BREAST AND PROSTATE CANCER

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Receptors

CC 001 ESTROGEN RECEPTOR AND PROGESTERONE RECEPTOR REGULATION, Benita S. Katzenellenbogen, Susan M. Aronica, Hyeseong Cho, Mary Herman, William L. Kraus, Pascale LeGoff, Peter NG, Farzad Pakdel, Joseph C. Reese, Cynthia H. Wooge and Carol K. Wrenn, University of Illinois, Department of Physiology & Biophysics, Urbana, IL 61801.

We are interested in identifying the factors that regulate the levels and activity of estrogen receptors (ER) and progesterone receptors (PR) in breast cancer cells since these will play an important role in determining cell responsiveness to estrogen (E) and progestin hormones. Studies on ER regulation of PR biosynthesis and ER regulation of transfected estrogen response element-CAT reporter plasmids indicate that some growth factors such as IGF-1 and agents that increase intracellular cAMP,in addition to estrogen, influence the ability of the ER to stimulate transcription. Protein kinase inhibitors and antiestrogens suppress the increases evoked by cAMP, IGF-1 and estrogen indicating the involvement of the ER and phosphorylation pathways. Direct phosphorylation studies show that many of these agents do alter the magnitude of ER phosphory-lation, although the role of phosphorylation in transcription regulation is not clear. E is a major regulator of ER levels, and the reduction in ER levels by E in most breast cancer cells at least in part reflects a decrease in ER gene transcription rate. Affinity labeling studies with covalently attaching estrogens (Es) and anti-estrogens (AEs) and the use of site directed mutants have identified cys 530 and cys 381 as sites of covalent labeling by aziridine analogs of Es and AEs. Site-directed mutagenesis of select amino acids in these regions of the ER hormone-binding domain, and random chemical mutagenesis of the ER cDNA with screening in yeast, followed by assessment of interesting mutants in mammalian cells, have provided information about regions of the receptor important in ligand binding and in discrimination between Es and AEs. Of note, some mutants which fail to respond to Es still respond to AEs and vice versa, and some mutants are even more sensitive to low concentrations of estrogen than is wild type receptor. Changes of specific amino acids near cys 530 result in changes in binding affinity of receptor for one category of ligands (Es) but not for another (AEs), resulting in a greatly reduced potency of Es in transactivation and a greatly enhanced sensitivity to AEs in suppression of E-stimulated transcription, suggesting that these amino acids near cys 530 are involved in receptor discrimination between Es and AEs. These studies provide evidence for multifactor regulation of ER bioactivity and are beginning to provide detailed information on residues that comprise the ER hormone binding pocket and amino acids important in discrimination between different categories of agonist and antagonist ligands.

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CC 002 DIFFERENTIAL EFFECTS OF ANTIESTROGENS ON ESTROGEN RECEPTOR DIMERISATION AND TURNOVER, Malcolm G. Parker, Sophie Dauvois, Nicola Arbuckle, Paul Danielian, and Roger White. Molecular Endocrinology Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.

The normal function of estrogen receptors as transcription factors can be inhibited by the binding of antiestrogens. Some, such as tamoxifen, not only act as antiestrogens but also behave as agonists depending upon the responsive gene and cell type. Others, such as ICI 164,384, are claimed to be "pure" antiestrogens. We have compared the effects of these two antiestrogens on estrogen receptor function. Previous work in our and other laboratories has shown that tamoxifen, like estradiol, promotes the DNA binding activity of estrogen receptors but fails to stimulate full transcriptional activity. Preliminary work with ICI 164,384 is rather more controversial. We have shown that the DNA binding activity of the receptor was inhibited by this antiestrogen, probably by interfering with dimerisation of the receptor. It appears that its ability to inhibit DNA binding in cell-free extracts depends on the binding conditions and this might be explained by variations in the stability of receptor dimers in different sources of receptor. Since it may not always be possible to dissociate pre-formed dimers and inhibit DNA binding in extracts we have investigated whether ICI 164,384 was able to prevent DNA binding in intact cells. Under conditions in which receptor mRNA was unaffected by ICI 164,384 we found that DNA binding was rapidly reduced as predicted but this was in fact due to a reduction in the cellular levels of receptor. The effect was dose dependent, reversible by estradiol and mediated by the hormone binding domain. Pulse chase experiments indicate that the half-life of the receptor is reduced from approximately 5 hours in the presence of estradiol to less than 1 hour by ICI 164,384. It seems likely that the increased turnover of the receptor is a consequence of impaired receptor dimerisation. ICI 164,384 also reduced cellular receptor levels in breast cancer cells (ZR 75-1) but the results are complicated by additional effects on estrogen receptor gene expression.

CC 003 GENETIC DIFFERENCES IN SUSCEPTIBILITY TO *ras+myc*-INDUCED CARCINOGENESIS IN RECONSTITUTED MOUSE PROSTATE INVOLVES MESENCHYMAL-EPITHELIAL INTERACTIONS. T.C. Thompson[1,2], T.L. Timme[1], S. Egawa[1], L. Truong[3], X. Lu[4], D.P. Lane[4], S.H. Park[1], E. Ripoll[1], A. Mottaz[1], K. Yoshida[1], D. Kadmon[1], and P.T. Scardino[1]. [1]Scott Department of Urology, Baylor College of Medicine, [2]Department of Cell Biology, Baylor College of Medicine, [3]Department of Pathology, Baylor College of Medicine, [4]Department of Biochemistry, University of Dundee.

Using a mouse prostate reconstitution (MPR) model system under conditions where the *ras* and *myc* oncogenes are introduced via a recombinant retrovirus into both the mesenchymal and epithelial compartments of the urogenital sinus, poorly differentiated prostate cancer is produced with high frequency (>90%) in inbred C57BL/6 mice. In contrast, similarly infected inbred Balb/c MPRs form benign prostatic hyperplasia that converts to cancer with low frequency (5%-10%). The observed strain-specific susceptibility to *ras+myc*-initiated carcinogenesis does not appear to result from differences in virus infectivity or viral gene expression but as demonstrated by southern blotting results from a marked reduction in both the number of *ras+myc*-initiated cells that undergo clonal expansion as well as the extent of clonal expansion in *ras+myc*-initiated Balb/c MPRs. Our genetic studies using *ras+myc*-sensitive C57BL/6 mice and resistant Balb/c mice are consistent with polygenic determinants for carcinogenesis which include a dominant growth suppressor activity. The transition from benign to malignant status in *ras+myc*-initiated epithelial cells is always associated with the induction of elevated transforming growth factor-beta 1 (TGF-81) expression in both C57BL/6 and Balb/c MPRs. However, we have also shown by restricting the oncogenes to the mesenchymal compartment that transforming growth factor-81 expression and a concomitant desmoplastic reaction is induced to a greater extent by *ras+myc* in C57BL/6 mesenchyme compared to that of Balb/c mice. These differences led to studies using heterologous MPRs composed of C57BL/6 mesenchyme and Balb/c epithelium and vice versa which clearly demonstrated that intrinsic properties of Balb/c mesenchyme can block the transition of *ras+myc*-initiated C57BL/6 epithelium from benign hyperplasia to malignant carcinoma.

Interestingly, chronic exposure to pharmacological levels of testosterone dramatically increased the incidence of *ras+myc*-induced carcinomas in resistant Balb/c mice from 6% (n=32) to 47% (n=17). Elevated levels of *jun*-B mRNA were markedly reduced in tumors promoted by testosterone compared to those which arose infrequently in the control *ras+myc* only group suggesting a possible tumor suppressor function for this gene under these conditions. In a large panel of cell lines derived from *ras+myc*-induced C57BL/6 carcinomas, we have demonstrated a second novel mechanism through which tumor suppressor activity may be eliminated. In 6 out of 7 tumor cell lines, apparent loss of p53 function was observed indicating this biological feature was highly selected for during progression. Overall, these studies indicate that the strain-specific response to *ras+myc*-induced carcinogenesis is manifest in the mesenchymal as well as the epithelial compartment and define a role for the mesenchyme in the rate of cancer progression in this model system. Interestingly, reduced levels of *jun*-B mRNA levels are associated with *ras+myc* + testosterone-induced Balb/c carcinomas and loss of p53 function appears to be associated with *ras+myc* only-induced C57BL/6 carcinomas. Further studies will elucidate the complex interrelationships between these candidate tumor suppressor genes and specific mesenchymal-epithelial interactions involved in carcinogenesis in this model system.

CC 004 THE ROLE OF TRANSFORMING GROWTH FACTOR β IN HUMAN PROSTATE CANCER, George Wilding, Jacque Mitchen, Ann-Li Cheng, Maureen Ripple, Randall Rago, Frank Mott and Chawnshang Chang, Department of Human Oncology, University of Wisconsin Comprehensive Cancer Center and the Wm. S. Middleton VA Hospital, Madison WI.

The growth of human prostate cancer and its relationship to the surrounding stroma are controlled by complex mechanisms. Growth factors such as TGF β and its family members appear to have crucial roles in these processes and are notable for their wide spectrum of biological effects. Since TGF β1 inhibits the growth of a variety of epithelial tumor cells in vitro, we examined the effects of TGF β1 on human prostate cancer cell lines for possible inhibitory activity (1). In summary, growth in monolayer was initially inhibited in a dose-response fashion in the two androgen independent cell lines PC3 and DU145. Anchorage-independent growth was inhibited to 55% and 16% control levels in PC3 and DU145, respectively. Scatchard analysis showed 1500 and 2900 TGF β binding sites on DU145 and PC3, respectively. High affinity sites could not be found on LNCaP cells. Analysis of conditioned media by immunoprecipitation and a radioreceptor assay showed secretion of TGF β1 into the media by DU145 and PC3 but not LNCaP. Northern analysis showed the presence of TGF β1 transcript in DU145 and PC3 but not LNCaP. These data indicate that TGF β1 might serve as an autocrine inhibitory factor in prostate cancer.

Injection of TGF β1 i.p. or s.c. into nude mice induces a generalized interstitial fibrosis and cachexia (2). Treatment of nude mice with TGF β1 via a s.c. infusion pump failed to inhibit the growth of subcutaneously implanted DU145 cells proximal or distal to the pump exit despite evidence that trichloroacetic acid precipitable ^{125}I-TGF β1 injected subcutaneously is taken up by the tumors. Since the inhibitory effects of TGF β1 on prostate cancer cells appears to diminish as the process of transformation progresses towards less differentiated states, the net effect of TGF β on prostate tumor growth may be positive.

Primary epithelial cultures derived from normal, BPH and cancerous prostate tissues were evaluated for their response to TGF β1. All of the primary cultures examined were inhibited by TGF β1 with an IC50 of approximately 10pM compared to the established lines where IC50's were 10 fold greater. No differential response to TGF β1 was observed for epithelial cells derived from the three tissue types. The inhibition by TGF β1 was not reversed by the addition of EGF or HBGF I. Flow cytometry showed an increase in the fraction of cells in G_1/G_0 with a decrease in the fraction of cells in S phase after TGF β1 treatment. Analysis of mRNA from these primary cultures revealed the presence of TGF β1,2&3 mRNA's in each of the cultures without differential expression according to the pathology of the tissue of origin. In situ hybridization of prostate tissue is now being performed to determine the cells of origin of the TGF β's.

Both the Rb and c-myc gene products have been implicated as possible mediators of TGF β's growth inhibitory effects. In our laboratory, in both PC3 and DU145 cells, TGF β1 (100pM) increased the steady state mRNA levels of c-myc and c-jun, with peaks achieved at 6-12 hrs and 1-2 hrs, respectively. The effects of the Rb protein and TGF β on the 5' upstream sequences of c-myc are now being investigated.

Androgens might play a critical role in the modulation of TGF β expression in prostate cancer cells. In LNCaP cells, which contain a mutated AR, TGF β3 expression increased 3x when placed in charcoal stripped serum for 7 days. TGF β2 expression was not detectable. By using a ribonuclease protection assay, 1 nM R1881 resulted in a transient diminished expression of TGF β3. We are examining the effects of AR on TGF β1 5' flanking sequences.

(1) Wilding G, Zugmaier G, Knabbe C, Flanders K, Gelmann E: Differential effects of transforming growth factor β on human prostate cancer cells in vitro. Molec Cell Endocrinol 62:79-87, 1989.
(2) Zugmaier G, Paik S, Wilding G, et al: Transforming growth factor β1 induces cachexia and systemic fibrosis without an antitumor effect in nude mice. Cancer Res 51:3590-3594, 1991.

Prostate Cancer B

CC 005 MODIFICATION OF DIFFERENTIATION AND NEOPLASTIC GROWTH IN A RAT PROSTATIC CARCINOMA BY INDUCTIVE MESENCHYME. G.R. Cunha and N. Hayashi, University of California, Anatomy Department, San Francisco, CA 94143

Mesenchymal-epithelial interactions have been shown to continue into adulthood. Alteration in the mesenchymal environment of an adult epithelium can have profound effects on morphogenesis, cytodifferentiation and function of an adult epithelium. For example, when adult epithelial cells from the ureter, epididymis, or ductus deferens are grown in association with neonatal seminal vesicle mesenchyme, seminal vesicle differentiation is induced morphologically and functionally with the expression of the complete spectrum of major seminal vesicle secretory proteins. Since adult epithelial cells are unquestionably capable of expressing alternative phenotypes when induced by embryonic or neonatal mesenchymes, this suggests that the stability of adult epithelial differentiation may be due to continuing influences of the adult stroma. A corollary to this concept suggests that emerging or established carcinomas might be also be altered by their connective tissue environment. To test this idea the effects of certain urogenital mesenchymes have been examined for their potential to alter the differentiation of the R3327 Dunning prostatic adenocarcinoma (DT)

The experimental model involved associating mesenchyme of the urogenital sinus (UGM) or seminal vesicle (SVM) with 0.5 mm^3 fragments of the DT and growing the resultant tissue recombinants in male nude mouse hosts for 1 month to assess epithelial differentiation. Such tissue recombinants were defined as 1°SVM+DT or 1°UGM+DT recombinants. Grafts of DT alone formed tumors containing small ducts lined by one or more layers of undifferentiated squamous or cuboidal epithelial cells characteristic of the DT. In grafts of 1°SVM+DT or 1°UGM+DT recombinations the mesenchyme induced the undifferentiated DT epithelial cells to differentiate into tall columnar secretory epithelial cells arranged into large cystic ducts. Interposed between these more normal ducts were regions that maintained the histopathology characteristic of the parental DT. In contrast to the differentiating effects of UGM and SVM, mesenchyme of the neonatal urinary bladder failed to induce changes in the DT.

To determine whether the mesenchyme-induced changes in histodifferentiation of the DT cells were coupled to changes in neoplastic growth, the large fluid-filled ducts of 1°SVM+DT recombinants were excised, dissected into 1 mm segments and either grafted to new hosts or associated with fresh mesenchyme to generate 2°SVM+DT recombinants that were in turn grown under the renal capsule of a second male host for 3 months. Secondary hosts were grafted with 3 one mm^3 pieces of the parental DT on one kidney and an equal number of either differentiated DT ducts or 2°SVM+DT recombinants on the opposite kidney. Grafts of cystic ducts of differentiated DT epithelial cells isolated from 1°SVM+DT recombinants exhibited modest growth while 2°SVM+DT recombinants grew little if at all. By contrast, grafts of the DT completely overgrew the host's kidney forming a single large tumorous mass. The apparent reduction in growth rate and loss of tumorigenesis of the SVM-induced DT epithelial cells was accompanied by an 8-fold reduction in ^{3}H-thymidine labelling index.

CC 006 ROLE OF NERVE GROWTH FACTOR-LIKE PROTEIN(S) IN THE PARACRINE REGULATION OF PROSTATE GROWTH. Daniel Djakiew, Department of Anatomy & Cell Biology, & Surgery (Division of Urology), Georgetown University Medical Center, 3900 Reservoir Rd., N.W., Washington, D.C. 20007.

Prostate cancer has become the most frequently diagnosed form of cancer in men throughout the U.S.A. Recently, our laboratory described a nerve growth factor (NGF)-like protein which modulates paracrine interactive growth between a human prostatic stromal cell (HPS) line and a human prostatic tumor cell (TSU-pr1) line. During these investigations we determined that secretory proteins from the HPS stimulated relative growth of the TSU-pr1 line in a dose dependent manner and that immunoneutralization of the HPS secretory protein with an antibody against NGF eliminated the growth stimulatory effect of the HPS protein. Immunofluorescence microscopy localized the NGF-like protein in secretory vesicles within the stromal cells. In addition, Western blots of the HPS protein identified two NGF-like immunoreactive bands of 65 kD and 42 kD. Neurite outgrowth assays of PC12 cells confirmed the presence of NGF-like activity in the HPS protein. In order to relate these results to prostatic adenocarcinomas in vivo we carried out immunohistochemical studies of the NGF-like protein and the low affinity NGF receptor (NGF-R) on frozen tissue sections. These results show that the paracrine NGF-like protein is localized predominantly to the stromal compartment, whereas the NGF-R localized exclusively to the epithelium of acini in the adenocarcinoma tissue sections. Hence, the NGF-like protein secreted by stromal cells may be interacting with an NGF-R on the adjacent epithelial cells in the mediation of prostatic growth. In order to investigate other functions of the NGF-like protein secreted by the HPS cells we carried chemotaxis assays in blind ending Boyden chambers. The results show that HPS protein stimulates chemotaxis of the TSU-pr1 cells in a dose dependent manner, maximal chemotaxis occurring at a concentration of approximately 50 μg/ml. Immunoneutralization of the maximal stimulatory dose of HPS protein with a monoclonal antibody or polyclonal antibody against NGF reduced chemotaxis to 43% and 36% of maximal levels, respectively. In conclusion, it appears that an NGF-like protein within the human prostate gland is secreted by stromal cells; that the adjacent epithelial cells contain a NGF receptor, and that this NGF-like factor is associated with the paracrine stimulation of epithelial cell growth and chemotaxis.

CC 007 MOLECULAR CHANGES ASSOCIATED WITH THE PROGRESSION OF PROSTATIC CANCER, John T. Isaacs[1], William B. Isaacs[1], Bob S Carter[1], Jonathan L Epstein[1], Constance A. Griffin[1], Tomohiko Ichikawa[1], Jack Schalken[2], and Carl Barrett[3]. [1]Johns Hopkins University School of Medicine, Baltimore, MD, [2]University Hospital, Nijmegen, The Netherlands, [3]National Institute of Environmental Health Science, Research Triangle Park, N.C.

