antibodies can additionally be used for immunostaining of tissue samples and for serum quantification by enzyme-linked immunoabsorbant assay (ELISA). Furthermore, these antibodies neutralized *gp30*-induced growth of erbB-2 overexpressing human breast adenocarcinoma cell lines. This finding suggests that *gp30* is indeed essential for receptor activation. Application of such antibodies include prognostic and diagnostic testing to help elucidate the importance of the *erb*B-2 ligands in the progression of breast cancer and moreover, may play a role in immunotherapy for cancers found to overexpress the *erb*B-2 oncogene.

Y 402 ANTISENSE-MEDIATED INHIBITION OF P120 EXPRESSION AND CELL GROWTH IN HUMAN BREAST CANCER CELL LINES, Freeman, J.W., Fonagy, A., Swiderski, C., Bennett, F*. and McGrath, P., Department of Surgery, University of Kentucky Medical Center, Lexington, KY 40536, and Isis Pharmaceuticals*, Carsbad, CA 92008

Proliferation-associated nucleolar antigen expression has been correlated with prognossis in breast cancer. The purpose of this study was to determine whether the expression level of P120 was related to proliferation properties and to determine the efficacy of P120 antisense therapy independent of P120 expression level. In this study the steady-state level of P120 protein and P120 mRNA was compared to the doubling time and S-phase fraction in six different human breast cancer cell lines. The level of P120 protein was highly correlative with the level of P120 mRNA (p=0.0001). There was also a significant positive correlation between the level of P120 protein/mRNA and doubling times (p=0.0008) or percent S-phase cells (p=0.0210). Treatment of the six cell lines with either of two separate P120 antisense oligomers inhibited cell growth in a dose dependent manner. Inhibiton of cell growth, P120 mRNA and P120 protein reached approximately 70% after 24 hrs treatment with 100 μM concentration of antisense oligomer. Slower growing cells that expressed less P120 required less oligomer for 50 % inhibition of growth. Antisense treatment of cells for 3 days resulted in a change in cell morphology and longer treatments of 5 days resulted in loss of cell viabilty. Corresponding non-sense oligomers had little or no effect on cell growth or viability. These studies indicate that P120 expression level is related to proliferation rate and that P120 may serve as a potential therapeutic target in breast cancer.

Y 403 COMPARATIVE GENOMIC HYBRIDIZATION (CGH) PREDICTS RECURRENCE IN NODE-NEGATIVE BREAST CANCER. Jorma Isola, Lisa Chu, Olli Kallioniemi, Suzanne Fuqua, Susan Hilsenbeck, Gary Clark, Kent Osborne, and Fred Waldman. Department of Laboratory Medicine, University of San Francisco,San Francisco, CA 94143 and University of Texas Health Science Center, San Antonio, TX

Identifying genetic defects that have the potential to predict tumor behavior is important in breast cancer because of the variability in clinical disease progression. With the aid of our new technique, comparative genomic hybridization (CGH), we investigated how relative DNA sequence copy number changes throughout the genome correlate with outcome of node-negative breast cancer patients. We compared 23 breast tumor samples from patients having no recurrence in 5 years (group 1) and 25 with having recurrence during the 5-yr follow-up (group2). All patients had 2-4 cm invasive cancers. CGH was done as described previously (Kallioniemi et al., Science, 1992;258:818-21). The total number of copy number aberrations was significantly correlated with recurrence (a median of 3/tumor in group 1 vs. 8/tumor in group 2, p=0.011, Mann-Whitney U-test). Of the individual genetic aberrations, high level gain of chr 8q (present in 14/48 cases) was significantly associated with recurrence (p=0.01, Fisher's exact test). C-MYC amplification pattern was seen only in three of these cases. Recently described amplification in chr 20q13 was found in 7/48 cases (15%), of which 6 had recurrence in <32 months. Aberrations in chrs 8q, 20q13, and 11q13 were partly additive in their prognostic significance. In conclusion, this pilot study suggests that CGH could provide valuable prognostic information in node-negative breast cancer. It also directs further studies to gene aberrations (such as 20q13 amplification) having high biological and clinical significance. Supported by NCI CA44768, CA 30195 and CA 585183.

Y 404 LOSS OF EXPRESSION OF A NF-1 LIKE GENE IN MAMMARY TUMORS DETECTED BY RNA FINGERPRINTING, Burkhard Jandrig, Kathrin Welzel, Hans Krause, Siegfried Scherneck, Department of Tumor Genetics, Max-Delbrück-Center for Molecular Medicine, 13122 Berlin, Germany

To test the possible involvement of tumorsuppressor-like genes in etiology of human mammary tumors we performed a search for differentially expressed mRNAs in normal and tumor specimens, respectively. By using the "differential display method" described by Liang & Pardee (Science 257 (1992) 967) we screened for the "absence" of specific bands in RT-PCR product patterns of tumor sample versus corresponding pattern of normal tissue on polyacrylamide gels.

After electrophoresis different band patterns were obtained. Bands, which occured in only one of the corresponding lanes, were excised, reamplified, cloned into a TA-vector, and sequenced. The cloned sequences were compared with known sequences in Genbank.

We found that in tumour tissues genes for ribosomal proteins (L17, S4) often are more expressed than in normal. Expressed sequence tags were identified to be more expressed in normal specimens (e.g. EST 00357, 00567, 02173). We also found expression of an neurofibromatosis protein (NF-1) like sequence in normal tissue that was lost in the corresponding tumor.

Now we are trying to identify full-length cDNAs to the expressed sequence tags as well as the NF-1 like sequence.

Y 405 MODULATION OF c-fos IN ANDROGEN-DEPENDENT AND ANDROGEN-INDEPENDENT CELL LINES BY PHORBOL ESTER, Arnon Krongrad, Department of Urology, University of Miami School of Medicine, Miami, FL 33101

We have shown previously that stable expression of androgen receptor in androgen-independent prostate cell line PPC-1 does not confer androgen growth sensitivity. These experiments also demonstrated that PPC-1 is relatively serum independent in its growth requirements. This led us to hypothesize that in association with androgen independence there was deregulation of serum-dependent genes.

To test the hypothesis we examined the expression of c-fos, a serum inducible gene, in two prostate derived cell lines. Phorbol ester (PMA) was used to induce expression of c-fos in PPC-1 and in androgen sensitive prostate line LNCaP. Following a two day serum deprivation (1% charcoal stripped serum), PMA was added (50 ng/ml) to the culture medium for variable periods. Immunoblots using affinity purified anti-fos (4) polyclonal rabbit antibody (Santa Cruz) revealed induction of c-fos protein by PMA in LNCaP. No protein was demonstrable either before or after stimulation with PMA in PPC-1. c-fos promoter activity in response to PMA was studied using luciferase reporter assays. Transient transfections revealed that in LNCaP the c-fos promoter was markedly inducible (800 fold). By contrast, PPC-1 basal expression of c-fos promoter was high and minimally inducible (0.3 fold).

It has previously been demonstrated that c-fos protein can markedly effect the ability of androgens to regulate target sequences through the androgen receptor. It is hypothesized that alteration of c-fos expression may not only be a marker of serum independence but may also explain the androgen independent phenotype of PPC-1.

Y 406 GENETIC ALTERATIONS IN LOCALIZED PROSTATE CANCER: IDENTIFICATION A COMMON REGION OF DELETION ON CHROMOSOME 18q,

R. Lidereau[1], A. Latil[1], JC. Baron[2], O. Cussenot[3], G. Fournier[4], T. Soussi[5], L. Boccon-Gibod[2], A. Le Duc[3] and J. Rouëssé[1], [1]Laboratory of Oncogenetic, Centre René Huguenin, F-92211 St-Cloud, France; [2]Department of Urology, CHU Bichat, F-75018 Paris, France; [3]Department of Urology, CHU Saint-Louis, F-75010 Paris, France; [4]Department of Urology, CHU A. Morvan, F-29609 Brest, France; [5]U 301 INSERM, Institut de Génétique Moléculaire, F-75010 Paris, France.

Accumulation of mutations in oncogenes and tumor suppressor genes transforms a normal cell into a malignant cell by allowing it to escape from normal control of growth. For prostate tumorigenesis, the current model defines specific mutations of the TP53 tumor suppressor gene and loss of heterozygosity (LOH) for loci on chromosomes 8p, 10q, 16q and 18q. In order to determine if alterations frequently found in other adenocarcinomas (breast, ovarian, gastric, colorectal) including losses of heterozygosity on chromosomes 1p, 3p, 7q, 11p, 17p, 17q and 18q, and amplification of c-myc, c-erbB-2/neu oncogenes and the 11q13 region are also involved in prostate cancer, we examined 21 localized early stage prostate tumors. We detected no amplification of the c-myc, c-erbB-2/neu proto-oncogenes and 11q13 region (int2/FGF3), or mutations of the TP53 gene. Allelic losses were found in chromosomal regions 10q (20%), 7q (33%) and 18q (33%). Furthermore, as the first step toward isolation of tumor suppressor genes on 18q, we used six polymorphic markers and identified a common region of deletion between the 18q centromere and the D18S19 locus.

Y 407 LOSS OF CHROMOSOME 8p LOCI IN PROSTATE CANCER: MAPPING BY QUANTITTATIVE ALLELIC IMBALANCE,

Donal MacGrogan[1], Alina Levy[1], David Bostwick[2], Michael Wagner[3], Dan Wells[3], and Robert Bookstein[1]. [1]Department of Molecular Biology, Canji Inc., San Diego, CA; [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, and [3]Department of Biology, University of Houston, Houston, TX.

A previous study of 18 primary or metastatic prostate cancers showed loss of genetic markers on chromosomes 8, 10, or 16 in more than 50% of cases (Bergerheim et al., Genes Chrom Cancer 3: 215-220, 1991). The small size and infiltrative nature of primary prostatic tumors have hindered efforts to assess allelic losses by traditional restriction fragment length polymorphism (RFLP) / Southern blotting methods. To improve the sensitivity and specificity of this analysis in early prostate cancer, we have amplified polymorphic microsatellite repeats by polymerase chain reaction (PCR), and have quantitated allelic imbalances with phosphor imaging technology. In this study, 63 primary prostate tumors and matched benign tissues obtained by radical prostatectomy were examined at 28 genetic loci on chromosome 8, all but five of which were located on the short arm. Twenty-nine (46%) of the 63 cases showed loss of at least one locus. Multiple adjacent loci, usually including the *LPL* and *MSR* genes in 8p22, were lost in 28 cases. In 10 of these, losses were observed at all informative loci on the p arm. In another 15 tumors, losses were restricted to subregions of the p arm by loci retained either distally toward the p terminus or proximally at the 8p12-8p21 border, or both. In 3 tumors, two discrete regions of loss were observed within 8p, separated by several retained loci. Allelic loss of 8p loci was associated with higher tumor grade. These data are complementary to previous reports of allelic deletions in colorectal, hepatocellular, and non-small cell lung cancers and suggest the existence of one or more pleotropic tumor suppressor genes on 8p.

Y 408 **GENETIC CHANGES IN HUMAN CARCINOMA OF THE PROSTATE,**

[1]Norman J Maitland, [1]Philippe Berthon, [1]Susan Murant and [2]Michael Stower *[1]Cancer Research Unit, Department of Biology, University of York, [2]York District Hospital, York United Kingdom.*

The major risk factor for human carcinoma of the prostate appears to be age. It is therefore likely that a multi-mutational mechanism will be important, as has been recognised in a number of other common human tumours. We have set out to overcome the problems associated with such a genetic analysis of prostate by combining a microdissection approach, taking normal and tumour cells from phenotyped and immunocytochemically typed thin sections, with the advantages offered by fluorescently labelled PCR primers to analyse gene loss and mutation.

Preliminary studies confirmed our initial suspicions that such analyses with whole biopsies from tumours or biopsies in which the tumour cell content carrying a specific mutation comprised less than 25% of the cell population, would produce false negative results. Precise microdissection, using an inverted microscope and a converted microinjection apparatus, permitted the selection of cell populations greater than 90% pure for any cell type, and greatly simplified the analysis of loss of heterozygosity (LOH) at chromosomal locations suspected of containing tumour suppressor genes. In addition the microdissected material provided an ideal substrate for the single strand conformational polymorphism analysis (SSCP)- based analysis of point mutations in known cancer associated genes.

Previous work in other laboratories has implicated a number of chromosomal locations, which may contain genes which are important in development of carcinoma of the prostate. Accordingly sets of PCR primers were prepared in both known locations, and spanning the suspected locations, initially chromosome 10q. PCR products, after amplification from matched normal and tumour material, were separated and analysed on an Applied Biosystems Model 373 Automated DNA Sequencer, equipped with Genescan 672 software. To further refine the analysis, the polymorphic products deriving from CA repeats were "polished" by a post PCR treatment with T4 DNA polymerase. Similarly, fluorescently labelled single strand products, produced from the p53 gene (amongst others), were also employed in SSCP to detect the frequency of point mutations in this key cancer associated gene. All experiments were carried out on coded material to ensure a completely unbiased analysis. The results of the accumulated mutational analysis on graded and staged material will be presented in full.

Y 409 USING THE RNA ARBITRARILY PRIMED POLYMERASE CHAIN REACTION (RAP-PCR) TO ANALYZE GENE EXPRESSION IN HUMAN BREAST CANCER CELLS LINES,

Douglas McKenzie and Robert Buchner, Stratagene Inc., La Jolla, CA 92037

Simple and reproducible fingerprints of complex genomes can be generated using a single oligodeoxynucleotide of arbitrary sequence in a low stringency polymerase chain reaction (PCR). The method, referred to as arbitrarily primed PCR (AP-PCR) (1), has been used to detect polymorphisms which have utility in genetic mapping, ecology, epidemiology, and phylogenetics. Recently, Welsh and colleagues described a variation of AP-PCR technique in which the arbitrary oligodeoxynucleotide was used in both first and second strand cDNA synthesis. PCR amplification was subsequently used to amplify the products (2). This method required only nanograms of RNA, and yielded reproducible PCR fingerprints for analysis of differential RNA expression between different populations. A similar approach using an anchored oligo-dT primer for first strand synthesis and a combination of anchored-dT and arbitrary oligodeoxynucleotide in the ensuing PCR has recently been reported (3).

In this abstract, we report on the utilization of RAP-PCR to examine differential gene expression in a series of human breast cancer cell lines. The primers utilized in these studies consist of two distinct types. The first are primers that are strictly arbitrary in nature and contain no obvious self-complementarity. Consequently, there is no apparent bias in the fingerprints which result from the use of these primers in RAP-PCR. The second group of primers consist of a series of primers designed to annneal to concensus oligodeoxynucleotide sequences contained within various protein families. In contrast to the first group of primers, these primers yield fingerprints that potentially contain information as to the spectrum of protein family members expressed in any given RNA population. In addition to allowing for the detection of differential RNA expression, these primers may allow for the identification of new members within interesting protein families.