Prostatic cancer varies widely in its clinical aggressiveness. In some patients, prostatic cancer metastasizes rapidly, killing the patient within one year of initial clinical presentation; other patients may live for many years with localized disease without apparent metastases. If prostatic cancer is truly localized, then radical prostatectomy can be used to cure the patient. In contrast, if a patient has metastatic prostatic cancer, systemic therapy is required. Although androgen ablation is standard therapy for metastatic prostatic cancer, this therapy is rarely curative. The major reason for this is that metastatic cancer within an individual patient is heterogeneous, including both androgen-dependent and -independent prostatic cancer cells, even before therapy is initiated. Thus, androgen ablation does not eliminate preexisting androgen-dependent cancer cells within the patient. What is urgently needed is an effective therapy for androgen-independent prostatic cancer cells which can be combined with any of the large variety of available forms of androgen ablation to affect all of the heterogeneous tumor cell populations present within an individual patient with prostatic cancer. Unfortunately, there are no currently utilized chemotherapeutic agents which can effectively control the growth of androgen-independent prostatic cancer cells.

Based upon these limitations, it is logical to suggest that aggressive screening of aging men for prostatic cancer should allow greater numbers of prostatic cancers to be diagnosed during a curable portion of their natural history. As logical as such an approach is, there are major problems with aggressive screening for prostatic cancer. Based on autopsy studies, 10% of men in the age range of 50-60 years and 50% of those 70-80 years of age have histological deposits of cancer within their prostates. Only a small subset of these histologically detectible prostatic cancers undergo all the malignant changes required to produce a clinically aggressive tumor. In addition, the majority of these histologically localized prostate tumors never undergo the malignant changes required to produce a life threatening disease despite host longevity and ample time for tumor growth. Thus, the majority of histologically localized prostatic cancers will remain subclinical and will never require treatment. Presently, it is not possible to predict which histologically localized cancers have undergone all of the steps needed for progression to clinical cancer and which have not (i.e. the natural history is unpredictable). Thus, the ability to predict which tumors have the capacity to manifest aggressive behavior requiring therapy becomes a critical issue as greater emphasis is placed upon screening for earlier detection of prostatic cancer.

In an effort to understand the molecular events leading to tumor progression, we have begun to analyze genetic changes in prostate cancers. These studies demonstrate that mutations and/or down-regulation in the p53, Rb, E-cadherin, and 5α-reductase genes, allelic loss of genes on chromosomes 10q, 11p, 16q, 8p, and 17p, and enhanced expression, but not mutations, of the ras family of oncogenes occur in prostatic cancer. The critical role and temporal sequence of any of these molecular change in prostatic cancer progression is just beginning to be clarified. Greater knowledge of these events may result in the ability to predict prostatic cancer aggressiveness.

During the progression of prostatic cancer, initially localized cancer cells can acquire the ability to metastasize to distant sites. Acquisition of metastatic ability by prostatic cancer cells involves not only increased gene expression but also decreased expression of metastasis suppressor genes. The conclusion is based upon the observation that when highly metastatic rat prostatic cancer cells are fused with non-metastatic rat prostatic cancer cells, the metastatic ability of the resultant somatic cell hybrid is suppressed without suppression of the tumorigenicity, if the hybrid cells retain all of the chromosomes from both of the parental lines. This suggests that for a prostatic cancer cell to become highly metastatic, metastatic suppressor gene(s) must be inactivated by either mutation, allelic loss of epigenetic inactivation (eg. hypermethylation). Additional studies using this rat hybrid system demonstrated that high metastatic ability is re-expressed in occasional hybrid segregates which minimally lose a single copy of a normal rat chromosome 2. Rat chromosome 2 thus appears to contain gene(s) which can suppress the metastatic ability of rat prostatic cancer cells. To determine the chromosomal location of human prostatic cancer metastasis suppressor gene(s), the technique of microcell-mediated chromosome transfer was used to introduce specific human chromosome into highly metastatic rat prostatic cancer cells. Introduction of human chromosome 11 into highly metastatic rat prostate cancer cells results in suppression of metastatic ability without suppression of the in vivo growth rate or tumorigenicity of the hybrid cells. Spontaneous deletion of portions of human chromosome 11 in some of the clones, delineated that the minimal portion of human chromosome 11 capable of suppressing prostatic cancer metastasis involves the region between 11p13-11p11.2 but does not include the Wilms' tumor-1 locus. (NIH CA 55231, CA 15416).

CC 008 AUTOCRINE AND PARACRINE GROWTH REGULATION OF HUMAN PROSTATE CARCINOMA CELLS: A NEW TARGET FOR THERAPY OF HUMAN PROSTATE CANCER?

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We have previously shown that different polyanions like suramin and dextransulfates partially antagonize androgen induced growth of androgen-responsive human prostate carcinoma cells through inactivation of secreted growth factors in a dose dependent and reversible fashion. Growth of androgen- independant human prostate carcinoma cells was also inhibited by polyanionic substances. These data suggest that part of androgen action is mediated through autocrine acting growth factors. On the basis of these findings, a new therapy of prostate cancer, the ***antipeptidergic*** approach, has been proposed which is directed against **autocrine** mechanisms of both androgen-dependent and -independent cells. Ongoing studies have allowed the identification of members of the TGFα-, TGFß-and FGF-family as androgen regulated secretory products of human prostate cancer cells in tissue culture as well as in endocrinologically characterized tissue from human prostate primary cancers and metastases. Whereas TGFßs are used as markers of (anti)androgen action FGFs might play an important role in the paracrine communication between the tumor cell and the surrounding stroma. Therefore, inhibition of **paracrine** mechanisms represents another target of the ***antipeptidergic*** approach of therapy. Supported by DFG

Clinical Aspects of Prostate and Breast Cancer

CC 009 GROWTH FACTORS AND ANGIOGENESIS, Adrian L Harris, Elizabeth Horak, Ken Smith, Russell Leek, Sue Lejeune, Michele Relf and Roy Bicknell, ICRF, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, U.K

Epidermal growth factor receptor (EGFr) and erbB2 expression in primary breast cancer has been associated with early recurrence and poor survival. Relative resistance to hormone therapy occurred, even in oestrogen receptor positive (ER+) cases, if either growth factor receptor was expressed. p53 expression assessed immunochemically was associated with EGFr+ and ER- cases, but not node status. Other members of the EGFr or its ligands were studied by Northern blots and immunochemistry. Amphiregulin was expressed in 37% of cases and was associated with node positive cases, but not with any other prognostic markers in primary cancers. Angiogenesis was quantified in a series of primary tumours after an initial screen of 10 monoclonal antibodies to various endothelial antigens. This was to relate angiogenesis to other variables and screen for expression of angiogenic growth factors. Vessel counts per high power field correlated highly with lymph node involvement. 4/66 with less than 120 vessels/mm^2 had node involvement, whereas 31/35 with more than 140 vessels/mm^2 had involved nodes. High angiogenesis score was not related to ER, EGFr, but it was to tumour size and poor differentiation. Once adjustment for node status was made, this association was not significant. These results suggest that there is a critical phase of angiogenesis development associated with node metastasis, and it is more likely to occur in poorly differentiated or larger tumours. Although p53, EGFr+, ER- tumours tend to be more aggressive, with high growth fractions, they are not associated with a higher incidence of node involvement or high angiogenesis scores. Thus other prognostic fadctors that may regulate angiogenesis need to be evaluted.

CC 010 CHALLENGES IN PROSTATE CANCER: ETIOLOGY, NATURAL HISTORY, EARLY DIAGNOSIS, AND MANAGEMENT, Patrick C. Walsh, The James Buchanan Brady Urological Institute, The Johns Hopkins Medical Institutions, Baltimore.

Familial Factors: A case controlled study to estimate the relative risk of developing prostate cancer on men with a positive family history was performed. Extensive cancer pedigrees were obtained on 691 men with prostate cancer and 640 spousal controls. Men with a father or brother affected were twice as likely to develop prostate cancer as men with no relatives affected; men with 2 or 3 first degree relatives affected had a 5 and 11-fold increased risk of developing prostate cancer.

Etiology: Androgens may play a role in the pathogenesis of prostatic cancer acting as promotors of disease many years prior to detection. To test this thesis we have evaluated androgen levels longitudinally in men from the Baltimore Longitudinal Study of Aging: 16 men with no prostatic disease by urologic history and exam; 20 men with a histologic diagnosis of benign prostatic hyperplasia (BPH) who had undergone simple prostatectomy; 18 men with a histologic diagnosis of prostatic cancer. An average of 6.5 determinations were made over 20 years prior to diagnosis. The mean age-adjusted free testosterone levels at 10-15 years before diagnosis for subjects with prostatic cancer were significantly higher than for controls and subjects with BPH. These data suggest that serum androgen levels may be important in the pathogenesis of prostatic cancer and may provide a marker for identification of individuals at risk for developing the disease.

Early Diagnosis: A longitudinal evaluation of prostate specific antigen (PSA) levels was performed in the above mentioned group of men from the Baltimore Longitudinal Study. There was a significant difference in the age adjusted rate of change in PSA between groups (prostate cancer > BPH > control, $p<0.05$). At a time when PSA levels did not differ between BPH and prostate cancer subjects (5 years before diagnosis), rate of change in PSA (0.75 ng/ml/year) was significantly greater in subjects with prostate cancer compared to controls or BPH groups. Furthermore, rate of change in PSA distinguished prostate cancer from BPH in control subjects with a specificity of 90 and 100% respectively.

Radical Prostatectomy: An anatomical approach to radical prostatectomy has been developed over the past 15 years. Based upon sound anatomical principles this technique has resulted in less intraoperative blood loss, improved urinary continence postoperatively, and preservation of sexual function in approximately 70% of men. Interim followup evaluation indicates excellent cancer control.

Oncogenes

CC 011 INTERMOLECULAR FEATURES OF RECEPTOR DRIVEN CELL GROWTH AND TRANSFORMATION AND NOVEL FORMS OF ANTI-TUMOR THERAPY, William Dougall, Xiaolan Qian, Valerie Brown, Maria Hellman, Uri Saragovi, Arabinda Samanta, James Davis, Charles LeVea, Makoto Katsumata, and Mark I. Greene, Division of Immunology and Center for Receptor Biology, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA .

The interaction of two receptor species, Neu and the epidermal growth factor receptor (EGFR) appears to be important for regulating their functions in cell growth and transformation. We have described a synergistic effect of Neu and EGFR on cellular transformation. The regions of the Neu receptor which determine its ability to physically associate with the EGFR, and the regions of Neu involved in ligand and anti-receptor antibody mediated dimerization and internalization have been examined. Heterodimerization between Neu and EGFR mediates different cell cycle and growth responses relative to cells expressing only one of these receptors.

The development of a large panel of monoclonal antibodies specific for various regions of the extracellular domains of these receptors has permitted attempts to optimize down modulation in vitro and in vivo. We have devised a unique transgenic model of tumor development (in which Neu and EGFR are co-expressed in the same cells) to study the effects of monoclonal antibody therapy in vivo. Down modulation by sets of antibodies that bind the distinct parts of the extracellular domain is being performed to develop clinically useful reagents. Through analysis of the structure of these monoclonal antibodies, we will develop organic mimetics of the interacting complementarity determining region of the antibodies. These mimetics will be used for receptor analysis and novel forms of therapeutics.

CC 012 THE ROLE OF THE TYPE I GROWTH FACTORS AND THEIR RECEPTORS IN HUMAN BREAST CANCER, William J. Gullick[1], Sally A. Prigent[1], Christine M. Hughes[1], and Nicholas R. Lemoine[2], ICRF Oncology Group, [1]Molecular Oncology Laboratory, [2]Molecular Pathology Laboratory, Hammersmith Hospital, Du Cane Road, London W12 0HS, U.K.

The type I family of protein tyrosine kinase growth factor receptors consist of EGF receptor, c-*erb*B-2 and c-*erb*B-3. Four ligands have been cloned and sequenced and partially characterised which bind to the EGF receptor. These are EGF, TGF-alpha, amphiregulin and heparin binding EGF. A fifth putative EGF-like growth factor called *cripto* has also recently been identified but it is not yet known if this binds to any of the type I receptors. As yet no ligand has been cloned which binds to the c-*erb*B-2 protein. It is not yet clear whether any of the known factors bind to the c-*erb*B-3 protein.

It is known or inferred that these systems which are present widely in normal human tissues are involved in growth regulation such as in embryogenesis and in wound healing. Since cancer is a disorder in which cells grow aberrantly, the structure and function of the type I receptors and ligands have been examined in tumours and cell lines and contrasted with the same systems in the normal cells from which the tumours arise. Alterations in both the structure and level of expression of the EGFR and c-*erb*B-2 protein have found consistently and frequently in common solid human tumours including breast cancer. We have now raised antibodies to additional members of the type I ligand/receptor family in order to explore if they are expressed and are functional in normal breast and if any alterations occur in their sites or levels of expression during breast cell transformation. Three antipeptide antibodies have been raised to the c-*erb*B-3 protein which detect its expression by Western blotting, immunoprecipitation and staining of formalin-fixed, paraffin-embedded tissues. Each gave the same pattern of staining localising the c-*erb*B-3 protein to normal breast myoepithelial cells. About 30% of breast cancers showed moderate to high levels of protein expression. Thirty six cases of breast cancer were examined by Southern blotting for alterations in gene copy number of c-*erb*B-3. All cases had a normal gene number suggesting that alterations in protein expression may be by increased transcription.

We also raised antipeptide antibodies to the amphiregulin and *cripto* proteins. Immunocytochemical staining of normal breast demonstrated low levels of expression of both proteins. Elevated expression was seen in breast cancers suggesting a role for these factors in cancer cell growth. In summary, we have demonstrated expression and possible tumour associated alterations of new members of the type I ligand/receptor family in breast cancer.

Stromal-Epithelial Interactions

CC 013 MAMMARY GLAND AS A MODEL FOR STUDIES OF EPITHELIAL MORPHOGENESIS AND FUNCTION: THE ROLE OF EXTRACELLULAR MATRIX (ECM) AND ECM-DEGRADING ENZYMES, Mina J. Bissell[1], Charles Streuli[1], Christian Schmidhauser[1], Rabih Talhouk[1,2], Zena Werb[2], [1]Lawrence Berkeley Laboratory, Berkeley, [2]UCSF, San Francisco.

In the last decade, it has been convincingly demonstrated that ECM, in general, and basement membrane (BM) components in particular, play a crucial role in maintenance of tissue-specific genes in the mammary gland and most probably other tissues. We have established a functional mammary epithelial culture system where we can study the molecular mechanisms of cell-cell and cell-ECM interactions. We have shown, in a single cell assay, that BM specifically regulates milk protein expression in the absence of cell-cell interaction and polarity (Streuli et al., J. Cell Biol. 1991, in press). The regulation is via ECM receptors (integrins) and is transcriptional (Schmidhauser et al., PNAS, **87**, 9118-9122, 1990).

We have identified an enhancer sequence (160 nucleotides) in the 5' region of β-casein gene that confers ECM-responsiveness and that appears to be tissue-specific.

To verify further the significance of ECM in vivo, we have mapped the developmental regulation of ECM-degrading proteases and their inhibitors in vivo (Talhouk et al., Development **112**, 439-449, 1991). Using implants and transgenic animals (with an activated form of rat stromelysin), we have shown that both morphology and function of the gland can be altered if the balance between the proteinases and their inhibitors is altered.

CC 014 **STROMAL GROWTH FACTOR EXPRESSION AND FUNCTION IN HUMAN BREAST CANCER.** Kevin J. Cullen[1], Christian Singer[1], Audrey A. Allison[1], Isabella Martire[1], Marc E. Lippman[1] and Helene S. Smith[2] [1]Vincent T. Lombardi Cancer Research Center, Georgetown University,Washington DC 20007 and [2]Geraldine Brush Cancer Research Institute, Pacific Presbyterian Medical Center, San Francisco, CA. 94115

Breast tumors are a complex mix of epithelial, stromal and vascular elements. While laboratory models of breast cancer have traditionally focused on studies of malignant epithelium, an increasing body of data over the last several years has begun to address the role of interactions between tumor epithelium and stroma in the genesis and proliferation of breast cancer. We examined primary cultures of breast fibroblasts derived from benign and malignant lesions for expression of various growth factors. All fibroblast cultures, regardless of whether they were derived from benign or malignant lesions expressed PDGF A chain, basic FGF, FGF-5 and TGF-ß1 mRNA. None expressed PDGF B chain or TGF-α mRNA. However, examination of mRNA expression for the insulin-like growth factors revealed 7/8 fibroblasts derived from benign lesions expressed IGF-I mRNA, while only 1/9 fibroblasts derived from malignancies expressed IGF-I mRNA. The opposite picture was seen for IGF-II mRNA expression, in which 1/9 benign derived fibroblasts expressed IGF-II mRNA, while 5/9 malignant derived fibroblasts expressed IGF-II. This correlated with previous in situ hybridization data, which showed IGF-I mRNA expression confined to the stroma of benign breast tissue. Thus there was an apparent dichotomy between IGF-I mRNA expression in the majority of fibroblasts derived from benign lesions and IGF-II mRNA expression in the majority of tumor derived fibroblasts.

We subsequently studied the IGF-I and IGF-II m-RNA expression in matched sets of fibroblasts from each of 6 patients using RNAse protection assays and PCR. These fibroblast sets included cells from breast tumors, macroscopically normal breast adjacent to the tumor and from uninvolved breast skin. Both assays revealed that all fibroblast cell lines from normal breast expressed easily detectable IGF-I mRNA while only 1 of 6 expressed detectable IGF-II. In contrast, 4 of 6 tumor fibroblasts expressed IGF-II mRNA, along with variable amounts of IGF-I. All 6 skin fibroblast cell lines expressed very high levels of IGF-II mRNA and small amounts of IGF-I mRNA. These results indicate that the production of IGF-I is typical for normal breast fibroblasts while tumor and skin fibroblasts express predominantly IGF-II. Since the insulin-like growth factors are potent mitogens for breast tumor epithelial cells, these data support the notion of a paracrine growth promoting role for the insulin-like growth factors in breast lesions, and suggest that IGF-II may be the more important growth promoter in breast cancers. Alternatively, IGF-II expression in tumor fibroblasts may be a marker of some other cellular event in neighboring tumor epithelium.

Co-culture experiments in contact-dependent and contact-independent systems show that breast tumor epithelial cells (MCF-7) are markedly stimulated in the presence of fibroblasts regardless of their source (breast tumor, normal breast or skin). This indicates that soluble factors produced by the fibroblasts are responsible for the mitogenic stimulus seen. Experiments with monclonal antibodies directed against the IGF's and other growth factors are currently underway to identify the ligands responsible for this stimulus.

CC 015 ROLE OF TENASCIN IN STROMAL REGULATION OF BREAST CANCER DEVELOPMENT, Teruyo Sakakura[1], Noriko Hiraiwa[1], Masanobu Obara[1], Yumiko Saga[1], Hirokatsu Kida[1], Tetsuya Tsukamoto[2], Akiko Okada[3], Yasuhiro Tomooka[1], and Moriaki Kusakabe[1], [1]Tsukuba Life Science Center, RIKEN, Tsukuba, [2]Aichi Cancer Center Research Institute, Nagoya.[3]Mie University School of Medicine, Tsu.

Tenascin(TN), an extracellular matrix glycoprotein(ECM), is a six-armed multidomain macromolecule composed of disulfide-linked subunits of 190-250kDa. A wide variety of tissues have so far been examined by immunohistochemistry and by in situ hybridization technique in human, mouse and chicken to demonstrate its characteristic expression with spatio-chronological restriction. Unlike other ECMs such as fibronectin, laminin and collagen, the localizations of TN protein or TN mRNA are highly specific in the stroma of growing and regenerating tissues with a few exceptions in the epithelia at very beginning of organogenesis. Although the patterns of distribution in the tissues suggest that TN is important in the developmental and inflammatory procedures, littles is known about the biological functions of TN. We have isolated mouse TN-encoding cDNA from a cDNA library of the 2H6GR mammary tumor cell line. TN mRNA expression, revealed by Northern blot analysis, was detected brain, skeletal muscle, digestive tract, urinary bladder, lung, kidney and thymus at perinatal stage and was down-regulated 1 month after birth in most tissues(1). By in situ hybridization, TNmRNA message was detected in the stroma of cancers. During mammary gland development, immunoreactive TN appears in the mesenchyme at 14 days of gestation, being localized within a limited area immediately surrounding the epithelium, then disappears in the stroma of elongating ducts and reappears in the dense stroma surrounding the juvenile endbuds, mammary cancers and pulmonary metastatic foci(2). When embryonic mammary epithelium is cultured on fat pad precursor tissue, TN accumulates in the dense stroma forming a halo surrounding the growing epithelium. These results suggest that TN synthesis is induced in the stroma as a consequence of interaction with embryonic or neoplastic epithelium. However, we found that the primary cultures of mouse mammary epithelial cells and human breast cancer cell lines, HBL100, MDA-MB-231 and MDA-MB-468, can produce TN in regular culture. Besides, MCF7 cells which express neither TN mRNA nor immunoreactive TN in vitro can express TN if co-cultured with embryonic fibroblasts. Therefore, even epithelial cells are capable to express TN in response to the environmental conditions. In culture, TN inhibits cell atttachment to fibronectin and to other ECMs and cell migration on them. Considering these results together, we propose that TN is expressed in the epithelium and/or mesenchyme induced by tissue interactions , accumulates in the stroma and probably inhibits movement of both epithelial and mesenchymal cells.

References

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Metastasis/Progression

CC 016 OVEREXPRESSION OF CATHEPSIN D IN BREAST CANCER CELLS : MECHANISM AND CONSEQUENCES, Henri Rochefort, Patrick Augereau, Pierre Briozzo, Jean-Paul Brouillet, Françoise Capony, Vincent Cavaillès, Chantal Escot, Marcel Garcia, Marc Mathieu, Thierry Maudelonde, Philippe Montcourrier, Isabelle Touitou, and Françoise Vignon, Hormones and Cancer Unit (U 148) INSERM, and Faculty of Medicine, University of Montpellier 1, 60 rue de Navacelles, 34090 Montpellier, France.

Cathepsin D, an acidic protease normally acting in lysosomes, is overproduced both in vitro and in vivo in most breast cancer cells. In MCF7 cells, cathepsin D is specifically and directly induced by estrogens and also induced by growth factors (EGF, IGF-I and bFGF), but this induction is dependent upon de novo protein synthesis. The mechanism of estrogen induction involves EREs located upstream from the gene. We cloned the promoter region (-4kb) of cathepsin D and found EREs located in the proximal 5' region of the gene. In MDA-MB231 and BT20 cells, cathepsin D is over-expressed but not regulated by estrogens. In breast cancer cells, the routing of pro-cathepsin D to lysosomes is altered compared to normal mammary epithelial cells in culture, leading to its increased secretion, possibly via saturation of the Man-6-P/IGFII receptor. Total cathepsin D concentration can be assayed by IRMA in breast cancer cytosol routinely prepared for receptor assays. Several retrospective clinical studies indicate a significant correlation between high cathepsin D concentrations in the cytosol of primary breast cancer and further development of clinical metastasis. High cathepsin D concentration in the primary tumor may be either a consequence, or more likely a cause, of metastasis. Pro-cathepsin D can be auto-activated to degrade extracellular matrix and to activate other pro-proteinases in acidic compartments. Transfection experiments using cDNA-cathepsin D expression vectors have shown that overexpression of cathepsin D in rat tumoral cells facilitates their metastatic potential in nude mice (Garcia et al., Oncogene 5:1809-1814, 1990). Recent data on the estrogen regulation of cathepsin D gene transcription and on the mechanism by which cathepsin D facilitates metastasis in nude mice will be given.

CC 017 A NEW MODEL FOR THE STUDY OF CARCINOMA CELL INVASION AND METASTASIS. Jean Paul Thiery, Brigitte Boyer, Annie Delouvée, Jacqueline Jouanneau, Pierre Savagner, Gordon Tucker, and Ana Maria Valles. Laboratoire de Physiopathologie du Développement, CNRS-Ecole Normale Superieure 46, rue d'Ulm, 75230 Paris Cedex 05 FRANCE.

We have made the hypothesis that some cells in carcinoma could invade and metastasize following a transient conversion to a fibroblast-like state. This hypothesis stems from multiple studies on epithelial-mesenchymal cell interconversions and on migratory events occurring during embryogenesis. A rat bladder carcinoma cell line has been used as a model system to study the mechanisms controlling the conversion of an epithelial to a migratory fibroblast-like state. Several collagens can trigger this morphological transformation through their interaction with a subset of b1-containing integrins, whereas the carcinoma cells attach and spread on fibronectins and laminin but remain as an epithelial sheet. A similar conversion is induced by acidic Fibroblast Growth Factor(aFGF) in subconfluent cultures while this multifunctional growth factor acts as a mitogen on high density cultures. In low density cultures, aFGF and several other growth factors acting through tyrosine kinase receptors induce a rapid internalization of desmosomes, a major adhesive structure of epithelia. The newly formed fibroblasts progressively lose their cytokeratins which are replaced by vimentin intermediate filaments. The transformation is fully reversible upon removal of the growth factor. Acidic FGF also triggers cell motility and production of gelatinases. On collagen substrates, the speed of locomotion is enhanced in the presence of acidic FGF and under these conditions the bladder carcinoma cells readily invade 3D collagen gels. The bladder carcinoma line can also become fibroblastic after transfection with an expression vector coding for acidic FGF, most likely through an autocrine mechanism. Clones producing the growth factor can efficiently invade rat bladders maintained in organotypic cultures. These clones grow much more rapidly in nude mice than the original cell line and micrometastases are detected in less than two weeks following subcutaneous injection. Thus, this model system may offer a unique opportunity to evaluate the role of the different adhesion modes and signalling factors in tumor progression leading to the metastatic phenotype

Growth Inhibitors/Suppressors

CC 018 ISOLATION AND PURIFICATION OF NEW GROWTH REGULATORY MOLECULES, G.J. Todaro[1], G.D. Plowman[2], and M. Shoyab[2], [1]Fred Hutchinson Cancer Research Center, Seattle, WA 98104 and [2]Bristol-Myers Squibb Co. Seattle.

The transformation of a cell from normal to neoplastic can involve the loss of normal growth control mechanisms, often mediated by polypeptide growth factors and their receptors. In our laboratory the search for novel growth factors has focused on molecules that are part of the cell's normal regulatory pathways in preference to those that are indiscriminately cytotoxic. New factors can be detected in the supernatant of established cell lines, such as mammary carcinomas or leukemias, following stimulation with phorbol ester. A battery of human tumor cells, including breast, colon, and lung carcinomas and melanomas, is used to screen for growth inhibitory activity. At the same time, the putative growth regulators are tested on normal cells, usually of mesenchymal origin, to demonstrate that the molecules have either no effect or a stimulatory effect on non-tumor cells.

Using this approach, amphiregulin (AR), a growth regulatory protein belonging to the EGF/TGF-α family, was initially isolated from phorbol ester-treated MCF-7 breast carcinoma cells. While AR stimulates growth in normal fibroblasts and keratinocytes, it was first identified for its inhibition of human tumor cell lines, including breast, ovarian and cervical carcinomas. A number of groups working in different systems have subsequently identified the molecule as keratinocyte autocrine factor (KAF), rat mammary carcinoma growth factor (RMGF), and rat schwannoma-derived growth factor (SDGF). AR is expressed in about 50% of human breast and colon tumors, with lower expression levels in normal tissues.

The epithelins, an unusual family of molecules with growth regulatory activity, were isolated on the basis of their ability to inhibit the growth of A431 cells, a human epidermal carcinoma of the vulva. The family consists of seven genes expressed as multiple repeats on the same message, each repeat containing a unique pattern of 12 cysteine residues. The two proteins isolated thus far regulate the growth of various epithelial cells, in some cases with antagonistic properties. Epithelin 1 stimulates the growth of mouse and human keratinocytes, but epithelin 2 inhibits this stimulation.

Growth Factors

CC 019 GROWTH STIMULATORY FACTORS IN BREAST CANCER, Robert B. Dickson, Mozeena Bano, Junichi Kurebayashi, Michael D. Johnson, Mary Beth Sabol, Barbara Ziff, William R. Kidwell, Francis G. Kern and Marc E. Lippman, Lombardi Cancer Research Center, Georgetown University, Washington, D.C. 20007.

Human milk contains many components important for nourishment of the baby. These include protein (including casein) carbohydrate,and vitamins. In addition, recent studies have determined that hormones (such as prolactin) and growth factors (such as TGFα) are also in milk, but their function is not yet fully determined. We have characterized a new growth factor in milk-mammary derived growth factor 1 (MDGF-1). We do not yet know the function of the growth factor for the newborn but current studies suggest that it might play a role in normal mammary growth and development and in the regulation of breast cancer. Not only is the growth factor found in milk but it is secreted by normal mammary epithelial cells in culture and by some breast cancer cells in culture. The factor has been purified to homogeneity from milk as well as primary human breast tumors; it appears identical from the two sources. When applied to normal or malignant mammary epithelial cells, this growth factor stimulates proliferation and protein synthesis. When applied to normal fibroblasts, the growth factor stimulates collagen I synthesis in the absence of proliferation. Preliminary evidence has begun to determine the structure of the growth factor and its receptor. Both appear unique, and not closely related to previously described growth factors and their receptors.[2-4]

MDGF-1 growth factor is acidic, pI of 4.8 and 62 kDa size as purified either from human milk or primary breast tumors. Sequencing of the amino terminal 17 amino acids of MDGF-1 from milk has revealed no homology with other growth factors and has afforded production of a high affinity antiserum to the factor. The antiserum can be used to purify the MDGF-1 activity and to recognize the molecule in western blot, ELISA immunohistochemistry, and immunoprecipitation protocols using milk or breast tumor sources. Further studies in normal mammary epithelial cells (184 strain obtained from Dr. Martha Stampfer, Berkeley) and a range of breast cancer lines have demonstrated that the growth factor it first synthesized as a 55 kDa precursor and then N-glycosylated to yield the final product.[2]

The factor has been iodinated and binding sites studied on responsive breast epithelial cell lines. MDGF-1 binds a 130 kDa putative receptor with nM affinity on responsive cell lines.[3] Interestingly, binding of MDGF-1 triggers rapid phosphorylation of a membrane associated protein of 180-200 kDa in size. The phosphorylation appears to be associated with tyrosine residues by antiphosphotyrosine western blot methodology.[4] Current studies are focused on determining the relationship between the receptor binding site and phosphoprotein, the full cDNA and protein sequence of the factor, and the expression of both factor and its receptor in breast cancer. At present both the factor and its receptor have been found in normal 184 human mammary epithelial cells and in the mille-derived HBL 100 human mammary cell line, suggesting the potential for autocrine function. The growth factor, but not the receptor, has been detected in hormone independent breast cancer cell lines, while the receptor has been detected in both hormone dependent and independent breast cancer cells.[3-4] The factor thus has the potential for paracrine, as well as autocrine function in epithelial-stromal communication in the normal and malignant gland.[1]

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CC 020 EPIDERMAL GROWTH FACTOR (EGF)-RELATED PEPTIDES IN BREAST AND COLORECTAL CANCER. David S. Salomon[1], Nicola Normanno[1], Nichloas Kenney[1], Toshiaki Saeki, Chen-Feng Qi[1], Nancy Kim[1], Fortunato Ciardiello[1], Kurt Stromberg[2], Gibbes Johnson[2], William J. Gullick[3], Greg Plowman[4], Eiichi Tahara[5], and George Todaro[6], [1]National Cancer Institute and [2]Food and Drug Administration, Bethesda, MD. 20892, [3]Imperial Cancer Research Fund, London, England, [4]Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, [5]Hiroshima University School of Medicine, Hiroshima, Japan and [6]Fred Hutchinson Cancer Research Center, Seattle.

Expression of specific subsets of growth factors and their cognate receptors in tumors may be important in regulating the growth of malignant cells through either autocrine and/or paracrine pathways. One growth factor that exhibits some of these characteristics is transforming growth factor α (TGFα) which is a member of the EGF family of proteins and which binds to the EGF receptor. TGFα mRNA and protein are found in approximately 50 to 70% of primary human breast tumors of which 70 to 80% of these tumors also express EGF receptors. In breast cancer cell lines that are estrogen-responsive, phsiological concentrations of 17β-estradiol (E2) can induce an increase in TGFα mRNA expression and protein production suggesting that the growth-promoting effects of E2 may be mediated in part through the intermediary action of this growth factor via an autocrine mechanism. This may be the case since infection of estrogen-responsive MCF-7 or ZR-75-1 cells with an amphotropic recombinant retrovirus containing a fragment of the human TGFα cDNA oriented in the 3' to 5' orientation to generate an antisense mRNA can significantly inhibit the induction of TGFα mRNA and protein in response to E2 and can partially block E2 stimulated anchorage-dependent and anchorage-independent growth in soft-agar. The expression of TGFα can also be modulated by specific oncogenes. For example, immortalized, non-transformed MCF-10A human mammary epithelial cells can be transformed by an activated c-Ha-*ras* protooncogene or by overexpression of the c-*erb* B-2 protooncogene. MCF-10A cells express approximately 3 x 10^5 EGF receptor sites/cell and require exogenous EGF for growth. Transformation of MCA-10A wells by c-Ha-*ras* but not by c-*erb* B-2 results in an increase in the production of TGFα and in a concomitant loss in the mitogenic responsiveness of these cells to exogenous EGF. Soft agar growth of the *ras* transformed cells can be partially blocked by treatment of these cells with either a TGFα neutralizing monoclonal antibody or with an EGF receptor blocking monoclonal antibody demonstrating that TGFα is able to function as one autocrine intermediary in the transformation pathway which is utilized by *ras* but not by *erb* B-2 in mammary epithelial cells. TGFα may also function as a dominantly acting oncogene in mammary epithelial cells that express a sufficient complement of functional EGF receptors because overexpression of TGFα in MCF-10A cells following infection with an amphotropic retroviral expression vector containing the human TGFα cDNA is able to transform these cells *in vitro*. However, neither TGFα, *ras* nor *erb* B-2 is able to elicit a fully malignant phenotype *in vivo* since none of these genes either alone or in combination were able to promote tumor formation in nude mice suggesting that the expression of an additional oncogene(s) or the loss of expression of a tumor suppressor gene is probable required to complete this process. Two other EGF-related proteins, amphiregulin (AR) and cripto, may also be important mitogens for mammary and colonic epithelial cells and might be involoved in the pathogenesis of breast and colorectal cancer. Approximatley 50% of primary human breast tumors and an equal percentage of benign breast lesions express AR mRNA and *erb* B-3, and EGF receptor-related proetin. MCF-10A cells also express a low level of AR mRNA and exogenous AR is as potent as EGF in stimulating the proliferation of these cells. Following transformation of MCF-10A cells with either a point-mutated c-Ha-*ras* or with c-*erb* B-2 gene, there is a 20-40-fold increase in the level of AR mRNA expression. A corresponding increase in AR protein production could also be detected immunocytochemically in these oncogene transformed cells particularly in the nucleus. AR and cripto mRNA are expressed in 60-70% of primary or metastatic human colorectal carcinomas but in only 2-7% of normal colon that was adjacent to carcinomas. In contrast, *erb* B-3 mRNA was detected in 55% of the carcinomas and in 22% of the normal colon samples.