(1) J. Welsh and M. McClelland. 1990. Nucleic Acids Res. 18:7213.
(2) J. Welsh, K. Chada, S.S. Dalal, R. Cheng, D. Ralph, and M. McClelland. 1992. Nucleic Acids Res. 20:4965-4970
(3) P. Liang and A.B. Pardee. 1992. Science 257:967-971.

Y 410 INDEPENDENT EXPRESSION OF *INT*-2 mRNA AND HERV-K *ENV* mRNA IN HUMAN BREAST TUMORS, Stephanie G. Poirier, Justin Poley, Kevin Cuddy, Isadore Brodsky, Jeffrey Brodsky and David Gillespie, Departments of Neoplastic Diseases and Surgery, Hahnemann University, Philadelphia, PA 19102

Human breast tumors carry genetic changes in oncogenes, mutations which are reflected in increased gene expression and/or increased gene number. However, the significance of these mutations in breast carcinogenesis remains unclear. In mice, some breast tumors are caused by overexpression of the *int* -2 gene, which is activated by insertion of the mouse mammary tumor virus (MMTV) promoter. In 20-30% of human breast tumors the *int* -2 gene is amplified and may also be overexpressed. Additionally, the human genome carries potentially active HERV-K proviruses that are genetically related to MMTV. It is possible that HERV-K retrotranspositions might activate *int* -2 expression . Since HERV-K retrotransposition would require at least its transient expression, a simple hypothesis is that HERV-K and *int* -2 gene mRNA would be found together in human breast tumors.

Accordingly, we measured the abundance of the mRNA of these two genes by capture-RT-PCR. Message RNA was captured from prepared breast tumors with an affinity membrane. Captured RNA was copied into cDNA by RT and the cDNA was amplified by PCR. Six out of 9 breast tumors expressed *int* -2, three at relatively high levels; including one fibroadenoma raising the question whether *int* -2 expression is significant in human breast carcinogenesis. In addition, two of the 9 breast tumors expressed Herv-K *env* , apparently independent of *int* -2 expression.

Y 411 **CLINICAL TRIALS OF TAMOXIFEN FOR PREVENTION OF BREAST CANCER** Trevor J Powles Royal Marsden Hospital Downs Road Sutton Surrey SM2 5PT UK Overview data of randomised trials of tamoxifen given as adjuvant therapy after primary treatment of breast cancer throughout the world, indicate that tamoxifen will reduce the risk of relapse by about 30% and mortality by about 20%, providing no other systemic therapy is given. This benefit continues for at least 10 years, and is equally effective in pre and postmenopausal women on no other systemic therapy. However, premenopausal women developing ovarian ablation on chemotherapy probably gain little extra adjuvant endocrine benefit by adding tamoxifen. Adjuvant tamoxifen reduces the risk of a new breast cancer in the contralateral breast by about 40%. Epidemiological and clinical data indicate that oestrogen may be critically involved in the promotion of a carcinogenically induced breast carcinoma. Clinical use of tamoxifen to treat breast cancer indicates that this drug is antiproliferative for the disease, with very low toxicity. In order to test whether breast cancer can be prevented by using tamoxifen we have undertaken a double blind, randomised, feasibility trial using tamoxifen 20 mgs per day versus placebo. We have been able to accrue over 1600 healthy women with a family history of breast cancer into this programme. Approximately 50% of eligible women accept randomisation into the programme, and compliance is maintained at about 80% at five years. Acute toxicity is similar for patients receiving tamoxifen or placebo, except for a significant increase of about 15% in hot flushes in tamoxifen treated women. There is a significant 15% reduction in total serum cholesterol in pre and postmenopausal women, especially in those who have a high initial level of cholesterol. This drop in total cholesterol is associated with a significant reduction in LDL-cholesterol and apolipoprotein B together with a non significant increase in HDL-cholesterol. This would indicate a potential reduction in coronary heart disease. We have also monitored bone density and various clotting factors and found no adverse effects. Sequential transvaginal ultrasound examination of the ovaries indicate an increased incidence of ovarian cysts for women on tamoxifen. We are now continuing accrual to this trial to 2500 healthy women at the Royal Marsden Hospital. These results indicate that it is safe to extend this trial to larger multicentre trials needed to detect relatively small prevention effects. Multicentre trials have started in Italy and in the USA, and have been approved in the UK, aimed at accruing over 50,000 women.

Y 412 THE OVEREXPRESSION OF A $172^{Arg\text{-}Leu}$ p53 MUTANT RESULTS IN ALTERED MAMMARY GLAND DEVELOPMENT IN TRANSGENIC MICE, Jeffrey M. Rosen, Baolin Li and Daniel Medina, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

While missense mutations of the p53 gene are the most commonly observed mutations in human cancer, it is unclear if individual mutations result in a gain of function and/or suppress transactivation of wild-type (wt) p53. To date, the function of mutant (mu) p53s has been studied primarily in transfected cell lines either lacking the wt p53 allele or containing other p53 mutations. In order to study the effects of specific p53 mutations in the mammary gland of transgenic mice, a point mutation was created on codon 172 (Arg-Leu, equivalent to aa 175 in human p53, a mutational hotspot in breast cancer) of a wt mouse p53 genomic minigene that was fused to the rat mammary specific whey acidic protein (WAP) promoter. Several independent lines of transgenic mice were generated that expressed the expected p53 mRNA transcript preferentially in the mammary gland at levels at least 10-100 fold greater than the wt p53 mRNA. The mu p53 protein was detected primarily in the cytoplasm by immunocytochemical analysis. Transgenic mice displayed different degrees of altered mammary gland development depending on the level of overexpresion of the mu p53. The most severe phenotype observed in one line of mice expressing the highest levels of mu p53 was the total failure to lactate, and the absence of appropriate alveolar development. Overexpression of this mu p53 appeared to induce apoptosis in the mammary gland of transgenic mice, and resulted in increased expression of mdm-2, hsp70, and histone H4 mRNAs, and a decrease in PCNA mRNA levels. These results suggest that individual mu p53s may exert very different properties, and in some cases may still retain some of the characteristics of wt p53. Despite these apparent wt features, lines of mice overexpressing this mu p53 appear to have an increased susceptibility to DMBA-induced mammary carcinogenesis. (Supported by NIH grant CA16303.)

Y 413 A GENOME-WIDE SURVEY OF GAINS AND LOSSES OF DNA SEQUENCES IN PROSTATE CANCER BY COMPARATIVE GENOMIC HYBRIDIZATION. T.Visakorpi[1],A.Kallioniemi[1],J.Isola[1,2], D.Pinkel[2] and OP. Kallioniemi[1]. Lab for Cancer Genetics, Dept Clin Chem, Tampere University Hospital, Box 2000, FIN-33521, Tampere Finland and Dept. Lab.Medicine, UC San Francisco, CA 94143-0808

Genetic changes associated with the development of prostate cancer are poorly known. Comparative genomic hybridization (CGH) allows detection and mapping of DNA sequence copy number changes anywhere in a tumor genome in a single hybridization (Kallioniemi et al, Science 258:818 1992). In CGH, differentially labeled tumor and normal DNA are hybridized to a normal metaphase spread. Losses and gains of DNA sequences are seen as variation in the binding of the two labeled DNAs along normal metaphase chromosomes. We have analyzed 3 prostate cancer cell lines, and 14 uncultured primary tumors by CGH. Two cell lines, PC-3 and DU145, showed amplification of DNA sequneces originating from 14q21-24. Primary tumors showed gain of the entire long arm of chromosome 8 in 29% of cases, but no regional amplifications. Otherwise, mainly deletions were observed in primary prostate cancers: 8p; 43% (common region p21-pter), 9q; 29% (q24-qter), 10q; 36%(q25-qter), 14q; 29% (q32-qter), 16q; 64% and 17p; 50%. CGH analysis facilitates analysis of the genetic progression of prostate cancer and highlights chromosomal regions that may contain important genes.

Y 414 MOLECULAR CYTOGENETIC ANALYSIS OF TWO NEW PROSTATE-CANCER XENOGRAFTS, B. Jill Williams[1], Emma Jones[1], William J. Ellis[2], Robert L. Vessella[2], Debra M. Sutkowski[3], James M. Kozlowski[3], Chung Lee[3], Arthur R. Brothman[1]. University of Utah, Salt Lake City, UT 84112[1]; University of Washington, Seattle, WA[2]; Northwestern University Medical School, Chicago, IL[3].

We have initiated molecular cytogenetic studies to characterize two new prostate-cancer xenografts, LuCAP 23 and RP22090. Both of these lines grow following serial passage in athymic (nu/nu) mice, but do not grow in long term culture. G-banded chromosomes were minimally informative, with a modal chromosome number of 78 for LuCAP and 70 for RP22090. However, the chromosomes appeared to be of human origin, which was confirmed for both xenografts using human and mouse genomic-painting probes for fluorescence in situ hybridization (FISH). In order to determine the number of copies of each chromosome present in the two xenografts, chromosome-specific FISH probes were used. We have completed counts of the modal number of sixteen chromosomes present in these xenografts, including chromosomes 1, 2, 3, 4, 6, 7, 8, 10, 11, 12, 15, 16, 17, 18, X, and Y. For LuCAP, there were two copies of the Y chromosome, three copies of 1, 4, and 15, and four copies of 7, 8, 10, 12, 16, 17, 18, and X. For RP22090, there was one copy of the Y chromosome, two copies of 8, 15, 16, 17, and X, three copies of chromosomes 1, and four copies of 4, 7, 10, and 18. From these data, we are not yet certain how to interpret gain or loss of whole chromosomes, or the presence of regions other than the centromere. In order to address this problem, we have measured gain and loss of non-centromeric chromosome-specific regions using comparative genomic hybridization (CGH). For example, we have detected apparent loss of the distal Y chromosome in RP22090 using CGH, although a single Y centromere is present in virtually all cells counted using FISH analysis. Region-specific gain and loss of other chromosomes has also been detected in both xenografts using CGH. Supported by R01-CA46269 (NCI).

Y 415 THE CALMODULIN-LIKE PROTEIN WITH REDUCED ABUNDANCE IN TRANSFORMED HUMAN MAMMARY AND PROSTATIC EPITHELIAL CELLS SELECTIVELY BINDS AND REGULATES MULTIFUNCTIONAL Ca^{+2}/CALMODULIN-DEPENDENT PROTEIN KINASE II, Paul Yaswen, Carl F. Edman, Samuel E. George, Anthony R. Means and Howard Schulman, Life Sciences Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720, Departments of Cardiology and Pharmacology, Duke University Medical Center, Durham, NC 27710 and Neurosciences Program, Stanford University School of Medicine, Stanford, CA 94305

Because of the critical role that calcium regulation has been shown to play in the growth and differentiation of epithelial cells, proteins involved in calcium mediated pathways are potential targets of carcinogenic changes. A comparison of transcripts expressed in normal vs. transformed human mammary epithelial cells (HMEC) has lead to the identification of a calmodulin-like protein (CLP) which is often downregulated or absent in tumorigenically transformed epithelial cells with respect to levels in normal HMEC. Unlike the ubiquitous distribution of calmodulin, CLP expression is normally restricted to certain epithelial cells, and appears to be modulated during differentiation. Since a large number of calcium regulated cellular activities are thought to be mediated by the calcium sensitive binding of calmodulin to regulatory sites on responsive enzymes, our current studies have been performed in order to identify potential cellular targets of CLP. We have found that CLP activates the multifunctional Ca^{+2}/calmodulin-dependent protein kinase II with kinetics which are identical to those of calmodulin itself. In contrast, other calmodulin dependent enzymes, cGMP phosphodiesterase and myosin light chain kinase, display much stronger activation by calmodulin. In fact, in the case of myosin light chain kinase, CLP competitively inhibits calmodulin activation of this enzyme. Thus, CLP may have evolved as a specific agonist which can activate certain calmodulin dependent enzymes, and as a specific antagonist which can inhibit other calmodulin dependent enzymes. The consequences of CLP depletion in normal and transformed cells are under investigation.

Diagnosis, Treatment, and Prevention I & II

Y 500 OESTROGENS ARE INACTIVATED BY NORMAL BUT NOT MALIGNANT BREAST EPITHELIAL CELLS, E. Anderson, N. Rati and *A. Howell, Departments of Clinical Research and *Medical Oncology, Christie Hospital NHS Trust, Manchester M20 9BX, UK.

To begin to investigate the possibility that there are differences in the mechanisms by which breast epithelial cells metabolise oestrogens and their precursors in women with or at risk of breast cancer, we have measured the inter-conversions of oestradiol (E_2) and oestrone (E_1) by an immortalised normal human breast epithelial cell line (MTSV1-7) and compared them to those in malignant breast cancer cell lines (MCF-7, ZR 75-1 and BT-20).

After incubation of the MTSV1-7 with $^3HE_1$ or $^3HE_2$ (1μCi/well) in serum-free conditions for 72h, 66.2±0.56% (mean±sem;n=3) of the added radioactivity remained in the culture medium after extraction with ethyl acetate. In contrast, when the MCF-7, ZR 75-1 or the BT-20 cells were incubated with $^3HE_1$ or $^3HE_2$ under the same conditions, 70-80% of the radioactivity was extracted into the organic solvent. Reverse phase HPLC analysis of the extracted steroids revealed that reduction (MCF-7 and ZR 75-1 cells) or oxidation (BT-20) by 17β-hydroxysteroid dehydrogenase (HSD) was the major route of oestrogen metabolism in the malignant cell lines. However, the major product of the MTSV1-7 cells was a highly polar derivative irrespective of the substrate used. This polar compound could be converted back to the parent oestrogen by treating the culture medium with sulphatase before extraction with the organic solvent indicating that the major route of oestrogen transformation in the MTSV1-7 cells is conjugation by sulphotransferase. The results suggest that the malignant breast epithelial cells are capable of converting less active to more active oestrogens that could stimulate proliferation in an autocrine fashion. The only route of oestrogen metabolism in the 'normal' cells, however, is conversion to inactive, water-soluble conjugates that may be more easily excreted. A decrease in the ability of breast epithelial cells to inactivate the high levels of oestrogen, to which they are normally exposed, may predispose to the development of cancer and this possibility is now under investigation.

Y 501 ANTI-GROWTH FACTOR RECEPTOR MONOCLONAL ANTIBODIES ENHANCE THE ANTITUMOR EFFECTS OF PACLITAXEL IN BREAST CANCER XENOGRAFTS. Jose Baselga, Larry Norton, Keren Coplan, Refaat Shalaby and John Mendelsohn. Memorial Sloan-Kettering Cancer Center, N.Y., N.Y. 10021. and Genentech Inc., South San Francisco, CA 94080.