Poster Session I

CC 100 ANDROGEN RECEPTOR GENE MUTATIONS IN HUMAN PROSTATE CANCER. Evelyn R. Barrack, Jay R. Newmark, Dianne O. Hardy, Bob S. Carter, Jonathan I. Epstein, William B. Isaacs, and Terry R. Brown. The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Androgen is required for the development of prostate cancer, but prostate cancer usually progresses from androgen-dependent to androgen-independent growth. Given the important role of the androgen receptor (AR) in signal transduction, we screened human prostate cancer tissues for the presence of somatic mutations in the AR gene. Genomic DNA was isolated from surgical specimens of 26 untreated stage B prostate cancers and 2 stage D2 prostate cancers that had failed hormonal therapy. The 4 exons (E,F,G,H) of the hormone binding domain (HBD) of the AR gene were amplified using the polymerase chain reaction. Each entire exon plus its intron/exon borders was amplified to allow detection of mutations in coding regions and potential splice site mutations. Amplified DNA fragments were analyzed by denaturing gradient gel electrophoresis (DGGE) in which DNA fragments that differ by only a single base differ in mobility. We detected a mutation in Exon E of the HBD in a patient with organ confined stage B prostate cancer (Gleason score 8). This mutation was present in 2 different pieces of the same tumor but was not detectable in adjacent normal prostate tissue. Normal prostate DNA (wild type AR) migrated in DGGE as a single band because there is only 1 allele (X chromosome) per cell. The tumor DNA migrated in DGGE as 4 bands (mutant, wild type, and 2 heteroduplex forms), indicating the presence of cells with mutant AR plus cells with wild type AR, and confirming that the tumor contained a somatic mutation. Direct sequencing of the PCR-amplified mutant DNA fragment revealed a G→A substitution in codon 730, resulting in an amino acid change from valine to methionine. Mutation of codon 730, located in a region of the HBD that is highly conserved among members of the steroid receptor family, may alter receptor function. A substantial number of cells with this mutation were present in the specimen (>20%), indicating that it occurred in cells that had a growth advantage. We conclude that somatic mutations of the AR gene occur in prostate cancer and are detectable in early stages of the disease, even prior to androgen ablation.
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CC 101 EXPRESSION OF EGF RECEPTOR, TGFα AND TGFβ IN NORMAL AND FIBROCYSTIC HUMAN BREAST, Sue A. Bartow, Marie T. Boyd, and Richard H. Hildebrandt, Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM 87131.

Expression of EGF receptor, TGFα and TGFβ was examined in a set of 78 human breast tissues. The tissue was obtained largely from forensic autopsy cases and included samples from female infants, children, adult women in both phases of the menstrual cycle, pregnant, post-partum and post-menopause. Among the samples were 16 cases of histologically proven proliferative and non-proliferative fibrocystic change. A few examples of fibroadenoma, phyllodes tumor, and gynecomastia were also studied. By northern analysis of extracted RNA, EGF receptor was expressed at low and fairly consistent levels in all samples except those of pregnant/post-partum women. In this group of women the expression was uniformly increased. TGFα was expressed at moderate and consistent levels in all adult groups, including pregnant/post-partum women. Expression of TGFα was lower in infants and children. TGFβ expression was relatively low and constant in all groups, including the putative stromal proliferative processes of fibroadenoma and phyllodes tumor. Interestingly in 5 examples of fibrocystic change the EGF receptor and TGFα expression was elevated. In 3 of the 5 ER expression, usually high normal in fibrocystic change, was extremely low. In the other 2 cases ER expression was intermediate. These results suggest that altered EGFR/TGFα expression may occasionally be involved in the process of fibrocystic change.

CC 102 BREAST TUMORS EXPRESS EMBP, A PROTEIN OF *PROSTATIC ORIGIN THAT SPECIFICALLY BINDS THE* ANTI-MITOTIC DRUG ESTRAMUSTINE, **Per Björk[1], Åke Borg[2], Mårten Fernö[2] and Sten Nilsson[3], Department of Immunology, Kabi Pharmacia Therapeutics, Lund[1], Department of Oncology, University Hospital, Lund[2], and Department of Oncology, Akademiska sjukhuset, Uppsala[3], Sweden**

Estramustine is the cytotoxic metabolite of estramustine phosphate (Estracyt[R], Emcyt[R]), which is used in the treatment of advanced prostatic carcinoma. Estramustine binding sites were previously demonstrated in 74/306 (24%) consecutive breast cancer biopsy samples using isoelectric focusing and were inversely correlated with estrogen and progesterone receptor levels. This study aimed at further characterization of these sites in breast tumors by a comparison with estramustine-binding protein (EMBP) in the prostate - a secretory protein with proteolytic activity, which is responsible for the selective binding and uptake of estramustine in prostate cancer. Immunohistochemical examination of breast cancer specimens showed a positive staining of varying intensity confined to the cytoplasm of malignant cells. Using a RIA, EMBP was demonstrated in 6/17 (35%) mammary tumors (range 11.3-2,660 ng/g). EMBP positive samples all displayed negative estrogen receptor values, which confirms the previously reported inverse relation between EMBP expression and high estrogen receptor levels of the tumor. Biochemical and immunochemical analysis revealed (1) an almost identical surface-charge distribution by Mono Q ion-exchange chromatography, (2) a similar native *Mr* of ~50.000 and (3) the same subunit composition by Western blot analysis under denaturing conditions. In an extended experiment on twenty breast cancer specimens, attempts were also made to correlate EMBP expression, determined by Western immunoblotting, with amplification of the c-*erb*B-2 proto-oncogen. In conclusion, our findings unequivocally demonstrate the expression of an EMBP-like protein in a subgroup of mammary tumors and suggest that monitoring of EMBP in breast cancer biopsy samples will be helpful in defining patients who may benefit from Estracyt therapy.

CC 103 THE DETERMINATION OF THE INTRACELLULAR PATHWAYS OF THE NEU ONCOGENE AND PROTO-ONCOGENE AND THE ELEMENTS WHICH REGULATE THESE PATHWAYS, Valerie I. Brown, Maria Hellman, Yasunori Mikami, and Mark I. Greene, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

The aberrant expression of the neu gene has been implicated in the development of certain neoplasias of the breast and pancreas. Our laboratory is interested in determining the molecular mechanisms of the role of this growth factor receptor in abnormal as well as normal cell growth, using the rat neu gene as our model system. To this end, we have shown a physical interaction between two growth factors, the product of the rat neu proto-oncogene (p185c-neu) and the EGF receptor. The overexpression of these two receptor kinases leads to the transformed phenotype. If either of these receptors is down-modulated by antibodies or ligands, the transformed phenotype is reversed. Also, we have shown that the internalization of p185neu, the product of the rat neu oncogene, reverses the transformed phenotype. We are in the process of determining the exact intracellular pathways taken by these receptors, and we are elucidating the regulatory elements of these processes by creating a panel of specific deletional and point mutations in p185c-neu and p185neu. These mutant cDNA's have been transfected into NIHw and NR6 cells and are being characterized. Understanding these pathways may lead to the development of novel therapeutic protocols to combat human malignancies.

CC 104 REGULATION OF TGF-ß1 EXPRESSION IN LNCAP ANDROGEN-RESPONSIVE HUMAN PROSTATIC CARCINOMA CELLS BY EGF, TGF-ßs AND DHT, David Danielpour, Seong-Jin Kim and Michael B. Sporn, Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892. Previous studies demonstrate both that 1) androgens negatively regulate transforming growth factor-ß1 (TGF-ß1) mRNA in rat ventral prostate, and that 2) TGF-ß1 is a potent inhibitor of prostate epithelial cell growth, suggesting that suppression of TGF-ß activity may mediate the androgen-responsive growth of normal and transformed prostatic epithelial cells. We investigated this possibility in LNCap human androgen-responsive prostatic carcinoma cells, using highly sensitive sandwich enzyme-linked immunoadsorbent assays for TGF-ßs 1 and 2. We were unable to detect the expression of these TGF-ßs in serum-free medium conditioned by LNCap cells under a variety of conditions even after maintaining cells in steroid-stripped serum for two weeks prior to conditioning, suggesting that the in vitro androgen-responsive growth in LNCap cells may not be mediated by inhibition of TGF-ß expression via an autocrine loop. Since these cells did not express any detectable TGF-ßs (< 0.2 pM) even after 3 days under confluent conditions we also studied the possible upregulation of TGF-ß expression in these cells. The addition of basic fibroblast growth factor, insulin, retinoic acid, TGF-ßs and dihydrotestosterone (DHT) either alone or together were ineffective in stimulating TGF-ß expression. Epidermal growth factor (EGF), however, gave detectable TGF-ß1 but not TGF-ß2 expression. In the presence of EGF, TGF-ß2 induced TGF-ß1 expression by over ten-fold, while none of the other above factors had any significant effect on the EGF-induced TGF-ß1 expression. The addition of 10 nM DHT significantly inhibited the levels of TGF-ß1 induced by EGF and TGF-ß2. Based on preliminary studies of TGF-ß1 mRNA induction and TGF-ß1 promoter-CAT expression, we speculate that transcription of TGF-ß1 in LNCap cells is shut off by potent transcription inhibitors that may be regulated by EGF, TGF-ßs and DHT.

CC 105 **CLONING AND EXPRESSION OF A DEVELOPMENTALLY REGULATED GROWTH FACTOR SECRETED FROM HUMAN BREAST CANCER CELLS.** **WENJING FANG**, [*]A.T. Riegel, [*]N. Hartmann, [**]A. Wellstein, [*]Lombardi Cancer Center and [**]Department of Pharmacology, Georgetown University, Washington, DC, 20007
We have previously detected an 18-kDa heparin-binding growth factor in the conditioned medium of a human breast cancer cell line, MDA-MB 231 (1). The N-terminal sequence was homologous to a developmentally regulated protein described as a heparin binding neurotrophic factor from bovine brain, a heparin binding growth associated molecule (HB-GAM) from perinatal rat brain and pleiotrophin (PTN) from bovine uterus (2).
We now describe further the characteristics of this factor. PolyA[+] RNA was isolated from MDA-MB 231 and reverse transcribed in vitro. The gene was amplified by PCR and cloned into a eukaryotic expression vector, pPRC/CMV (Invitrogen, CA). Based on the DNA sequencing results, one construct (pPTNwt) contains the wild type cDNA and the ORF while another construct with a frame shift mutation (pPTNmu) contains a premature stop codon and codes for a small non-functional fragment of PTN. Western blot analysis of the conditioned media obtained from transient expression experiments with pPTNwt or pPTNmu showed an 18-kDa band in the conditioned medium of pPTNwt transfected cells but not in that with pPTNmu. The protein bound to a heparin-Sepharose column and was eluted from the column by 2 M NaCl. Mitogenic activity for an epithelial cell line (SW-13) and fetal bovine heart endothelial cells was observed with the wild type construct only. Stable SW-13 pPTNwt transfectants formed > 4 fold more colonies than stable pPTNmu SW-13 transfectants. A 1.4kb mRNA was detected in several breast cancer cell lines and some prostatic and melanoma cell lines. The expression of this gene was shown to be the highest in melanoma cells and inducible with retinoic acid. We conclude that PTN belongs to a family of developmentally regulated cytokines and can act as an angiogenesis factor during tumor growth.
(1)Wellstein et al., JBC in press; (2)Merenmies and Rauvala, JBC, 1990, 265:16721; LI et al., Science, 1990, 250:1690.

CC 106 LONG-TERM OVARIECTOMY (MENOPAUSE) AND ALTERED MAMMARY GLAND HORMONAL RESPONSIVENESS, Sandra Z. Haslam, Katherine A. Nummy, Laura J. Counterman and Paul Corbier, Department of Physiology, Michigan State University, East Lansing, MI 48824
Most women develop breast cancer postmenopausally however, the role of menopause per se in breast cancer etiology is not understood. Using the mouse mammary gland as a model system, the present studies were undertaken to determine if hormonal responsiveness of the mammary gland is somehow altered under menopausal conditions. Mice 10 weeks or 9 months of age were ovariectomized (OVX) for 1 or 5 weeks before assessing hormonal responsiveness to estrogen (E) and/or progesterone (P). Regardless of age, mice that had been OVX for 5 weeks exhibited a 2-fold greater epithelial proliferative response to E; no increased response to P was detected. Stromal cells exhibited a 2-fold greater proliferative response to both E and P. ER concentration and subcellular distribution was not altered by long-term OVX. However, an analysis of the distribution of the growth inhibitor, TGF-ß1 revealed that after long-term OVX, treatment with E resulted in a significant reduction in stromal cell TGF-ß. These results suggest that as a result of long-term OVX and possibly menopause the mammary gland can exhibit a heightened sensitivity to mitogenic factors possibly through reducing the effects of growth inhibitors.

CC 107 MECHANISM OF NEU PROTO-ONCOGENE OVER-EXPRESSION IN BREAST CANCER. Mien-Chie Hung, Susan J. Miller, Ting-Chung Suen, Department of Tumor Biology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030. The human *neu* (HER-2/c-erbB-2) gene has been found to be overexpressed in many human tumors, including breast cancer. Furthermore, overexpression of *neu* has been shown to be a significant predictor of both overall survival and time to relapse for breast cancer patients, suggesting that overexpression of *neu* may play an important role in the pathogenesis of breast cancer. Therefore, studies on the mechanisms resulting in *neu* overexpression may provide insight for the pathogenesis of tumors overexpressing *neu*.In order to investigate these mechanisms, we have begun to characterize breast cancer cell lines that have 8 to 128 fold higher levels of *neu* mRNA compared to a control line, MCF-7. Gene amplification in these cells (2-4 fold) cannot fully account for these mRNA levels. Nuclear run-on assays show that the transcription rate across the *neu* gene in MDA-MB-453 and BT-483 cells is higher than in MCF-7 cells by 15 and 9 fold, respectively. In these same cell lines, transient transfection assays using the *neu* promoter to drive expression of the reporter gene, chloramphenicol acetyltransferase (CAT) have shown that MDA-MB-453 cells likely contain an altered factor that acts *in trans* to increase promoter activity while overexpression of *neu* in BT-483 cells is likely due to alterations present in cis elements. Furthermore, since transcriptional deregulation alone cannot explain the extreme level of *neu* mRNA copies in these cells, activations at the post-transcriptional level are also likely present. In order to address this question, *neu* mRNA stability is being analyzed. Our preliminary results indicate that the *neu* mRNA half-life in BT-474 cells seems to be higher than that in the MCF-7 line. In conclusion, our studies have provided evidence that in addition to gene amplification, transcriptional and post-transcriptional upregulation can be involved in *neu* overexpression in breast cancer cells.

CC 108 MODULATION OF THE *erbB*-2 RECEPTOR ACTIVATION BY ITS LIGAND (gp30) WITH THE HEPARIN ANALOGUE PENTOSAN POLYSULFATE. **Lupu R., Simpson S., Cho C., Colomer R., and Lippman M.E.** Lombardi Cancer Research Center, Georgetown University, 3800 Reservoir Rd, Washington DC, 20007. The *erbB*-2 oncogene encodes a 185 kDa transmembrane protein which is a growth factor receptor. We have identified and purified a heparin-binding growth factor (gp30) which is secreted by human breast cancer cells (MDA-MB-231). We have previously described that this protein (gp30) is a ligand for the *erbB*-2 receptor and that it can modulate proliferation of cells with *erbB*-2 amplification. We have also reported that soluble *erbB*-2 extracellular domain (ECD) can inhibit colony formation of cells overexpressing *erbB*-2 receptor. Moreover, stimulating concentrations of gp30 can reverse the anti-proliferative effect of soluble *erbB*-2 ECD. In the present study, we report the effects of a heparin-analogue, Pentosan polysulfate (PPS) on established human breast cancer cell lines in vitro. In proliferation assays, we have shown that gp30 stimulated proliferation when used at low concentrations, while at higher concentrations proliferation is inhibited in both anchorage dependent and independent growth. In order to block the growth effects of the *erbB*-2 ligand, we performed growth assays in the presence of PPS. When PPS was added in combination with gp30, the cellular effects of gp30 were completely abolished, whereas PPS alone at the same concentration did not have any anti-proliferative effects. Furthermore, activation of $p185^{erbB\text{-}2}$ by exogenous gp30 as detected by tyrosine phosphorylation assays, was significantly blocked by PPS. In addition, PPS blocked constitutive *erbB*-2 phosphorylation levels. In conclusion, a heparin-analogue compound is able to block activation of *erbB*-2 by its gp30 ligand and prevents the growth effects of the ligand. Interference of erbB-2 activation with its ligand could provide a significant tool for therapeutic applications.

CC 109 Differential Regulation of EGF-Receptors on Hormone-dependent and Hormone-independent Human Breast Cancer Cells. Heinz Mueller, Rong Liu, Petra Loop, and Urs Eppenberger. University Hospital Basel, Dept. Research, 4031 Basel, Switzerland.

Regulation of cellular responses to the binding of a hormone to surface receptors include the adjustment of the density of surface receptors to the immediate needs of the cell. Attempts to understand the regulation of EGF receptors have been focused on the control of functional states and their interconversion. A high and a low affinity state of the EGF receptor was described on separate cell lines; i.e. hormone-dependent cells (MCF-7) expose receptors with a relative high affinity (K_d = 1x10-10M), whereas hormone-independent cells (MDA-MB-231) have low-affinity surface receptors (K_d = 8x10-10M). Thus, these two cell lines represent excellent tools to study differences in high and low affinity states of the EGF receptor and possibly their interconversion. First, we studied the binding kinetics of the EGF receptors by using fluorescein-labeled EGF in flow cytometry experiments. Kinetic studies revealed that the association rates as well as the dissociation rates were slower on cells with low affinity receptors (MDA-231) by an order of magnitude . The slower association rate may be explained by a restricted access to the binding sites for the ligand. However, the slower dissociation rate may reflect an altered conformation of the receptor as a function of a change in the phosphorylation state on the cytoplasmic side. Incubating cells in the presence of EGF induced a regulation of surface receptors; MCF-7 cells exhibited a net up-regulation of about 20-30% of EGF surface receptors, whereas MDA-231 cells showed a dramatic decrease (50-70%) of their surface receptors in about 10-15 min at 37°C when measured with a monoclonal antibody against the extracellular domain of the receptor. This difference is not due to different down regulation capabilities of the two cell types, as was demonstrated by measuring internalization of receptor-EGF complexes using the fluorescein-labeled EGF. Kinetics of internalization of fluorescent EGF was comparable for both cell types. Thus, the difference in regulation is due to varying up-regulation and probably to differences in the synthesis of new EGF receptors.

CC 110 P53, C-ERBB-2, THE EGFR AND KI67 STAINING IN THE BENIGN AND MALIGNANT PROSTATE, *David E. Neal*, K. Mellon, S. Thompson, M. Robinson, C. H. W. Horne +, D. P. Lane *, A. L. Harris #. Departments of Urology and Pathology, Freeman Hospital, Newcastle upon Tyne NE7 7DN and + University of Newcastle upon Tyne. UK. * CRC Cell Transformation Group, University of Dundee. # ICRF Unit, Churchill Hospital, Oxford, UK.

Mutation of the p53 gene is a common genetic abnormality in solid human tumours, but no data have been reported in prostate cancer and it has not been correlated with cell cycling. Down regulation of the EGFr occurs in prostate cancer, but no data have been reported for c-erbB-2. 34 men with benign prostatic hyperplasia (BPH) and 29 with prostate cancer (CaP) were studied by immunohistochemistry using antibodies against mutated p53, c-erbB-2, the EGFr and Ki67.

Staining for p53 was found in 5 of 29 CaP (mean 21% $\pm$ 7% of cells stained in the +ve tumours), but no staining was found in BPH ($P<0.05$). 22 of 29 CaP (76%) contained Ki-67 immuno-reactivity (mean % of cells stained = 1.9%; range 0.2% to 9.1%) compared with 10 of 34 (29%) BPH (mean score = 0.19%; range 0.1% to 0.7%; $P<0.001$). The mean Ki-67 score in CaP positive for p53 (4.3%) was greater that found in CaP negative for p53 (1.2%; $P < 0.02$). The epithelium in BPH was stained positively for c-erbB-2 in 15% (5/34) and for the EGFr in 88% (30/34); whereas CaP stained strongly for c-erbB-2 in 17% (5/29) and for the EGFr in only 17% (5/29). CaP was associated with a significant decrease in EGFr staining ($P<0.0001$) and a significant increase in p53 staining ($P<0.05$) and Ki67 staining ($P<0.001$). No significant relationship was observed between tumour stage and grade and expression of p53, c-erbB-2, the EGFr or Ki67 staining.

These findings demonstrate that altered staining for p53, the EGFr and Ki67 occurs in prostate cancer, but is not associated stage or grade. The use of p53, EGFr and Ki-67 staining may allow development of new prognostic factors.

CC 111 STABLE TRANSFECTION OF THE ESTROGEN RECEPTOR INTO A HORMONE-UNRESPONSIVE HUMAN BREAST CANCER CELL LINE, Françoise Vignon, Gilles Freiss, Christine Prébois and Henri Rochefort, INSERM Unit 148 on "Hormones and Cancer", Montpellier, France.

In an attempt to reverse ER negative aggressive breast cancer cells into ER positive less aggressive cells, we have transfected ER gene into the ER- human breast cancer cell line MDA MB-231. The non-mutated HEGO human ER expression vector (human ER cDNA inserted into pSG1 vector, donated by P. Chambon, France) was introduced into MDA MB-231 cells by co-transfection with a Hygromycin-B resistance plasmid (PY3). Mock-transfectants were parallely established by co-transfection with pSG1 and PY3 vectors. A number of resistant clones were isolated and ER expression was evaluated by whole cell binding assay with ^{3}H-estradiol (E_2) or by enzyme immunoassay on cell cytosols. The levels of expressed ER reached 75 fmoles/mg cytosol protein in HC1 and HE5 HEGO-transfected positive clones whereas they remained negative in control pSG1 transfectants (PB4 and PB5). Scatchard analysis of binding assays demonstrated the presence of high affinity binding sites with a dissociation constant of 0.08 - 0.8 nM at 37°C. The expression of ER was stable over 1 year of continuous clones culture. Functional receptor activity was demonstrated by restoring estrogen regulation of exogenous or endogenous genes : i. transient transfection of a plasmid containing the chloramphenicol acetyl transferase (CAT) gene linked to the vitellogenin estrogen responsive element (vit-tk-CAT plasmid) was accompanied by stimulation with E_2 and inhibition by partial (hydroxy - tamoxifen) or pure (ICI 164,384) antiestrogens in HC1 and HE5 positive clones ; ii. endogenous Cathepsin D gene, which is constitutively expressed in ER- cells, was estrogen regulated in HC1 and HE5 clones both at the mRNA and protein levels. Other endogenous genes (pS2, IGF-II, EGF-R, TGFα...) were differently affected. General cellular response could also be modulated since the proliferation of two ER+ clones was dose-dependently stimulated **in vitro** by estradiol.

In conclusion, transfection of non-mutated ER is able to restore estrogen regulation of some endogenous genes and of cellular growth in ER- human breast cancer cells.

Poster Session II

CC 200 PROGNOSTIC SIGNIFICANCE OF ANTIGENIC HETEROGENEITY, GLEASON GRADE AND PLOIDY OF LYMPH NODE METASTASES IN PATIENTS WITH PROSTATE CANCER.

Michel Bazinet, Seif M. Hamdy, Louis R. Bégin, Robert A. Stephenson, and William R. Fair. From the departments of Urology and Pathology, McGill University, Montreal, Canada, University of Utah Health Sciences Center, Salt Lake City, Utah; and Memorial Sloan-Kettering Cancer Center, New York, NY.

We retrospectively evaluated 51 prostate cancer patients found to have pelvic lymph node metastases at the time of pelvic lymphadenectomy and 125I implantation. All patients were followed till death or for a minimum of 70 months. Rabbit polyclonal anti-PSA, anti-PAP, anti-PSP-94, and the mouse TURP-27 monoclonal antibodies were used in immunohistochemical evaluation of the metastatic lesions. In addition, Gleason grade and ploidy were evaluated and correlated.

No tumor with a Gleason grade of less than 7 could be found in the metastatic lymph nodes. Patients whose tumor had a primary Gleason pattern of 5 present had significantly shorter time to progrssion ($p=0.003$), disease specific survival ($p=0.009$) and overall survival ($p=0.003$).

Flowcytometry of the lymph node metastases showed that diploid tumors had a statisically longer time to progression ($p=0.016$) and overall survival ($p=0.045$) than aneuploid tumors.

In the PSA heterogeneity study, those patients whose tumors showed reactivity in more than 75% of the cancer cells, had a statistically significant better survival than those who had less than 75% of the cancer cells expressing PSA ($p=0.0006$ log rank test). Means of overall survival±SEM were 71.5±5 and 34.9±5.5 months respectively. Similar correlations were found with disease-specific survival and time to progression. Patterns of PAP expression and TURP-27 reactivity were not prognostically informative, whereas PSP-94 expression may add some additional information.

These data suggest that evaluation of tissue PSA heterogeneity in lymph node metastases may provide additional prognostic information in prostate cancer patients. Better prediction of individual prognosis may be obtained with the combined use of Gleason grade, flowcytometry and PSA expression. Multivariante analysis of these prognostic indicators showed that the presence of Gleason pattern 5 in lymph node metastases was the strongest predictor of survival in this group of patients.

CC 201 PRESERVATION OF APOPTOTIC POTENTIAL IN ANDROGEN-DEPENDENT TUMORS BY INTERMITTENT ANDROGEN WITHDRAWAL,

Nicholas Bruchovsky, Koichiro Akakura, S. Larry Goldenberg and Paul S. Rennie, Department of Cancer Endocrinology, British Columbia Cancer Agency, Vancouver, B.C., Canada, V5Z 4E6

Since post-castration progression of tumors to an androgen-independent state appears to be linked to the cessation of androgen-induced differentiation of tumorigenic stem cells, we hypothesized that the replacement of androgens at the end of a period of apoptotic regression might result in the regeneration of differentiated tumor cells with further apoptotic potential. To determine the effect of intermittent exposure of androgens on the androgen-dependent Shionogi carcinoma, the tumor was transplanted into a succession of male mice, each of which was castrated when the estimated tumor weight became approximately 3 g. After the tumor had regressed to 30% of the original weight, it was transplanted into the next non-castrated male. This cycle of transplantation and castration-induced apoptosis was repeated successfully 3 times before growth became androgen-independent during the fourth cycle. In 4 of stage C and 3 of stage D patients with prostatic carcinoma, androgen withdrawal was initiated with cyproterone acetate (100 mg/day) and diethylstilbestrol (0.1 mg/day) and then maintained with cyproterone acetate in combination with the LHRH agonist, goserelin acetate (3.6 mg/month). After 6 or more months of suppression of serum prostate specific antigen into the normal range, treatment was interrupted for 2 to 11 months. Following recovery of testicular function, androgen withdrawal therapy was resumed when serum prostate specific antigen increased to a level of approximately 20 µg/L, and consecutively repeated to a total of 2 to 4 times over treatment periods of 21-47 months with no loss of androgen dependence. These results show that in both experimental and clinical carcinomas, apoptosis can be induced multiple times using sequential courses of androgen withdrawal and replacement.

CC 202 SEGREGATION AND LINKAGE ANALYSES OF HUMAN PROSTATE CANCER, Bob S. Carter, Terri H. Beaty, Gary D. Steinberg, Barton Childs, G. Steven Bova, William B. Isaacs, and Patrick C. Walsh, Departments of Epidemiology, Urology, and Pediatrics, Johns Hopkins Medical Institutions, Baltimore, MD 21205

Previous studies have demonstrated familial clustering of prostate cancer. To define the nature of this familial aggregation and to assess whether Mendelian inheritance can explain prostate cancer clustering, proportional hazards and complex segregation analyses were performed on 691 families ascertained through a single prostate cancer proband. The proportional hazards analyses revealed that two factors, early age at onset of disease in the proband and multiple affected family members were important determinants of risk of prostate cancer in these families. Furthermore, complex segregation analyses revealed that this clustering can be best explained by autosomal dominant inheritance of a rare ($q=0.0030$) high risk allele, which leads to an early onset of prostate cancer. The estimated cumulative risk of prostate cancer for carriers revealed that the allele was highly penetrant: by age 85, 88% of carriers compared to only 5% of non-carriers are projected to be affected with prostate cancer. The best fitting autosomal dominant model further suggested that this inherited form of prostate cancer accounts for a significant proportion of early onset disease, but overall is responsible for a small proportion of prostate cancer occurrence (9% by age 85). These data provide evidence that prostate cancer is inherited in Mendelian fashion in a subset of families, and provide a foundation for gene mapping studies of inherited prostate cancer. We have obtained DNA samples from seventeen families in which multiple family members are affected with prostate cancer. We are currently performing linkage studies (affected-pedigree-member method) in these families in an effort to map genes involved in inherited prostate cancer. Characterization of such genes could provide important insight into the development of this disease in general. (Support by NIH grant CA09314)

CC 203 DETECTION OF PROSTATE SPECIFIC ANTIGEN (PSA) WITH MONOCLONAL ANTIBODIES DETECTED AGAINST NATIVE AND DENATURED PSA, Rudolf Dernick[1], Folkert Donn[2], Astrid Leuner[1], Thomas Quack[1,2] and Uwe Tessmer[1]. 1. Heinrich-Pette-Institute at the University of Hamburg, and 2. Department of Urology, Marienkrankenhaus, Hamburg, Germany.

Monoclonal antibodies (mAbs) are generally prepared against native proteins. Therefore they usually react with conformational epitopes on the native protein. Many detection systems, however, are performed with denatured proteins, e.g. Western blotting after SDS-PAGE. We have therefore prepared native PSA by chromatographic methods and denatured PSA by preparative SDS-PAGE and electro-elution. These PSA preps were used for the production of murine mAbs and rabbit polyclonal antisera, which allowed the detection of native and denatured PSA by various techniques. These antibodies were also used to determine the antigenic epitopes on PSA in native and denatured structures. They detected at least three different PSA entities differing in molecular weight and a number of PSA degradation products useful for epitope mapping. A method was established for the renaturation of the biological activity of PSA. mAbs against denatured proteins are expected to detect also truncated PSA molecules and their fusion products, obtained from genetically expressed PSA in bacteria.

CC 204 EXPRESSION OF RELAXIN GENES IN HUMAN PROSTATE CANCER CELLS. Deborah J. Hansell, Kevin J. Cullen and H. James Voeller, Pacific Biomedical Research Center, University of Hawaii, Honolulu HI 96822 and Lombardi Cancer Research Center, Georgetown University Medical Center, Washington D.C. 20007.

Relaxin (Rlx) is a member of the insulin and insulin-like growth factor family, and two genes, H1 and H2, have been identified. The hormone has been deduced to act in a paracrine manner in the pregnant female: local production in the decidua and local action on the fetal membranes induces collagenolysis. The function of Rlx in the male reproductive system remains to be clarified. Rlx has been shown by immunocytochemistry, Northern hybridization and cDNA cloning in the benign hyperplastic (BPH) prostate. In order to dissect the mechanisms by which expression of these Rlx genes is regulated *in vivo* we identified human prostate cancer cell lines producing H1 and H2 Rlx mRNAs using RNase protection assays. Both prostate specimens with BPH and prostate cancer cell lines were examined in relation to the established source of Rlx, the human corpus luteum. A number of transcripts were detected. An elevated expression of full length H1 and H2 (432 and 425 bp protected fragments respectively) Rlx transcripts in the LNCaP cell line was found when compared to BPH RNA. In addition, a number of smaller transcripts of approximately 270 bp with the H1 probe and 270 and 310 bp with the H2 probe were found. No full-length or shorter transcripts of either gene were observed in the androgen-dependent DU-145 and PC-3 cell lines. The nature of these smaller fragments is being further investigated by sequencing.

In conclusion, the mechanisms responsible for these multiple mRNA transcripts are unknown, but may include alternative transcription initiation and/or alternative RNA processing. LNCaP cells represent a potential model for studying an autocrine/paracrine role for Rlx in tumor growth. (This work was supported in part by UH Fujio Matsuda Fellowship to DH, and Grant G12RR03061 under the Research Centers in Minority Institutions Program of NIH).

CC 205 STRUCTURE AND FUNCTION OF THE ACIDIC FIBROBLAST GROWTH FACTOR I GENE IN THE ANDROGEN RESPONSIVE PROSTATE CANCER CELL LNCAP AND HAMSTER DDT1 CELL. Stephen E. Harris[1], Jeffrey A. Hall[2], Zing X. Rong[2], Ing-Ming Chu[3], Marie A. Harris[1]. Division of Endocrinology and Metabolism, University of Texas Health Science Center[1] at San Antonio, Texas, 78284-7877; W. Alton Jones Cell Science Center[2], Lake Placid, NY 12946; Department of Internal Medicine, Ohio State University[3], Columbus, OH 43210

Heparin-binding (fibroblast)-growth factor I, aFGF, is a major mitogen produced by prostate cancer cells, such as human LNCAP and the androgen-responsive hamster ductus-deferens tumor cell line, DDT1. We have been using these two cell lines to dissect out the mechanisms of steroid induced growth, using the aFGF/HBGF1 gene as a model for an androgen induced growth factor in human prostate cancer. Both the hamster DDT1 and human LNCAP aFGF transcription units and 5'-flanking regions have been isolated. A major complication in characterizing the aFGF gene arose when it became apparent that cloned aFGF cDNAs from different tissues, cells, and organs contained different 5'-non coding exons and identical coding exons. 5 different human 5'-non-coding (5'-NC) exons have so far been identified, suggesting the possibility of cell and tissue specific promoters for aFGF gene.

We have shown that the hamster DDT1 5'-NC exon and flanking region is homologous to the 5'-NC exon and flanking region utilized in androgen responsive human prostate LNCAP cells. This aFGF 5' NC exon utilized in human prostate cancer cells is different from the 5' NC exons utilized in brain, kidney, heart tissue.

Using aFGF-5'-flanking-CAT constructions, transfected into DDT1 cells, we have identified a region in the 5'-flanking region that is transcriptionally responsive to androgens and to aFGF. By sequence analysis of the 5'-flanking region and androgen responsiveness, we propose that both androgens and aFGF can activate a cascade of early growth response gene transcription factors such as C-fos, Egr-1 and Nlur 77 that are directly involved in aFGF transcription.

CC 206 DETECTION OF BREAST TUMOR CELLS BEFORE AND AFTER PHOTODYNAMIC THERAPY, **Ann Hornby, David Mitchell, Peter Cheung, Julia Levy and Robert McMaster, Department of Medical Genetics, University of British Columbia, Canada.** Autologous bone marrow transplant is becoming an accepted part of the treatment regime in patients with advanced metastatic breast cancer. One limitation of this treatment is the prevalence of bone metastases and individual cancer cells in the bone marrow of these women which will be reintroduced into the patient. Consequently, there is a need for *ex vivo* elimination of tumor cells from the bone marrow prior to transplant.

Cells (cell lines, bone marrow, or peripheral blood leukocytes) were treated with varying concentrations of the photosensitive drug, benzoporphyrin derivative (BPD), then exposed to a light source. This treatment resulted in preferential killing of tumor cells. Treated cells were then assessed for the presence of viable tumor cells utilizing PCR. The expression of breast-specific keratins and epithelial mucin RNA were detected with a sensitivity of one tumor cell in 10^6 normal cells.

Our data indicate that this may be an effective method for purging metastasized breast tumor cells from bone marrow prior to autologous bone marrow transplant.

CC 207 MATRIX GLA PROTEIN EXPRESSION IN TUMOR PROGRESSION, Eleni N. Levedakou, Torsten Strohmeyer*, Peter Effert* and Edison Liu, Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, NC 27599
*Urologische Klinik der Heinrich-Heine-Universität, Moorenstrasse 5, 4000 Düsseldorf, Germany

Matrix Gla protein (MGP), a protein of the bone and cartilage (Cancela et al., J. Biol. Chem. 265, 15040, 1990), was found to be highly expressed in prostatic and renal cell primary carcinomas. Carcinomas of the prostate exhibited variably high MGP mRNA as compared to prostate cancer cell lines derived from metastatic tumors of the lymph node (ILN and DUPRO) and brain (DU145), where MGP mRNA was not detectable. We also noticed that in one patient, the level of MGP mRNA of the primary tumor was at least four times higher when compared to the lymph node metastasis from the same patient. Analysis of a panel of 28 primary renal cell carcinomas revealed that 71% were overexpressing MGP as compared with matched normal kidney tissues. Additionally, we observed an inverse correlation between MGP expression ratio (tumor/normal) and tumor grade ($.01<p\leq.025$) as well as clinical stage ($.02<p\leq.05$). Taken together these observations suggest that the loss of MGP expression occurs during late tumor progression and metastasis.