Chemotherapy synergizes with anti-growth factor receptor monoclonal antibodies (ARMAs) in the treatment of human breast tumor xenografts in nude mice (J. Natl. Cancer Inst. 85:1327, 1993). Paclitaxel is active against human breast cancer and inhibits DNA synthesis mediated by epidermal growth factor receptor (EGFR). MDA-468 cells, which overexpress EGFR, were grown subcutaneously (s.c.) to a mean size of 200 mm^3. Animals then received paclitaxel alone (40 mg/kg i.v.) or anti-EGFR ARMA 528 (2 mg i.p. 2 x week) with respectively, 49% and 30% growth inhibition by day 39. Paclitaxel plus ARMAs 528 caused a 93% inhibition. We then analyzed the effects of paclitaxel in combination with ARMAs directed against the HER2 receptor, closely related to the EGFR. BT-474 cells, which express high levels of HER2, were grown s.c. to a mean size of 200 mm^3. In a pilot study anti-HER2 ARMA 4D5 alone (range 30 to 0.1 mg/kg i.p. 2 x week) resulted in a dose dependent inhibition of growth with tumor eradication in 30-50% of the animals at a dose level ≥10 mg/kg. For the combination studies a dose of 4D5 that resulted in a modest inhibition of growth was chosen. By day 36, treatment with anti-HER2 ARMA 4D5 alone (.3mg/kg i.p. 2 x week) produced a 35% growth inhibition, paclitaxel alone (20 mg/kg i.v.) resulted in 35% inhibition and doxorubicin alone (10 mg/kg i.p.) in 27 % inhibition of growth. The combined treatment with paclitaxel plus 4D5 resulted in a major antitumor activity with 93% inhibition of growth. This result was markedly better than doxorubicin plus 4D5 (70% inhibition). Thus, equipotent doses of paclitaxel and doxorubicin differed in their combined effect with ARMAs, which suggests synergy between paclitaxel and 4D5. ARMAs did not increase the toxicity of paclitaxel in animals as determined by animal survival and weight loss. The antitumor effects of paclitaxel can be markedly enhanced by the addition of ARMAs. Mechanisms are being explored.

Y 502 GENISTEIN: A PROTEIN-TYROSINE KINASE INHIBITOR INHIBITS THE GROWTH OF PROSTATE CANCER CELLS, Raymond C. Bergan, Edward Kyle, Len Neckers, Clinical Pharmacology Branch, NCI, NIH, Bethesda, MD 20892

Genistein, a known protein-tyrosine kinase (PTK) inhibitor *in vitro*, was found to be cytostatic to prostate cancer cells with an IC_{50} of less than 50 uM. Morphologically, there was a marked flattening of the cells associated with stronger adhesion to a solid support. Associated with these changes, we observed an increase in focal adhesion kinase PTK activity and no gross reduction in cellular phosphotyrosine content. Genistein affected the more metastatic PC3M cell line similarly to the PC3 cells, from which they were derived. In summary, genistein appears to be acting as a differentiation agent on prostate cancer cells. Its activity in the micromolar range makes it ideal for development as a pharmaceutical agent.

Y 503 MECHANISMS OF TUMOR PROGRESSION DURING INTERMITTENT ANDROGEN SUPPRESSION.

N. Bruchovsky, K. Akakura, P. S. Rennie and S. L. Goldenberg. Department of Cancer Endocrinology, B.C. Cancer Agency, Vancouver, Canada. The effect of intermittent androgen suppression (IAS) on the androgen-dependent (AD) Shionogi carcinoma was studied in terms of time to androgen-independence, change in the proportion of tumorigenic stem cells and altered expression of the apoptosis related gene, TRPM-2. The AD tumor was transplanted into a succession of male mice, each of which was castrated when the estimated tumor weight became about 3 g. After 4-5 cycles of transplantation and castration-induced apoptosis, the tumor abruptly progressed to an androgen-independent (AI) state. The emergence of AI malignancy was delayed from a mean of 50 days after monocastration to 116 days after IAS. The proportion of total stem cells was constant during the first 3 cycles but increased sharply between the 3rd and 4th cycles; this was associated with a 65-fold increase in the subpopulation of AI stem cells. TRPM-2 which codes for the anti-cytolytic protein, clusterin, was constitutively overexpressed in all tumors after initial androgen deprivation even in the absence of any overt signs of apoptosis. Furthermore, clusterin was increasingly localized in the cell nucleus with each repeated cycle. These observations suggest that deregulated expression of TRPM-2 and nuclear localization of clusterin serve to inhibit early events in the apoptotic process and thereby enhance the survival of stem cells.

Supported by MRC of Canada

Y 504 GENE THERAPY FOR HUMAN PROSTATE CANCER: EFFICACY IN ANIMAL MODELS AND FEASIBILITY FOR HUMAN TRIALS. Michael Carducci, Martin Sanda, Sujatha Ayyagari, William Kim, Larry Cohen, Richard Mulligan, Drew Pardoll, and Jonathan Simons, The Johns Hopkins Oncology Center, The Brady Urological Institute, Baltimore, MD 21287

Effective therapies for advanced prostate cancer are needed. The potential efficacy of gene-modified immunotherapy for prostate cancer was tested in rats with pre-established, anaplastic, hormone refractory Dunning prostate cancer deposits. MAT-LyLu tumors implanted in one limb were treated by vaccination in the contralateral limb with irradiated MAT-LyLu cells transduced to secrete recombinant human GM-CSF gene product. Rats treated with MAT-LyLu cells secreting 22ng GM-CSF/10^6cells/24hrs demonstrated longer disease-free survival than controls (p=0.009). Although all animals treated at this dose eventually developed tumor, the anti-tumor effect was confirmed by significantly smaller tumor size in animals receiving MFG-GM-CSF gene therapy. Control animals receiving untransduced tumor vaccine and either locally injected soluble GM-CSF or co-injected non-specific immuno-adjuvant (C.parvum) demonstrated no treatment benefit. To determine if tumor-bearing animals could be cured by increasing GM-CSF secretion by the gene-modified vaccine cells, a second experiment evaluated treatment with gene-modified vaccine cells secreting higher levels of GM-CSF (140ng/10^6cells/24hrs). 30% of treated animals were cured as shown by >130 day disease-free survival, while those treated animals that developed tumors again survived longer than controls (p=0.001). The feasibility of applying such gene therapy dose escalation to patient therapy was then evaluated. First, the established human prostate cancer cell line PPC-1 was transduced to secrete GM-CSF. Multiple transductions led to increase of GM-CSF secretion from 17 to 270 ng/10^6cells/24hrs. Repeated transduction of three consecutive patient-derived primary culture prostate cancer cells showed that increasing rates of GM-CSF secretion could be achieved in a clinical setting as well. These studies demonstrate the efficacy of gene-modified immunotherapy using MFG-GM-CSF in the Dunning model of prostate cancer, and the ability to escalate gene-dose in potential human vaccine cells, achieving pre-clinically effective levels of immunostimulatory cytokine secretion. The preclinical basis for gene-modified immunotherapy of human prostate cancer is thus demonstrated.

Y 505 **NOVEL RECOMBINANT EPITOPE ASSAYS (REAs) FOR BREAST EPITHELIAL ANTIGENS IN CIRCULATION,** Roberto L. Ceriani, Radwan Kiwan, Edward W. Blank, and Jerry A. Peterson, Cancer Research Fund of Contra Costa, Walnut Creek, CA 94596.

Breast epithelial antigens are found in the circulation of breast cancer patients and are used in their follow up. Current assays for one of these antigens the breast epithelial mucin (BEM), rely on a double determinant configuration. They have demonstrated medium to low sensitivities, once specificities are kept high. To overcome this drawback, competitive recombinant epitope assays (REAs) are proposed here to determine levels of BEM in human serum. These REAs employ fusion proteins carrying the epitope of the antibody(s) participating in the assay, and different anti-breast mucin monoclonal antibody(s). The configuration of the REAs include a novel purification step for the epitope used in this competitive procedure. Results obtained from a model panel of sera from normal female subjects and patients with active breast cancer, show that, keeping the specificity to 100%, a high level of sensitivity can be obtained by the simultaneous use of REAs.