CC 208 THE NUCLEAR MATRIX AS A TARGET FOR PROSTATE CANCER CHEMOTHERAPY: ESTRAMUSTINE AND ETOPOSIDE INTERACT AT THE LEVEL OF THE NUCLEAR MATRIX TO INHIBIT PROSTATE CANCER GROWTH. Kenneth J. Pienta. Meyer L. Prentis Comprehensive Cancer Center, Wayne State Univ. Sch. of Med., Detroit, MI 48201 Metastatic prostate cancer which is refractory to hormone therapy remains an incurable disease without effective therapy. Novel treatment strategies are clearly needed. A common event in prostate cancer carcinmogenesis appears to be an alteration in nuclear morphology. Nuclear morphology is controlled in part by the nuclear matrix, the dynamic RNA-protein network of the nucleus that provides structural support to the nucleus and which plays an important role in DNA replication and gene expression. We have begun to investigate the nuclear matrix as a target for prostate cancer chemotherapy. It was postulated that estramustine phosphate, an estradiol-nitrogen mustard conjugate which binds to the nuclear matrix, might enhance the cytotoxicity of etoposide, a topoisomerase II inhibitor which acts at the level of the nuclear matrix. In vitro, estramustine synergized the cytotoxic effects of etoposide on the Dunning R3327 prostate adenocarcinoma anaplastic, metastatic subline MAT-LyLu (MLL). Estramustine appears to alter the protein composition of the nuclear matrix as investigated by high resolution two dimensional electrophoresis. By alkaline elution assay, estramustine does not cause significant DNA strand breakage but does increase the amount of etoposide-induced strand breakage. Estramustine increases the amount of topoisomerase II-DNA crosslinking induced by etoposide. In a nascent DNA synthesis assay, estramustine and etoposide act synergistically to selectively inhibit new DNA synthesis on the nuclear matrix. In vivo, estramustine and etoposide acted synergistically to inhibit MLL growth in the male Copenhagen rat model. Together, these data have formed the basis of a Phase II clinical trial to examine the effect of estramustine and etoposide in patients with stage D hormone-refractory prostate cancer.

CC 209 ACTIVATION OF *INT-5* IN MOUSE AND HUMAN MAMMARY TUMORS, Tekmal R. Rao[1], Charles M. McGrath[2], Vincent L. Morris[3] and Doug A. Gray[4]. [1]Department of Obstetrics and Gynecology, The University of Texas Health Science Center, San Antonio, TX 78284-7836; [2]Grace Bio-Oncology Laboratory and Institute, 900 Auburn Road, Pontiac, MI 48057; [3]Department of Microbiology and Immunology, University of Western Ontario, London, Canada N6A 5C1; [4]Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5.

Our recent studies have resulted in identifying and cloning *int-5* genomic DNA, a unique and highly conserved novel mouse mammary tumor virus integration locus gene from the BALB/c precancerous D2 hyperplastic alveolar nodule (HAN). *Int-5* is different from other *int* genes, not only because of its localization on chromosome 9, but also because it has been found in precancerous, rather than cancerous neoplasms and it has been found in chemically- rather than virus-induced neoplasms. Compared to other *int* genes, *int-5* cloned from precancerous HAN is expressed in a variety of normal tissues such as liver, and many others of multiple germ-line derivation, including the D2HAN and normal mammary gland. The expression of *int-5* in D2 tumors is 10 fold greater than in normal mammary gland, indicating *int-5* is overexpressed in mammary tumors (Oncogene Res. 6:53-63, 1991). Our present studies with human normal breast tissue, breast tumors and breast cancer cell lines using mouse *int-5* DNA probe clearly indicate its overexpression in almost 40% of the breast tumors and breast cancer cell lines tested, compared to normal breast tissue and normal breast epithelial cell line. The DNA from the same samples was tested for a possible *int-5* gene amplification; none of these samples showed any amplification of *int-5* gene. These results suggest *int-5* involvement in human carcinogenesis, and indicate multiple mechanisms for the activation of this gene, different from those observed with *int-2* involved in breast cancer.

CC 210 RELAXIN GENE EXPRESSION IN HUMAN NORMAL AND NEOPLASTIC BREAST TISSUE. Lily S. Tashima, Gwen Mazoujian and Gillian Bryant-Greenwood. Pacific Biomedical Research Center, Dept of Anatomy and Reproductive Biology, University of Hawaii, Honolulu HI 96822 and Dept of Pathology, Barnes Hospital, Washington University, St. Louis MO 63110.

Relaxin is a polypeptide hormone belonging to the insulin and insulin-like growth factor family. It is traditionally produced by the ovary but has recently been detected by immunohistochemistry in normal and neoplastic human breast tissue (Lancet 335:298, 1990). Human relaxin is a product of two genes, H1 and H2. Only the H2 gene is expressed in the corpus luteum of the ovary whereas both H1 and H2 are expressed in the decidua, trophoblast and prostate gland. Accordingly, we have studied relaxin gene expression in normal and neoplastic breast tissue.

Total RNA was extracted, and first strand cDNAs were prepared. This was used in the polymerase chain reaction (PCR) with relaxin H1 and H2 specific primers and actin primers to verify the integrity of the cDNAs. There was no expression of either H1 or H2 relaxin genes in 55% of 9(5/9) normal breast tissue samples, whereas 11% (1/9) showed H2 but no H1 relaxin and (33%) 3/9 showed the expression of both H1 and H2 relaxin genes. Of 12 neoplastic breast tissue samples studied, 25% (3/12) showed H2 relaxin but no H1 expression and 75% (9/12) showed both H1 and H2 relaxin expression. Southern blot analysis of the PCR products with an internal relaxin 48 mer oligonucleotide, and restriction enzyme digestion with *Hpa*II and *Hpa*I which are specific for H1 and H2 relaxin genes respectively verified the specificity of the PCR relaxin fragments.

In conclusion, these results show that H2 relaxin is the predominant form expressed in normal and neoplastic breast tissue. In addition, both H1 and H2 relaxin gene expression is elevated in neoplastic breast tissue. The role of relaxin in the breast is unknown but may have an autocrine/ paracrine function in this tissue. (This work was supported by Grant G12RR03061 under the Research Centers in Minority Institutions Program of NIH)

CC 211 BASEMENT MEMBRANE INVASION / MIGRATION STIMULATED BY THE *erbB*-2 RECEPTOR LIGAND (gp30) IN HUMAN BREAST CANCER CELL LINES. **Erik W. Thompson, Jeffrey Torri, Gloria Arand, Marc E. Lippman, and Ruth Lupu.** Lombardi Cancer Research Center, Georgetown University, 3800 Reservoir Rd, Washington DC, 20007.

The *erbB*-2 oncogene encodes a 185 kDa transmembrane growth factor receptor which has prognostic significance in breast cancer. We have identified and purified a heparin-binding growth factor (gp30) which is a ligand for the *erbB*-2 receptor that can modulate proliferation of cells with *erbB*-2 amplification. Using the Boyden chamber chemoinvasion and migration assays, we sought to examine the potential involvement of basement membrane invasiveness in the *erbB*-2-associated poor survival. Interestingly, we found that human breast cancer cell lines which overexpress the *erbB*-2 oncogenes (SK-Br-3, MDA-MB-453, BT474) are relatively uninvasive in these assays. However, in the presence of the gp30 ligand, a dose-dependent increase of up to five-fold in chemoinvasive activity was observed. Ligand-stimulated invasiveness of SK-Br-3 cells, which express moderate levels of EGF-R and very high levels of *erbB*-2, was not blocked by anti-EGF-R antisera, suggesting that the ligand effect is mediated by the *erbB*-2 receptor. Migration analysis in Boyden chambers lacking the basement-membrane-like matrigel barrier indicated a parallel induction of migration by ligand in these cells, and this may facilitate the increased invasiveness. These observations suggest that ligand-induced migration/invasiveness may contribute to the *erbB*-2-associated poor prognosis in human breast cancer, and may help to explain the paradoxical localization of the *erbB*-2 oncogene in both *in situ* and invasive components of human breast cancer.

CC 212 CHARACTERIZATION AND EXPRESSION OF THE ANDROGEN RECEPTOR GENE IN HUMAN PROSTATIC CARCINOMA AND HUMAN PROSTATIC CARCINOMA CELL LINES. Theodorus H. van der Kwast, Hein F.B.M. Sleddens, Fritz H. Schröder, Wim Boersma, Albert O. Brinkmann and Jan Trapman, Depts. of Pathology, Endocrinology and Reproduction, and Urology, Erasmus University Rotterdam, and Dept. of Immunology, MBL/TNO, Rijswijk, The Netherlands.
The expression of the human androgen receptor (hAR) protein in prostatic carcinomas was investigated by immunohistochemistry with monoclonal antibody F39.4 specific for a unique epitope of the N-terminal domain of the AR. It was found that in prostatic carcinomas derived from prostatectomy specimens (stage <T3) the majority of tumor cells was hAR positive. Similarly, most progessively growing prostatic carcinomas derived from patients treated by androgen ablation therapy largely consisted of hAR positive tumor cells (1). Two of these hAR positive rapidly proliferating androgen-insensitive prostatic carcinomas were examined for alterations in exons 2-8 of the hAR gene. However, no mutations were found. In 7 human prostatic carcinoma cell lines, the length of the polymorphic (CAG)-repeat (encoding a poly-glutamine stretch) in exon 1 was determined. In 4 samples the length of the repeat fell within the range 19 to 22. In 3 others, (CAG)-repeats of 25 to 27 were detected. The latter finding suggests genomic instability of this region in the prostatic carcinoma cell lines.

1) Th.H. van der Kwast, J. Schalken, J.A. Ruizeveld de Winter, C.C.J. van Vroonhoven, E. Mulder, W. Boersma and J. Trapman. Androgen receptors in endocrine-therapy-resistant human prostate cancer. Int. J. Cancer 48: 189-193, 1991.

CC 213 THE GENE OF HUMAN PROSTATIC ACID PHOSPHATASE, Pirkko Vihko, Pirjo Virkkunen and Elli Birr, Biocenter and Department of Clinical Chemistry, University of Oulu, SF-90220 Oulu, Finland.
The prostate gland is a useful model to study androgen action, as the development and maintenance of prostatic morphology and function is dependent on the availability of androgens. We have previously cloned cDNAs encoding prostatic acid phosphatase (PAP) and prostate specific antigen (PSA), two major prostate specific secretory proteins. We have demonstrated that the levels of mRNAs coding for PAP and PSA are conversely regulated by androgens in LNCaP cell line, PAP mRNA is downregulated and PSA mRNA is upregulated by androgens. In order to elucidate the negative androgen regulation of human PAP gene, we have isolated genomic DNA clones of PAP. A genomic lambda DNA library and genomic cosmid DNA library were screened with two PAP cDNA probes. Four positive clones were subcloned and further characterized by a restriction enzyme mapping and a sequence analysis. The gene of PAP is over 20 kb of size. So far, we have sequenced 8 exons, but the gene is likely to contain 10 of them. In the promoter region, a putative TATA box, TAAATA sequence, and the CAAT sequence were located at the positions -77 and -91 respectively. In addition, three complementary GC boxes were identified at the positions -235, -67 and +13. The transcription initiation site was located at the position -50 by primer extension analysis and S1 nuclease mapping. Further characterization of the 5' flanking sequence and studies of the promoter activity are going on.

CC 214 A NOVEL NUCLEAR PHOSPHOPROTEIN IDENTIFIES A SUBCLASS OF BASAL STEM CELLS IN RAT VENTRAL PROSTATE, Loren D. Walensky, Tseng-Hui T. Chen, Donald S. Coffey, Tzyy-Choou Wu, and Gary R. Pasternack, Departments of Pathology and Urology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205
The association between the expression of a novel 32 KD nuclear phosphoprotein (pp32) and cell proliferation was investigated in the regenerating prostate. pp32 is constitutively expressed at high levels in populations of neoplastic B cell lines but only focally in normal tissues as in intestinal crypt epithelial cells. Antibromodeoxyuridine monoclonals, recombinant anti-pp32 polyclonals, and *in situ* hybridization were used to monitor DNA synthesis and pp32 expression, respectively, in rat ventral prostate epithelium following castration and androgen restoration. Forty-five day castrates retained only 6% of the epithelial cells of intact controls. Upon exogenous testosterone replacement, the remaining epithelial cells in the castrate are capable of gland restoration; it is not known whether all or a particular subclass are responsible for the intense proliferative activity. We found the epithelia in castrates to be divided into 2 populations, with 55% of the cells immunohistochemically positive for pp32. In intact animals pp32 is localized primarily to basal cells and some columnar cells. These results support the stem cell hypothesis of Isaacs and Coffey that suggests an amplifying cell population in castrates, originating from a very limited population of basal/stem cells and serving to amplify the total number of cells through androgen-induced transient self-renewal and clonal expansion. *In situ* RNA hybridization showed increased message expression in castrates and focally increased expression in proliferative areas after testosterone replacement. We propose that pp32 is a new nuclear marker for prostate amplifying cells, a special subpopulation of the basal cells. An amplifying cell-specific role for pp32 may account for the differential expression of pp32 in castrates, the dilution of anti-pp32 labeled cells by unlabeled transit cells upon prostate regeneration, and pp32 expression in basal and columnar epithelial cells.

Poster Session III

CC 300 ROLE OF GROWTH FACTORS AND ß$_1$ INTEGRIN-CELL MATRIX INTERACTIONS IN PROLIFERATION AND METASTASIS OF A MURINE MAMMARY CARCINOMA.

Bruce Elliott[1], Arne Östman[2], Bengt Westermark[3], Peter Ekblom[4] and Kristofer Rubin[1]. [1]Dept. of Medical and Physiological Chemistry, and [2]Ludwig Institute for Cancer Research, BMC, and [3]Dept. of Pathology, and [4]Dept. of Zoophysiology, University of Uppsala, Box 575, S-751 23, SWEDEN.

We have examined the role of growth factors and extracellular matrix (ECM)-ß$_1$ integrin interactions in the growth and metastasis of a murine mammary carcinoma, SP1, and a stable highly metastatic variant, SP1-3M. Rabbit anti-ß$_1$ integrin IgG, but not preimmune IgG, inhibited the formation of pulmonary macrometastases from primary intramammary tumors of SP1, but not SP1-3M cells. Primary tumor growth and the formation of micrometastases, detected by immunofluoresence with anti-tenascin antibodies, were unaffected by anti-ß$_1$ integrin IgG; these findings suggest that growth of SP1 cells that seed and proliferate in target organs are more dependent on ß$_1$ integrin interactions than SP1-3M cells. We therefore compared the role of ß$_1$ integrin/ECM interactions in growth factor-dependent proliferation and cell adhesion of SP1 and SP1-3M cells. A strong proliferative response to basic fibroblast growth factor (bFGF) occurred within 12 to 24 h with SP1 and SP1-3M cells cultured on plastic, or on substrata consisting of collagen type I or fibronectin. A 12 to 24 h proliferative response to platelet-derived growth factor-BB (PDGF-BB) occurred on fibronectin, but not on collagen type I. Without exogenous growth factors, both cell lines adhered to fibronectin and laminin. SP1-3M cells did not bind to collagen type I, whereas SP1 cells did. Binding to all three substrata was inhibited by anti-ß$_1$ integrin IgG; suggesting that the primary adhesion to these substrata is mediated by ß$_1$ integrins. Under serum-starved conditions, SP1 and SP1-3M cells showed similar integrin patterns following immunoprecipitation by anti-ß$_1$ integrin IgG. bFGF stimulated increased adhesion and spreading of both SP1 and SP1-3M cells to collagen type I within 24 h, whereas PDGF-BB was less capable of this effect. Interestingly, SP1-3M cells, but not SP1 cells, displayed a late (3 day) proliferative response to PDGF-BB on collagen type I. Our results suggest that ß$_1$ integrin-ECM interactions can modulate the proliferation and metastasis of SP1 cells, whereas a metastatic variant, SP1-3M, is less restricted by exogenous ECM components. (Supported by the Swedish Cancer Foundation).

CC 301 AN IMMUNOHISTOCHEMICAL STUDY OF EGFR AND *Neu* IN STAGE A PROSTATE CARCINOMA.

Fox SB, Persad R, Kore RN, Collins CC. Dept of Pathology, University of Sheffield Medical School, Sheffield, U.K.

Prostatic carcinoma is the commonest cancer in men. In the UK, when diagnosed incidentally treatment is deferred since most are expected to die of intercurrent disease. However, a significant proportion of these aged under 70 show tumor progression even when in the best prognostic group. Thus, classification of these stage A tumors by conventional histopathology is of little help in determining prognosis or therapy. The availability of new markers that can be applied to routinely processed material may help to resolve this problem. We focused on the tyrosine kinase oncogenes EGFR and *neu* which are prognostically useful in other hormone responsive tumors. EGF is a transforming growth factor which acts on EGFR to regulate normal cell growth and differentiation. EGF is a potent prostatic cell mitogen and both EGF and *neu* have been detected in the diseased prostate. *In vitro* assays of EGFR in prostate cancer have given conflicting results probably due to heterogeneity of the tissue examined. Similar inconsistencies have been reported for *neu*. We have circumvented this by using immunohistochemistry and the well characterised antibodies 12E (EGFR) and CB11 (*neu*). We examined archival tissue from 36 stage A prostatic carcinomas in men under 70 yrs for whom follow up was available. Our results are presented in the table.

	EGFR+ve	Neu +ve	Both+ve
Tumor	39%	47%	19%
Non-neoplastic epithelium	89%	25%	25%

Immunoreactivity was usually focal and in contrast to other reports was consistently more intense in the non-neoplastic than neoplastic epithelium. Although no corellation was observed with survival both EGFR and *Neu* might be important in the development of both prostatic hyperplasia and cancer.

CC 302 RB GENE EXPRESSION IN HUMAN MAMMARY CELLS TRANSFORMED BY THE SV40 LARGE T ANTIGEN, Gérard Goubin[1], Jérôme Lebeau[1], Fabien Calvo[3], Bernard Dutrillaux[2], [1] Laboratoire d'Oncogenèse, and [2] Section de Biologie, CNRS URA 620, Institut Curie, 26 rue d'Ulm, F-75231 PARIS Cedex 05. [3] Laboratoire de Pharmacologie, Institut de Génétique Moléculaire, Hôpital Saint Louis, F-75475 PARIS Cedex 10.

We have transfected primary cultures of mammary cells derived from reduction mammoplasties by a replication-defective mutant of SV40. Three cell lines were generated from different specimens. Subcutaneous injection of one of these cell lines (S2T2) into nude mice produced slow growing tumors while the two others were not tumorigenic. Several of the grafted tumors were propagated *in vitro* and characterized for the retinoblastoma susceptibility gene (RB) expression. Half of them retained the high level of gene expression observed in the parental S2T2 cell line, while the others showed very low levels of messenger RNA and p105RB. Although the association of the RB gene product with the SV40 large T antigen was reported to be sufficient to inactivate its function, the loss of expression was found to be associated with the loss of a RB allele. All the tumors exhibited a similar tumoriginicity when grafted into athymic mice. In contrast, the doubling time in culture was markedly increased in tumors expressing low level of the RB gene product. These results suggest that in SV40 transformed mammary cells, loss of RB gene expression is associated with an increased proliferation in culture but not with tumoriginicity. This model system could be useful to investigate the mechanism of RB gene inactivation found in 20-30% of human breast tumors.

CC 303 INDUCTION OF MAMMARY TUMORS IN TRANSGENIC MICE EXPRESSING THE UNACTIVATED C-NEU ONCOGENE, Chantale Guy, Michael Schaller, Thomas Parsons , Robert D. Cardiff, and William J. Muller, The Institute for molecular biology and biotechnology, McMaster University, 1280 Main W., Hamilton, Ontario, Canada, L8S 1T7.

Overexpression and amplification of the c-neu (c-erbB-2) proto-oncogene has been correlated with clinical progression in a large percentage of human breast cancers. In order to directly assess the tumorigenic potential of the c-neu proto-oncogene in the mammary epithelium we have created several strains of transgenic mice that carry the unactivated c-neu oncogene under the transcriptional control of the mouse mammary tumor virus (MMTV) promoter/enhancer. By contrast to the rapid tumor progression observed in transgenic strains bearing the MMTV/ activated c-neu transgene, transgenic mice overexpressing the unactivated c-neu allele develop solitary mammary tumors that occur after a long latency. Examination of transgene expression by RNAse protection and Western analyses indicated that the tumors express 20 to 50 fold greater levels of the c-neu transgene product in tumors compared to the adjacent normal epithelium which correlated with the appearance of several novel tyrosine-phosphorylated proteins. Taken together, these results argue that elevated levels of c-neu kinase activity is required to convert the primary epithelial cell to the malignant phenotype.

CC 304 ASSOCIATION OF P53 EXPRESSION WITH OTHER PROGNOSTIC FACTORS AND LONG-TERM SURVIVAL IN NODE-NEGATIVE BREAST CANCER
J.Isola, T.Visakorpi, K.Holli and O-P Kallioniemi, Dept. Biomed. Sciences, University of Tampere, Tampere, Finland. Mutations of the p53 tumor suppressor gene often result in the accumulation of large amounts of abnormal p53 protein in the cell nucleus. We studied the biological and prognostic significance of p53 protein expression immunohistochemically with a CM-1 antibody in 290 node negative breast cancers. Nuclear immunoreactivity was found in 79 tumors (27%) with 41 (14%) showing high level (>20% immunopositive cells) and 38 (13%) low level immunoreactivity. High level p53 staining was significantly associated ($p<0.0001$) with poor histologic grade and low estrogen receptor content, c-erbB-2 overexpression and high tumor proliferation rate as defined by flow cytometric s-phase analysis. Tumors with weak immunoreactivity showed none of these associations. Eight-year survival of patients with high level p53 expression was 56%, significantly different from those with weak (75%) or no (81%) p53 immunoreactivity. Tumors showing neither p53 nor c-erbB-2 positivity had a very favorable 8-year survival (83%), whereas tumors with strong p53 overexpression had poor prognosis regardless of the c-erbB-2 status. A multivariate Cox regression analysis in 269 patients indicated that high-level p53 expression ($p=0.0002$), tumor size ($p=0.016$) and c-erbB-2 overexpression ($p=0.09$) had independent prognostic value. However, when s-phase was also included in the Cox analysis (evaluable in 228 cases) both p53 and c-erbB-2 were removed from the model indicating that the prognostic impact of these genetic aberrations is mainly mediated by their effects on tumor cell proliferation rate.

CC 305 THE ROLE OF CATHEPSIN D IN THE INVASIVE PHENOTYPE OF MCF-7 CELLS IN THE BOYDEN CHAMBER INVASION ASSAY. M.D. Johnson, J. Torri, M.E. Lippman, and R.B. Dickson, Lombardi Cancer Center, Georgetown University, Washington, DC 20007. The aspartyl protease cathepsin D has been shown to be a marker of poor prognosis when found at high levels in primary breast tumors. It has been suggested that this is because the production of cathepsin D increases the invasive potential of the tumor cells thus increasing the probability of metastasis. We have therefore conducted experiments to determine if secreted cathepsin D makes a significant contribution to the invasive phenotype of the breast cancer cell line MCF-7 in the Boyden chamber assay of invasion. Nine single cell clones were prepared, by limiting dilution, from a bulk population of MCF-7 cells. The amount of cathepsin D secreted by each clone was then determined by labelling the cells with ^{35}S methionine, immunoprecipitating the cathepsin D from the conditioned media and running the precipitates on gels that were subsequently processed for fluorography. The behavior of each clone was also determined in the Boyden chamber assay, in which the ability of a cell to invade through an artificial basement membrane is measured. Repetition of both assays produced consistent results. The levels of cathepsin D secretion and the invasive potential of the 9 clones were spread over a six fold range, but there was no correlation found between cathepsin secretion and invasive behavior. Invasion assays were also conducted in the presence of concentrations of the aspartyl protease inhibitor, pepstatin, shown to block the proteolytic action of cathepsin D. The presence of pepstatin had no impact on the invasiveness of cells in this assay. These data suggest that cathepsin D secretion is not an important determinant of invasiveness in this system. It is, however, possible that cathepsin D is required for invasion, but is not secreted at a low enough level to be rate-limiting for any of the clones. Also, in the "micro environment" speculated to exist at the invading front of a cancer cell, the pepstatin may not have reached the concentration required to inhibit the enzyme. Further experiments will be conducted to address these possibilities.

CC 306 K-RAS MUTATION IN PRIMARY TUMORS OF RAT PROSTATE INDUCED BY N-NITROSO-N-METHYL UREA,
Kenji Kadomatsu, Mario A. Anzano, Joseph M. Smith and Michael B. Sporn, Laboratory of Chemoprevention, NCI, Bethesda, MD 20892
Ras mutation has been implicated in chemically induced tumors in animals and in various human tumors. We study the prevalence of ras mutation in primary tumors of rat prostate initiated by intravenous injection of N-nitroso-N-methyl urea (30 mg/Kg) and promoted by subcutaneous implantation of testosterone propionate (40 mg) every 2-3 months in Lobund/Wistar rats. Incidence of primary tumor of the prostate at 9-10 months following carcinogen treatment was 60%. DNA and RNA were extracted from prostate tumor samples. Polymerase chain reaction (PCR) of exons I and II of Ha-ras and Ki-ras genes was performed utilizing specific primers, and amplified PCR products were characterized using single strand conformation polymorphism analysis. Point mutation alters the secondary structure of single strand PCR products allowing detection as mobility shifts on non-denaturing gels. Screening of 12 prostate tumors showed mobility shifts in Ki-ras but not Ha-ras from 10 tumor specimens but not in saline or testosterone-treated controls. Sequence analysis of asymmetric PCR products showed point mutation at codon 12 of Ki-ras (G->A transition). We suggest that Ki-ras mutation may play a role in prostate carcinogenesis.

CC 307 ANALYSIS OF C-ERBB-2 AMPLIFICATION IN BREAST CANCER BY FLUORESCENCE IN SITU HYBRIDIZATION,
Olli-P. Kallioniemi, Anne Kallioniemi, Wayne Kurisu, Ann Thor, Ling-Chun Chen, Helene S. Smith, Fred Waldman, Dan Pinkel, Joe W. Gray, Division of Molecular Cytometry, Department of Laboratory Medicine, University of California, San Francisco, CA 94143-0808
We have developed a new method based on fluorescence in situ hybridization (FISH) for analysis of actual c-erbB-2 oncogene copy numbers, the level of amplification (c-erbB-2 copy number relative to chromosome 17 centromere copy number) and the distribution of amplified genes in breast cancer cell lines and uncultured primary breast carcinomas. Relative c-erbB-2 copy number determined by FISH in 10 breast cancer cell lines showed an excellent correlation ($r=0.98$) with Southern blot results. Mean c-erbB-2 copy numbers in 44 primary tumors were also closely associated with slot blot results of amplification and immunohistochemical detection of overexpression. In all primary tumors with >2-fold amplification (10/44) a high degree of intratumor heterogeneity in gene copy numbers was found with some tumor cells showing 1-4 clusters of at least 25-50 (occasionally over 100) c-erbB-2 copies and others having only 2-5 c-erbB-2 copies per cell. Tumors that did not show amplification by FISH (34/44) had an average of 1-5 c-erbB-2 copies scattered randomly in the nuclei and lacked cells with high copy levels. Metaphase analysis of cell lines showed that c-erbB-2 amplification always took place in chromosomes with less than 50% of the clusters being located on chromosome 17. Similar results were obtained from the evaluation of rare metaphase cells in primary tumors. Also the distribution of c-erbB-2 copies in the interphase nuclei suggested intrachromosomal amplification.
Assessment of c-erbB-2 amplification by FISH may improve prognostic assessments based on the pattern of amplification and detection of heavily amplified tumor cell subpopulations.

CC 308 DISTRIBUTION OF BASEMENT MEMBRANE COMPONENTS IN NORMAL AND MALIGNANT HUMAN PROSTATE. **J. David Knox, Virginia C. Clark, Anne E. Cress*, and Raymond B. Nagle.** Departments of Pathology and Radiation Oncology*, College of Medicine, University of Arizona, Tucson, AZ 85724.

In general basement membranes regulate cell attachment, growth, and differentiation, as well as acting as passive molecular sieves. Basement membranes are able to act as physical barriers to the passage of neoplastic cells and the alteration of basement membrane composition has been shown to modulate the invasive and metastatic properties of neoplastic cells. Our earlier studies using EM have demonstrated decreasing amounts of basement membrane with increasing grade of prostatic carcinoma (Fuchs and Nagle, 1989). In this study antibodies specific for the extracellular matrix components, type IV collagen, laminin, entactin/nidogen, fibronectin, vitronectin, and tenascin are used to analyze 20 snap frozen human prostate carcinomas. As determined by immunohistochemical staining, the composition of the basement membranes of normal prostate glands consist primarily of laminin, type IV collagen, and entactin with variable amounts of tenascin. The basement membranes of invading carcinoma of low grade have a similar composition, but with increasing grade there is a loss of type IV collagen and tenascin. (Supported in part by ACS grant PDT-388.)

CC 309 A COMPLEX MORPHOGENETIC RESPONSE EVOKED IN PROSTATE CELLS IN RESPONSE TO AN EXTRACELLULAR MATRIX SURFACE, Carolyn C. Lamb, Darius J. Bagli, and Michael R. Freeman, Pediatric Urology Research, Children's Hospital and the Joint Center for Radiation Therapy, Harvard Medical School, Boston, MA 02115

The molecular mediators of dissemination of prostatic metastases are largely unknown. However, it has been established that some regulatory signals affecting prostate cells reside in the extracellular matrix (ECM). We have isolated and characterized a transformed rate prostate cell line, NbMC-2, that is highly responsive to ECM from the EHS mouse tumor (Matrigel). Defined plating conditions have been established in which EHS matrix evokes a complex morphogenetic response in NbMC-2 cells after plating on the matrix surface. Plating single-cell suspensions of NbMC-2 cells onto centrifuged 500 um surfaces of EHS matrix resulted in the formation of multicellular aggregates over a 10 day period. Time-lapse photomicrography and measurements of [3H]-thymidine incorporation into acid-precipitable material indicated that aggregation results from directed cell movement as well as cell division. Aggregation did not occur on laminin, fibronectin, collagen I or collagen IV surfaces, nor on agarose gel-ECM mixtures of these proteins, suggesting that this response is specific to an EHS matrix substrate. Following aggregation, fibroblastic cells observed by microscopy to be invading the gel, repeatedly arose in focal arrays from approximately 50% of the aggregates. Subcultures enriched in fibroblastic cells were obtained by removing the ECM and associated cells and expanding the cell populations which had invaded the matrix and attached to the culture dish surface. Analysis of these subcultures indicated that the fibroblastic morphology observed in the ECM cultures was preserved with serial passage, suggesting that a stable transformation from epithelial to fibroblastic cells had occurred. Single-cell NbMC-2 clones derived by transfection with a G418 antibiotic resistance plasmid retained the aggregation response of the parental cells and gave rise to fibroblastic derivatives at frequencies approaching the uncloned population. These data suggest that EHS matrix surfaces are capable of eliciting a morphogenetic response involving discrete and sequential cell behavior and resulting in a stable cell transformation. Analysis of cytoskeleton and cell adhesion protein expression by immunofluorescence indicate that NbMC-2 cells express cytokeratin filaments as punctate, juxtanuclear structures and do not localize the E-cadherin epithelial junction protein to cell-cell boundaries. These observations indicate that NbMC-2 cells, which express a conventional epithelial morphology, express constitutive defects in normal epithelial subcellular organization. These molecular abnormalities may be related to the latent capacity for a morphogenetic response to an EHS matrix surface exhibited by NbMC-2 cells.

CC 310 DEVELOPMENT AND CHARACTERIZATION OF A CIS-DIAMMINEDICHLOROPLATINUM RESISTANT HUMAN OVARIAN CELL LINE EXPRESSING c-erbB-2 Beatrice C. Langton, Michael P. Longhi and Jian-Ai Xuan. Department of Cell Biology and Immunology, Berlex Biosciences, Alameda, CA 94501.

The c-erbB-2 protein (gp185) is a transmembrane growth factor receptor which, when overexpressed in breast and ovarian cancers, may contribute to disease progression. Further, expression of high levels of this protein in some cell lines leads to a tumorigenic phenotype. We have developed an anti-c-erbB-2 monoclonal antibody, TAb 250, that inhibits breast and ovarian cell lines overexpressing c-erbB-2 and enhances the inhibitory activity of cisplatin (CDDP) against these cell lines both in vitro and in vivo. To assess whether the development of CDDP-resistance would alter the efficacy of the antibody/drug combination treatment, an SKOV-3 (6×10^5-1.5×10^6 TAb 250 binding sites/cell) parental ovarian cell line (P) was made resistant by exposure to escalating doses of CDDP. Populations were treated at intervals of one to two weeks for 24-48 hr and surviving clones were allowed to grow to confluence at each dosage level before retreatment or dose escalation. Surviving populations were isolated from 10, 20, 27, 33, and 40 μM dose levels. SKOV-3(P) cells were 12-fold more sensitive to cisplatin than the SKOV-3(R) cells surviving the 40μM treatment with an IC_{50} of 10μM and 120μM respectively. The effects of drug resistance on c-erbB-2 expression and tumorigenicity were analyzed. Binding studies using ^{125}I-TAb 250 and flow sorting analysis indicated a decrease in TAb 250 binding sites per cell as the SKOV-3 population became increasingly resistant to cisplatin. We will report on the effects of TAb 250 on these CDDP-resistant cells, the ability of these cells to grow as xenografts in nude mice and the implications of these results for the combination therapy.

CC 311 MAMMARY GLAND INVOLUTION-RELATED GENES ISOLATED BY DIFFERENTIAL SCREENING OF cDNA LIBRARIES. Feng Li, Robert Strange, Ann-Catherine Andres and Robert R. Friis, Laboratory for Clinical and Experimental Research, University of Berne, CH-3004 BERN, Switzerland.

During post-lactational involution the mammary gland undergoes a widespread epithelial cell death and dramatic tissue remodelling. In previous studies, we have detected patterns of gene expression and non-random degradation of DNA that correlate with the morphological changes observed during mouse mammary gland involution. These observations were consistent with the existence of an apoptotic process.

We have used differential screening of cDNA libraries from lactating and involuting mouse mammary glands in order to isolate the following genes involved in the involution process. All exhibit greatly increased expression between 1 and 4 days post lactation. These genes are associated with: 1.) **cell death**: SGP-2 (a protein expressed in other systems undergoing apoptosis; 2.) **tissue remodelling**: Tissue inhibitor of metalloproteinase (TIMP), MI-24 (a mouse Transin-related protease) and Osteopontin (a calcium-binding protein implicated in bone remodelling and expressed in Ha-ras oncogene-induced tumors); 3.) **iron-transport**: ferritin and transferrin and 4.) **lipid metabolism and adipocyte-differentiation**: stearoyl-coA desaturase. Interestingly, two genes of the tissue remodelling category were sharply up-regulated in H-ras-induced, but not in c-myc-induced tumors.

CC 312 ***RAS* AND *P53* MUTATIONS IN HUMAN PROSTATIC CANCER.**
Caroline Moyret [1], Raoul Mazars [1], Jean-Yves Soret [2], Alain Daver [2], Philippe Jeanteur [1] and Charles Theillet [1]. 1 UA CNRS1191, Laboratoire de Biochimie Centre Paul Lamarque, 34095 Montpellier, FRANCE, 2 Service d'Urologie, CHRU Angers, FRANCE.

Prostatic tumors were investigated for point mutations affecting the *P53* and *RAS* genes. Recent advances in understanding the molecular genetics of solid tumors, have shown that activation of oncogenes and inactivation of tumor suppressor genes play an essential role in the pathogenesis of human cancer.
Very little is known about genetic disorders involved in prostate tumorigenesis. Recently, some authors have shown that point mutations in *RAS* genes are infrequent by studying DNAs from prostatic tumors with allele-specific oligonucleotide probe hybridization, screening codons 12 , 13 and 61.
Using the *SSCP* method (Single Strand Conformation Polymorphism), we have studied 24 adenomas and 25 carcinomas of the prostate, ranging stages A through D and Gleason scores 2 through 9, for the presence of mutations in the first and second exons of the *H, K* and *N RAS* genes, and in the exons *2, 5, 6, 7, 8* and *9* of the *P53* gene.
The analysis was performed on DNA extracted from frozen tissue sections. This approach was chosen because of the heterogeneous nature of prostate tissue which could cause a dilution of mutant signal.
The following results were obtained: (i) concerning the mutation in the three *RAS* genes, 3/24 (12 %) of the presented adenomas were mutated in the first exon of *N RAS* gene while 3/25 (12%) of carcinomas exhibited a mutation in the exon 2 of *K RAS* gene. (ii) concerning the *P53* gene, 1/24 adenomas presented a silent mutation in the exon *8* while 6/25 carcinomas presented mutations in exons *2, 5, 7* and *9*. There is no apparent association between the presence of mutations in *RAS* and *P53* genes and the grade of the prostate tumors. In all samples showing a *P53* mutation both the wild type and mutated alleles were present. The maintained presence of the wild type allele could be due to the heterogeneous cellular composition of the prostate tumors analyzed, but it could also mean that the tumor retains both *P53* alleles, suggesting that a mutated *P53* allele does not necessarily need the loss of its normal counterpart to be phenotypically expressed.
Thus, albeit at a low incidence, prostatic cancer presents inactivating mutations in the *P53* gene. It is interessing to note that these mutations occured exclusively in prostatic carcinomas. Our data concerning activation of *RAS* genes by point mutations is in general agreement with published results showing that they are rare events in this cancer. It is, however, interessing to see that the mutations found in our series do not belong to the classical types defined as oncogenic.