These REAs have as their basis the use of different epitopes of the breast epithelial mucin; these epitopes differ in the carbohydrate contribution to their structure. The assay configuration of these REAs represent a new approach, (where inexhaustible genetically engineered antigens are used), resulting in the ability to run simultaneously assays for different epitopes of a differentiation-sensitive breast antigen. The ability of the REAs to test for a breast cancer marker at different stages in its molecular maturation could explain the present results where a very low number of sera are found to be false negative. Results obtained indicate that the REAs provide an improved ability to detect breast cancer patients with identifiable tumor load and could form the basis for a population screening concept. Supported by NIH, NCI R01-CA39932.

Y 506 ASSESSMENT OF BREAST CANCER RESPONSE TO THERAPY BY CONTRAST ENHANCED MRI AT HIGH SPATIAL AND TEMPORAL RESOLUTION, H. Degani, E. Furman, R. Margalit, A. Horowitz*, I. Goldberg*, S. Fields+, R. Catane+, J.M. Gomori+, A. Libove+, G. Zajicek and T. Peretz, Weizmann Institute of Science, Rehovot, *Sheba Medical Center, Tel Hashomer, +Hadassah Medical Center, Jerusalem, Israel.

Chemotherapy and hormonal manipulation are the modalities by which it is possible to alter the natural course of breast cancer. In any stage of the disease, it is of utmost importance to be able to assess rapidly the effectiveness of therapy. In this report we present the use of contrast enhanced MRI to verify the response to tamoxifen of MCF7 tumors in nude mice and the response to preoperative chemotherapy in breast cancer patients. The animal studies were performed on a Bruker 4.7 Tesla spectrometer using fast 2D gradient echo (GE) and spin echo (SE) sequences with spatial resolution ranging between 1×0.14×0.14mm to 1.5×0.14×0.39mm and temporal resolution of 10 sec (GE) and 1.5 min (SE). Patients were scanned with an Elscint 2 Tesla spectrometer using 3D GE sequence with spatial resolution of 1.2×0.8×0.8mm and temporal resolution of 2 to 4 min. Images were recorded prior to and after administration of Gadolinium-DTPA. By evaluating signal enhancement at various regions of the tumor it was possible to discern changes that indicate response before observing any change in tumor size. Tamoxifen treatment of MCF7 tumors led within 1-2 weeks to central necrosis surrounded by regions showing fast contrast enhancement due to rapid leakage from microvessels. This was verified in computed maps of initial enhancement rates and by statistical analysis of the results which indicated 100%-150% increase in tumor enhancement within 7-14 days of treatment (% enhancement = [intensity(0)-intensity(t)]/intensity(0), where 0 and t indicate before (0) and after (t) contrast injection). Thus response to tamoxifen appeared to induce leakage from tumor microvessels thus diminishing their capacity to further deliver nutrients and oxygen into inner parts. In patients the MRI changes correlating with eventual tumor response to chemotherapy were a slower and lower signal enhancement. Before the administration of chemotherapy the maximal contrast enhancement of tumor areas reached ~70% within 0-2 minutes, while after successful chemotherapy the maximal contrast enhancement reached ~30% and only after 6-8 minutes. MRI may become an important tool for early demonstration of breast cancer response to treatment.

Y 507 MODULATION OF p185*c-erbB-2* EXPRESSION AND TUMORIGENIC GROWTH BY ANTI-RECEPTOR MONOCLONAL ANTIBODIES, William C. Dougall, Norman C. Peterson, and Mark I. Greene, Center for Receptor Biology and Division of Immunology, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

We have developed monoclonal antibodies (mAbs) reactive with the extracellular domain of the human p185*c-erbB-2* and rat p185*neu* protein. This protein is a 185 kDa cell-surface growth factor receptor/tyrosine kinase which is often overexpressed in human adenocarcinomas of the breast, pancreas and ovary. Treatment of NIH 3T3 cells overexpressing human p185*c-erbB-2* protein (LTR-2 cells) with mAb 7.16.4 (IgG2A) caused a rapid down modulation of cell-surface levels of p185*c-erbB-2*. Cell-surface expression of p185*c-erbB-2* was reduced 50% within 30 min of mAb treatment (10 ug/ml), and 70% by 1 hr. p185*c-erbB-2* was not activated by antibody treatment within 4 hrs. as determined by phosphotyrosine assays. We have also examined the effect of mAb treatment on p185*c-erbB-2* protein stability by metabolic labeling and pulse-chase experiments. Furthermore, treatment of LTR-2 cells with mAb 7.16.4 inhibited the anchorage-independent growth of these cells in a dose-dependent manner. Growth of LTR-2 cells in soft agar was inhibited by more than 90% using mAb 7.16.4. *In vivo* administration of anti-receptor antibodies significantly inhibited the tumorigenic growth of *c-erbB-2*-transformed cells implanted in *nu/nu* mice. We have also cloned the cDNA encoding the anti-receptor immunoglobulin, which will allow expression of smaller antibody fragments and the design of peptides and small molecules representing the antibody complementarity determining regions. These antibodies and small antibody molecules hold promise in the quantitiation and identification of p185c-erbB-2 expression in human neoplasias, and in the elucidation of the mechanism of p185c-erbB-2-mediated cellular transformation

Y 508 ADIPOCYTES STIMULATE ANCHORAGE-INDEPENDENT GROWTH OF A BREAST CARCINOMA: ROLE OF FIBRONECTIN MATRIX B.E. Elliott, R. Saulnier, B. Bhardwaj, R. Lall, D. Leopold, N. Rahimi, and L. Maxwell, Cancer Res. Lab. Queen's Univ., Kingston, ON, CANADA, K7L 3N6.

We have previously demonstrated the capacity of adipose tissue to support *in vivo* growth and metastasis of a murine mammary carcinoma, SP1 (Elliott *et al.* I.J. Cancer 51:416-424, 1992). Adipocytes are a dominant cell type in mammary stroma; we therefore examined the capacity of an adipocyte cell line (3T3-L1) to stimulate growth of SP1 cells *in vitro*. Proliferation of SP1 cells occurred in the presence of adipocytes (induced 3T3-L1 cells) or conditioned media from adipocytes, but not from preadipocytes (uninduced 3T3-L1 cells) or fibroblasts. To determine whether the above adipocyte interactions contribute to the tumorigenic phenotype, we examined anchorage-independent growth of SP1 cells as colonies in 0.36 % agar, separated from feeder cells by 0.6 % agar. Without feeder cells, no colonies grew at FCS concentrations between 0.01% and 1%. Monolayers of 3T3-L1 adipocytes stimulated anchorage-independent growth of SP1 cells more effectively than uninduced 3T3-L1 preadipocytes or fibroblasts. In SP1 colonies, fibronectin appeared to be deposited extracellularly in the form of microfibrils; no fibrils were observed with SP1 cells grown in monolayers, although some fibronectin was expressed intracellularly. We therefore investigated the role of fibronectin fibrils in the formation of SP1 colonies in FCS-supplemented cultures. In cultures with fibronectin-depleted FCS (7%), very few colonies formed; addition of 5 or 10 μg/ml of fibronectin reconstituted colony growth. Addition of 1-10 μg/ml of the 70 kd amino-terminal fragment of fibronectin, which blocks fibronectin fibril formation, inhibited SP1 colony formation. Immunoprecipitation of ^{125}I surface-labelled cells with anti-integrin antibodies demonstrated that the $\alpha_5\beta_1$ fibronectin receptor is the most abundant integrin type expressed on SP1 cells: there were lower amounts of $\alpha_v\beta_1$, $\alpha_6\beta_1$, and $\alpha_3\beta_1$, and no detectable amounts of $\alpha_1\beta_1$ or $\alpha_2\beta_1$. Together, these findings indicate that augmentation of the fibronectin adhesion system, in particular fibronectin deposition, may be one important regulatory event in adipocyte-mediated anchorage-independent growth of SP1 cells. (Supported by grants from NCI(C) and MRC).

Y 509 RADIOIMMUNOTHERAPY OF NUDE MICE BEARING A HUMAN HER2/NEU POSITIVE TUMOR UTILIZING THE α-EMITTING RADIONUCLIDE-CONJUGATED MONOCLONAL ANTIBODY ^{212}Pb-DOTA-AE1, Eva Horak,¶ Frank Hartmann¶, Kayhan Garmestani#, Chuanchu Wu#, Otto A. Gansow#, Nicholas F. Landolfi*, Thomas A. Waldmann¶. ¶Metabolism Branch, National Cancer Institute, NIH, Bethesda, MD 20892; #Radiation Oncology Branch, NCI; *Protein Design Labs, Mountain View, CA 94043.

Alpha particle emission with its high energy transfer and short range of action represents an attractive candidate for receptor targeted radioimmunotherapy of neoplastic diseases. We have evaluated the efficacy of alpha particle labeled murine anti-HER2/neu monoclonal antibody (^{212}Pb-AE1) in the prevention of development and in the therapy of established SK-OV-3 tumors expressing HER2/neu receptors in nude mice. Tumor development was prevented in 100 percent of animals that were injected with SK-OV-3 subcutaneously when treated 3 days subsequently with 20 μCi of ^{212}Pb-AE1 intravenously. In contrast treatment with equally labeled irrelevant antibody, unlabeled AE1 antibody and no treatment was not effective . Moreover this therapy was not effective with large established tumors. Thus monoclonal antibodies armed with the alpha emitting radionuclide ^{212}Pb may be of value in the adjuvant therapy of tumor micrometastasis.

Y 510 IMPROVEMENTS IN CONTINENCE USING DORSAL VENOUS LIGATION WITH ANTERIOR URETHROPEXY IN RADICAL RETROPUBIC PROSTATECTOMY Jeffrey A. Jones, Michael Straub, Jonathan Vordermark, O. Stalcup, Victor Ortiz, Lynn Avant. Division of Urology, Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, Texas 79415.

Methods to facilitate the vesico-urethral anastomosis and to enhance recovery of urinary continence are constantly being sought in patients undergoing radical prostatectomy(RRP) for prostate adenocarcinoma. The Deep Dorsal Vein Ligator(DDVL) was used to facilitate the vesico-urethral anastomosis, and to improve functional urethral length in patients undergoing RRP. The procedure is performed by dilating the urethra to 28 French. Then the puboprostatic ligaments and endopelvic fascia are incised, and the lateral prostate mobilized. The Dorsal Venous Complex is secured utilizing the DDVL after placing the ligating device in the urethra and delivering the needles lateral to the deep dorsal venous complex. The suture is tied and the anterior wall of the urethra is divided sharply. A free needle is placed on one limb of the absorbable ligating suture and is suspended from the inferior surface of the pubic symphysis periosteum, to complete the anterior urethropexy(AUP). The vesico-urethral anastomosis is then performed with six interrupted absorbable sutures after the prostatectomy is completed. This technique has been evaluated in twelve patients with the DDVL+AUP and in nine control RRP patients with pre- and post- operative endoscopic visualization and fluorovideourodynamics + urethral pressure profilemetry. The functional urethral length with this method has increased from an average of 1.8cm with standard methods to 3.0cm when utilizing the urethropexy. The mean time to continence has decreased from four months with standard methods to less than 1 month with the DDVL+AUP. All patients with functional urethral lengths(FUL) less than 1.5 cm are wet, while all with FUL greater than 2.8cm are dry. The DDVL + anterior urethropexy has facilitated the vesico-urethral anastomosis and improved continence in patients undergoing RRP.

Y 511 EXPRESSION OF HUMAN PROSTATE SPECIFIC ANTIGEN IN A TRANSFECTED MURINE ADENOCARCINOMA CELL LINE: DEVELOPMENT OF A MURINE TUMOR MODEL FOR IMMUNOTHERAPY OF PROSTATE CANCER. Joan F. Karr, Judith Kantor, Patricia Horan Hand, Chrysanthi Paranavitana, and Jeffrey Schlom. Laboratory of Tumor Immunology and Biology, National Cancer Institute, NIH, Bethesda, Maryland 20892.

Prostate specific antigen (PSA) is a glycoprotein of 33,000 - 34,000 daltons that is secreted exclusively by prostatic acinar cells and ductal epithelium. PSA, which belongs to a family of serine proteases, is frequently present in elevated levels in the serum of men with prostatic cancer. For this reason, PSA may be a potential target for specific immunotherapy of prostatic carcinomas. Rodent model studies involving human PSA as a target for active specific immunotherapy cannot be carried out since PSA is not expressed in rodent tumors. In order to develop such a model, we have transfected a C57BL/6 murine colon adenocarcinoma cell line, MC-38, with a retroviral vector containing cDNA encoding the human PSA gene. Genetic analysis of a PSA secreting clone, MC-38/PSA, demonstrated that the PSA gene had been stably integrated into the MC-38 genome. PSA expressed by these cells was analyzed by immuno-chemical and immunohistological methods. *In vivo*, MC-38/PSA grows as a subcutaneous tumor in male and female mice. MC-38/PSA has been characterized with respect to growth rate, expression of PSA in tumors and sera, and development of host immune response. It is proposed that this tumor model may be employed in the design of potential therapeutic vaccines and protocols to study immunotherapy responses directed against prostatic carcinoma expressing PSA.

Y 512 TUMOR SPECIFIC CYTOTOXIC T LYMPHOCYTES DERIVED FROM TUMOR INFILTRATING LYMPHOCYTES AND PERIPHERAL BLOOD LYMPHOCYTES BY *IN VITRO* SENSITIZATION WITH GENE MODIFIED AUTOLOGOUS TUMOR IN BREAST CARCINOMA. Lydia Kilinski, Deepa Murugesh, Elisa Brunette, Sohel Talib, Bharati Sanjanwala, Farshid Oshidari, Jane Lebkowski, Thomas B. Okarma and Ramila Philip. Applied Immune Sciences, Inc., Santa Clara, CA 950504

Recent interest in tumor immunotherapy has focused on the study and therapeutic exploitation of tumor infiltrating lymphocytes (TIL). TIL, being the immune system's representatives at the tumor site, appear to be enriched with cytotoxic and helper lymphocytes with MHC restricted specificity for autologous tumor. When removed from the immunosuppressive tumor microenvironment and activated and expanded with rIL-2, both tumor specific and MHC unrestricted cytotoxic lymphocytes were generated. We have explored the use of adeno-associated viral plasmid DNA:cationic liposome complexes to introduce an IL-2 gene in primary breast tumor cells to use them for *in vitro* sensitization of T cells. We have isolated tumor associated T cells and expanded them in rIL-2, and subsequently stimulated them with autologous tumor or an IL-2 gene transfected tumor *in vitro*. Phenotype, proliferation, cytotoxicity and T cell receptor restriction have been analyzed using these stimulated T cell populations. These analyses have demonstrated that although TIL are retrieved from the tumor, long term culture in IL-2 induces polyclonal expansion and loss of tumor specificity. However, when the expanded T cells were stimulated with autologous tumor, the specificity was enhanced and rescued. The specificity of TIL stimulated by an IL-2 gene modified tumor was greater than unmodified tumor when compared to day-0 specificity as demonstrated by TCR repertoires. Efficient gene transfer protocols for primary tumor cells and T cells will also be discussed.

Y 513 **RELEVANCE OF MULTIPLE BIOLOGICAL PARAMETERS IN BREAST CANCER PROGNOSIS,** S. Scholl[1], F. Beuvon[1], C. Pallud[2], K. Hacene[2], W. Gullick[3], P. Pouillart[1] and R. Lidereau[2], Département de Médecine , Institut Curie, Paris FRANCE (1), Centre René Huguenin, St Cloud, France,(2) and ICRF Oncology Group, Hammersmith Hospital, London, U.K (3)

A multitude of clinical, pathological and biological parameters have been reliably associated with prognosis in breast cancer patients in the past and much interest has been engendered by multifactorial computing methods attempting to elaborate higher prognostic accuracy as well as being of clinical utility for treatment selection. Clinical, pathological and a number of more recent biological parameters were evaluated retrospectively in a population of 196 breast cancer patients who had been treated with first line surgery and followed for a median of 7.3 years. Tumor size, menopausal status, type of treatment as well as tumor grade, pathological tumor size, node invasion, the presence of vascular emboli and steroid hormone receptor status were evaluated together with gene amplifications (by Southern blot) of neu, c-myc and int2 and overexpression (by immuno- histochemistry, IHC) of neu, EGF receptor, CSF-1 and CSF-1 receptor. The presence and abundance of inflammatory cell infiltrates (T,B cells and monocytes) was also evaluated by IHC. The risk of recurrence and death as tested in univariate and multivariate analyses was consistantly and independantly higher in patients with positive axillary nodes and whose tumours showed evidence of int2 gene amplification, of overexpression of neu at the cellular membrane, of vascular invasion by tumour cells and of abundant CD45RO+ T cells infiltrates. A prognostic index was calculated for each patient by adding the relative risk associated with each of these five parameters and risk profiles were established in order of increasing risk. Survival curves drawn for three groups of high, intermediate or lowest risk show a highly significantly poorer survival for the highest risk group.

The model we present, although far from the complexity of a 'neuronal network' analysis, does discriminate effectively between low, moderate and high risk groups. Future prospective studies should test these independant prognostic markers together with more recently established markers in an attempt to determine not only the most proficient but also the most cost-effective 'prognostikit' for breast cancer patients as well as to design new therapeutic approaches geared to specific disregulations of a given tumour.

Late Abstracts

CLONING AND CHARACTERIZATION OF A PUTATIVE METASTASIS SUPPRESSOR GENE ON HUMAN CHROMOSOME 11p11.2-13 FOR PROSTATIC CANCER, J. Carl Barrett, Jin-Tang Dong, Pattie W. Lamb, Carrie W. Rinker-Schaeffer and John T. Isaacs, Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, Research Triangle Park, NC, 27709 and Johns Hopkins Oncology Center, Baltimore, MD, 21231

Metastasis is the major cause of death for prostatic cancer (PC) patients. The progression of prostate cancer involves both the loss of metastasis suppressor functions and the activation of oncogenes. We have previously shown that human chromosome 11p11.2-13 is capable of suppressing metastatic ability of a rat PC cell line (Cancer Res., 52, 3486, 1992). The present study was carried out to isolate the responsible gene. Alu-PCR was performed on the hybrid containing human chromosome 11p11.2-13 and the PCR products were used to screen a cDNA library of the same hybrid. Several human cDNA clones from the hybrid have been identified. One of them, named JTD-161, detects a single human mRNA species of 2.4 kb. It predicts a 267 amino acid protein which contains 4 putative transmembrane domains and 3 potential N-linked glycosylation sites, suggesting that it may be distributed on cellular membrane and play a role in the branching of oligosaccharides, which are associated with metastasis. JTD161 is down-regulated in metastatic cancer cell lines examined, while its expression in metastasis-suppressed PC cells and normal prostate tissues is quite strong, suggesting its possible role in metastasis suppression. JTD161 has been transfected into the parental rat PC cell line and preliminary results indicate suppression of metastasis.

THROMBOSPONDIN OVEREXPRESSION IN THE HUMAN MDA-MB-435 BREAST CARCINOMA CELL LINE SUPPRESSES TUMOR GROWTH AND METASTATIC POTENTIAL, Debra L. Weinstat-Saslow, Vivian S. Zabrenetzky, David D. Roberts and Patricia S. Steeg, Laboratory of Pathology, National Cancer Institute, Bethesda, MD 20892

Thrombospondin (TSP-1) is an extracellular matrix glycoprotein that participates in multiple aspects of the metastatic process, including the modulation of cell adhesion, migration, and division. In the case of endothelial cells, TSP-1 inhibits cell proliferation, cord formation, bFGF-induced migration, and angiogenesis. To test the effect of tumor cell overexpression of TSP-1 on tumor growth, metastasis, and/or angiogenesis, we transfected the MDA-MB-435 breast cell line with the human TSP-1 cDNA (*THBS-1*) linked to a constitutive viral promoter. Two clones overexpressing *THBS-1* mRNA and TSP-1 protein were identified, TH29, which produced ~10-fold greater TSP-1 levels than control-transfected cells, and TH26, which produced ~50-fold greater levels than control transfectants. These cell lines showed no significant differences in cell morphology, growth, or colonization *in vitro* compared to control cell lines. Injection of TH26 and TH29 cells into the mammary fat pad of nude mice reproducibly resulted in markedly reduced primary tumor incidence and size compared to injection of control cells. The incidence of pulmonary metastases in each animal, determined by microscopic examination of stained lung sections, was ~50% in mice injected with control-transfected cells and ~25% in mice injected with *THBS-1*-transfected cells. Preliminary data from immunohistochemical staining of sections of primary tumors with endothelial cell-specific antibodies indicated that tumors from mice injected with TH26 cells had less capillaries, suggesting a possible reduction in angiogenesis. Furthermore, bovine aortic endothelial cells were less motile in the presence of conditioned media from TH26 and TH29 cells compared to control-transfected cells. Thus, overexpression of TSP-1 significantly inhibited MDA-MB-435 metastatic potential *in vivo* as well as primary tumor incidence and size, and the data are consistent with the hypothesis that TSP-1 is acting through its angiostatic properties.

STEROID RESISTANT ZR-I CELLS PRODUCE CONDITIONED MEDIA THAT INTERFERES WITH THE GROWTH INHIBITION OF TAMOXIFEN, Anne-Marie Maddox, University of Arkansas Medical School, Division of Hematology/Oncology, Little Rock, Arkansas 72205

Hormone sensitive ZR-75-I cells were made resistant to the anti-estrogens, tamoxifen and LY117018. These cloned resistant variants were stable in culture for greater than six months. Estrogen and progesterone receptors could not be measured in these resistant lines by radioligand assay. Expression of the early response genes, jun and fos after treatment with estrogen or tamoxifen was similar in the hormone sensitive and the resistant lines. The oncogene, cMyc, failed to increase in response to estrogen and decrease with tamoxifen treatment in the resistant variants. Conditioned media (serum free and stripped serum) from these resistant variants interfered with the growth inhibition of tamoxifen treatment on hormone dependent ZR-75-I cells. The factor or factors produced by the variants responsible for the interference of tamoxifen mediated growth inhibition have not been identified. Epidermal growth factor and alpha-transforming growth factor produce a similar antagonism to the action of tamoxifen on ZR-75-I and may be involved.