CC 313 IDENTIFICATION OF TUMOR SPECIFIC RFLP BANDS IN HUMAN CANCERS USING A HARVEY-ras PROBE,
Richard A. Nakashima, Xin Li, Abraham A. Alecozay*, Donald W. Mittanck, Andrew Simmons and W. Christian Wigley, Dept. of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX 79409 and *Dept. of OB/GYN, Univ. of Kansas School of Medicine, Wichita, KA 67214.
We have performed RFLP analysis on 23 primary human tumors and 19 control normal human tissues, using Mbo I digested DNA and a human Harvey-ras probe. Results show that a band of 3.0 kb apparent size is present in over fifty percent of tumors examined, and absent in all control human tissues tested. Results with a matched pair of prostate carcinoma and control prostate tissue taken from the same donor show that the 3.0 kb tumor specific H-ras band was present in tumor DNA and missing in control DNA from the same individual. Comparison of RFLP patterns generated with Mbo I and Sau 3AI, two isoschizomers of different methylation sensitivity, suggest that the presence of the 3.0 kb band is not due to differential methylation in tumor and normal human DNA. The polymorphism appears to be associated with the VNTR region located downstream from the H-ras coding sequence. The tumor-specific 3.0 kb band has been observed in 8 of 16 endometrial carcinomas, 2 of 2 prostate carcinomas, 1 of 2 adenocarcinomas of the lung, and 1 of 1 primary adenocarcinoma of the GI tract that had metastasized to the ovary. These results are consistent with a somatic mutation of H-ras occurring in a wide variety of primary human tumors, with about a fifty percent incidence of the tumor marker. It should be noted that this polymorphism is independent of changes in codons 12 and 61, and has been observed in tumor types that were reported to have a zero incidence of activating mutations in these codons. (Supported by Grant #003644-010 from the Advanced Research Program, State of Texas)

CC 314 p185$^{c\text{-}neu}$ AND EGF RECEPTOR ASSOCIATE INTO A NOVEL STRUCTURE COMPOSED OF ACTIVATED KINASES,
Xiaolan Qian[1], Stuart J. Decker[2], and Mark I. Greene[1], [1] Division of Immunology and Center for Receptor Biology, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia PA 19104-6082, [2] Parke-Davis Pharmaceutical Research Division, 2800 Plymouth Rd., Ann Arbor, MI 48106
The protein product of the neu proto-oncogene, p185$^{c\text{-}neu}$, which is often overexpressed in human , breast and pancreatic tumors, is structurally similar to the epidermal growth factor receptor (EGFr). Slight overexpression of these two receptor tyrosine kinases, but not either separately, leads to transformation and tumorigenicity. Heterodimerization of p185$^{c\text{-}neu}$ and EGFr occurs in M1 cells which express both receptors. We have characterized the physical and functional interaction between EGFr and p185$^{c\text{-}neu}$ in M1 cells. We identified the two components of the heterodimer, EGFr and p185$^{c\text{-}neu}$, by 2-D gel analysis and co-immunoprecipitations. Analysis of this association with non-denaturing detergents and in the absence of cross-linkers indicates that non-covalent interactions are primarily responsible for heterodimer formation. The reversible heterodimerization was promoted by ligands binding to their cognate receptors. Functionally, the heterodimer is a highly active protein kinase for both receptor auto- and exogenous substrate phosphoylation in vitro. The isolated heterodimer was highly phosphorylated on tyrosine residues in vivo. These results indicated that the physical association between EGFr and p185$^{c\text{-}neu}$ is of functional significance and defines enzymatic features of receptor complex formation.

CC 315 IGF-I AND IGF-II EXPRESSION IN FIBROBLASTS DERIVED FROM TUMOR, NORMAL BREAST AND SKIN OF BREAST CANCER PATIENTS.
Christian Singer, Helene S. Smith*, Marc E. Lippman and Kevin J. Cullen, Lombardi Cancer Center, Georgetown University, Washington, DC and *Geraldine Brush Cancer Research Institute of the Medical Research Institute San Francisco, CA.

Insulin-like Growth Factors are known to be mitogenic for a number of breast cancer epithelial cell lines *in vitro*. Previous experiments utilizing RNAse protection assays have shown that the majority of fibroblasts derived from benign breast lesions expressed IGF-I mRNA but no IGF-II mRNA, whereas the majority of fibroblasts originating from malignant tumors express IGF-II, but not IGF-I.[1)]

We studied the IGF-I and IGF-II mRNA expression in matched sets of fibroblasts from each of 6 patients using RNAse protection assays and PCR. These fibroblast sets included cells from breast tumors, macroscopically normal breast adjacent to the tumor and from uninvolved breast skin. Both experiments revealed that 6 out of 6 fibroblast cell lines from peripheral normal breast expressed easily detectable mRNA for IGF-I while only 1 out of 6 expressed IGF-II. All 6 skin fibroblast cell lines expressed very high levels of IGF-II mRNA and small amounts of IGF-I mRNA. 4 of 6 tumor fibroblasts expressed IGF-II, but in addition all expressed variable amounts of IGF-I. These results indicate that the production of IGF-I is typical for normal breast fibroblasts. However, skin fibroblasts and tumor-derived fibroblasts express predominantly IGF-II. This suggests that the IGF-II expressed in tumor fibroblasts is serving a paracrine growth promoting role in breast cancers. Alternatively, the transition to IGF-II expression in tumor fibroblasts may be a marker of some other cellular event occuring in neighboring tumor epithelial cells.

(1) Cullen et al., Cancer Research, 1991, 51: 4978

CC 316 Cloning of TIMP-1 Human Genomic Regulatory-Promoter.
Mark E. Stearns and Min Wang. Department of Pathology, Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, PA 19129

We have recently cloned the human genomic TIMP-1 gene (16.5Kb) including the coding and regulatory-promoter regions from highly non-invasive PC-3 human prostate sublines (see Wang and Stearns, 1991, Differentiation, in press, for characterization of the sublines). We have characterized the regulatory region by restriction endonuclease mapping and identification of potential regulatory sequences based on gel retardation assays. Currently we are subcloning DNA fragments from the promoter region for sequencing and transfection analysis. These experiments should demonstrate if independent regulatory sequences (and factors) control TIMP-1 or TIMP-2 synthesis.
Supported by NIH-NCI grant CA 45425 to MES.
Cloning of TIMP-1 Human Genomic Regulatory-Promoter.
Mark E. Stearns and Min Wang. Department of Pathology, Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, PA 19129

CC 317 P53 ACCUMULATION IN PROSTATIC CARCINOMA
Tapio Visakorpi, Olli-P. Kallioniemi, Asko Heikkinen, Timo Koivula and Jorma Isola, Tampere University Hospital, SF-33520, Tampere, Finland.
Mutations of the p53 tumor suppressor gene are known to result in the accumulation of abnormal p53 proteins in the tumor cell nuclei. We analyzed the level of p53 protein accumulation in 137 paraffin-embedded primary prostatic carcinomas by immunohistochemistry with a new polyclonal CM-1 antibody to study the role of p53 aberrations in the progression of prostatic neoplasia. Accumulation of p53 protein was found in 23 (17%) carcinomas, whereas normal and hyperplastic prostatic tissues were always negative. Eight (6%) carcinomas showed intense immunostaining in more than 20% of carcinoma cells whereas 15 (11%) had lower levels of immunostaining. Tumors with high level p53 accumulation were often histologically poorly differentiated, DNA aneuploid and had about two times higher cell proliferation rate (determinated by flow cytometric S-phase analysis and proliferative cell nuclear antigen immunohistochemistry) in comparison to p53 negative cases. Low level p53 accumulation was associated only with DNA aneuploidy. Accumulation of p53 protein was not significantly related to tumor size, stage or age of patient. High level p53 immunoreactivity predicted poor 10-year progression-free ($p<0.01$) and prostatic carcinoma spesific survival ($p<0.01$) with about ten-fold relative risk of death as compared with p53 negative cases. Low level p53 immunoreactivity did not have any prognostic value. In conclusion, high level p53 accumulation was found in a small subgroup of aggressive, highly proliferative prostatic carcinoma suggesting that p53 mutations may contibute to the progression of some prostatic carcinomas.

CC 318 **Androgen Receptor Regulation by Retinoic Acid in the Human Prostate Cancer Cell Line LNCaP.**
Charles Y.-F. Young and Donald J. Tindall, Depts. of Urology and Biochemistry/Molecular Biology, Mayo Clinic, Rochester, MN 55905.
The proliferation of prostate cells is highly dependent upon androgen stimulation, presumably via its cognate receptor. Recent studies have demonstrated that retinoic acid (RA) can repress the growth of primary human prostatic epithelial cells. Therefore, we have investigated the effects of RA on the expression of the androgen receptor (AR) in the human prostatic adenocarcinoma cell line LNCaP. LNCaP cells were incubated with RA (10^{-10}-10^{-5}M) in the presence of androgen ($6.4x10^{-10}$M) for 5 days; Trypan blue exclusion was used to assess cell viability. We found that RA inhibits the growth of LNCaP cells in a dose-dependent manner. Using a radio-ligand binding assay at 37°C, we found that the AR protein is increased after 24 hr incubation with 6 nM dihydrotestosterone (DHT) about two-fold above that of cells in the absence of DHT. Moreover, retinoic acid inhibited this androgen-induced increase in a dose-dependent manner, with maximum inhibition being achieved at $1x10^{-6}$M. However, the inhibition was never lower than that of control cells. Western blot analysis with a monoclonal anti-AR antibody confirmed these results. Interestingly, the affinity of AR for the synthetic androgen, mibolerone, as determined by Scatchard analysis was also decreased 2-3 fold by RA treatment. These findings, in conjunction with the observations that AR mRNA is down-regulated by androgens in LNCaP cells, suggest that the alterations in AR protein concentration and affinity are at the posttranslational level. Moreover, they imply that RA is capable of modulating the sensitivity of human prostate cancer cells to androgens. (Supported in part by NIH grants #CA32387 and #DK41995.)

Poster Session IV

CC 400 HUMAN MULTI-DRUG RESISTANT CANCER CELLS INDUCED TO END-STAGE CELL DIFFERENTIATION: SYNERGY BETWEEN GROWTH FACTORS AND CHEMOTHERAPEUTICS, Edwin W. Ades and James M. Pruckler, Biological Products Branch, Scientific Resources Program, National Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA 30333

Malignant cells have been induced to express a program of terminal differentiation by exposure to various chemical agents. The molecular mechanisms of the actions of these compounds have been studied, and changes of expression of protooncogenes, activation of protein kinase C and induction of cytoplasmic trans-acting factor(s) have all been identified and demonstrated. This study examined the effects of 15 different recombinant growth factors on end-stage cell differentiation of drug-resistant tumor cells. Cells were additionally examined for reversal of drug resistance. A combination of the differentiation agent hexamethylene bisacetamide and the inhibiting effects of TGF-B_1 demonstrated a synergistic interaction, resulting in a cell death rate of 80%. Interleukin-1, gamma-interferon, tumor necrosis factor and TGF-B_1 had enhanced but variable inhibitory effects on the cell death rate. A combination of growth factor(s) and chemotherapeutic agent demonstrated variable synergism. Potentiation of human natural killer cell activity towards multi-drug resistant cells was observed only with recombinant Interleukin-2. These data support the concept of programmed cell death and suggest that drug-resistant tumor cells may be susceptible to the combination of cytokines, differentiating agents and/or chemotherapeutic agents.

CC 401 INDUCTION OF TRANSFORMING GROWTH FACTOR-ß1 AND ß3 IN RAT PROSTATE FOLLOWING CASTRATION, Mario A. Anzano, Joseph M. Smith, Bryan K. McCune, David Danielpour and Michael B. Sporn, Laboratory of Chemoprevention, NCI, Bethesda, MD 20892

Transforming growth factor-ßs (TGF-ßs) are biologically active molecules that modulate cellular growth. Castration of 3 month old Lobund/Wistar rats causes progressive atrophy of the prostate with time. Following castration, expression of TGF-ß1 message (Kyprianou, N. and Isaacs, J., Mol. Endocrinology, 3, 1515-1522, 1989) and TGF-ß receptors (Kyprianou et. al., Endocrinology, 123, 2124-2131, 1988) is induced in rat ventral prostate. Expression of various TGF-ß subtypes was analyzed by northern hybridization using c-DNA probes of rat TGF-ß1 and mouse TGF-ß2 and ß3 and by immunocytochemical staining of histological sections using TGF-ß1, ß2 and ß3 specific antibodies in different regions of the rat prostate (lateral, ventral and dorsal) at various times following castration (0, 3, 6 and 9 days). Marked elevation of TGF-ß1 and ß3 but not ß2 message was observed at day 3, 6 and 9 following castration in lateral and ventral lobes with minor changes in the dorsal region. Immunocytochemical staining showed localization of TGF-ß1 mainly in the acinar cells and in blood vessels. TGF-ß3 is localized in basal area and very prominent in cells undergoing apoptosis. Our results suggest that TGF-ß1 and ß3 are are involved in programmed cell death in rat prostate following castration.

CC 402 A TRANSFORMING GROWTH FACTOR ß 1-INDUCED GENE ENCODES A NOVEL DNA-BINDING PROTEIN WITH ZINC FINGER STRUCTURE, J. Albert Fernandez-Pol, Dennis J. Klos, Paul D. Hamilton, and Vera M. Schuette, Laboratory of Molecular Oncology, DVA Medical Center, and Department of Medicine, St. Louis University, St. Louis, MO 63106

Transforming growth factor ß 1 (TGFß 1) is a member of a family of polypeptides that regulate cellular growth and differentiation. A human mammary carcinoma cell line that is partially responsive to the antiproliferative effect of TGFß 1 was examined for differential gene expression patterns that may be related to the mechanism of action of TGFß 1. Differential hybridization was used to screen a complementary DNA library constructed from mammary carcinoma MDA-468 cells treated with TGFß 1 and cycloheximide. One of the complementary DNA clones that was induced by TGFß 1 was found to have a nucleotide sequence that predicts a 10-kDal protein with homology to transcriptional regulatory proteins. This clone, MPS-1, contains one "zinc finger" domain similar to those present in *Xenopus laevis* TFIIIA and other DNA-binding proteins. The mRNA for MPS-1 was induced in MDA-468 cells by TGFß 1 in the presence of epidermal growth factor or retinoic acid. The MPS-1 gene is expressed at relatively high levels in several human carcinoma cell lines, particularly those derived from ectodermal layers, and at higher levels in melanomas (ontogenically of neural origin). In contrast, the MPS-1 mRNA is expressed at low levels in normal cells such as Wi-38 human lung diploid fibroblasts in culture. Additional data indicate that the MPS-1 protein may play a role in transcriptional regulation and also as a potentially important mediator of cellular proliferative responses to various growth factors and other environmental signals.

CC 403 INCREASED BONE RESORPTION BY FACTOR(S) PRODUCED BY THE S115 MOUSE MAMMARY TUMOR CELLS, Pirkko L. Härkönen, Ritva Hannuniemi and H. Kalervo Väänänen, Department of Anatomy, University of Turku, Leiras Biomedical Research Center, Turku, Department of Anatomy, University of Oulu, Finland

We have studied the ability of the androgen- and glucocorticoid regulated Shionogi 115 mouse mammary tumor cells (S115 cells) to stimulate bone resorption. A mouse calvarial resorption assay was used to estimate the bone resorption activity of the factor(s) produced by the S115 cells to the culture medium.

The S115 cells grown to near confluency in the DMEM containing 2 % fetal calf serum and 10 nM testosterone (T) were conditioned in serum-free DMEM in the presence of T for 24 h. The centrifuged and filtered conditioned medium (CM) was incubated with precultured and ^{45}Ca-prelabelled bone explants for 72 h in different combinations with the CMRL1066 medium. The addition of CM caused an up to 4-fold increase to the basal resorption rate in a concentration dependent manner. The resorption activity of CM was not abolished by heating. The time course studies showed that the stimulation of bone resorption by CM was slower than that by PTH, which suggests different modes of CM and PTH action. PTHrP immunoreactivity could not be shown by a specific radioimmunoassay of the S115 CM. The addition of anti PTHrP antibody or a PTHrP antagonist (Asn^{10}, Leu^{11}-PTHrP 7-34 amide) to calvarial cultures failed to block the effects which also suggested that other factors than PTHrP alone may be involved in the stimulation of bone resorption by the S115 CM. The inclusion of 1 µM indomethacin in the culture medium of S115 cells or in the resorption assay did not affect the production or action of the stimulatory activity of CM. The addition of calcitonin or an osteoclast inhibitor clodronate (100 µM) inhibited the resorption activity.

The results show that S115 mouse mammary tumor cells stimulate bone resorption by secreting factor(s) which influence osteoclasts by a mechanism which is independent of prostaglandin production.

CC 404 DEVELOPMENT, CHARACTERIZATION, AND APPLICATIONS OF MULTIPLE MONOCLONAL ANTIBODIES SPECIFIC FOR THE ECTODOMAIN OF C-ERBB-2. Maria Hellman, Uri Saragovi, Valerie Brown, Mark I. Greene, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Amplification of c-erbB-2 (a 185kDa Type I hormone receptor) occurs in high frequency in some human adenocarcinomas and has been shown to be involved in the transformation of NIH/3T3 fibroblast cells. Our laboratory is interested in the effects of specific antibodies on the transforming abilities of this protein. We have previously shown that mAb specific for ectodomains of p185neu (the rat homologue of c-erbB-2) can lead to reversion of the malignant phenotype of p185-transformed mouse cells *in vivo* and *in vitro*.

In order to develop similar therapeutic agents in the human system, we have developed a panel of 16 ectodomain specific c-erbB-2 monoclonal antibodies. Each Ab binds LTR-1/erB-2 cells (a high expressor of c-erbB-2) in flow cytometric analysis and is able to precipitate the protein. 50% of the Abs are useful in western blotting and the majority stain SKBRIII and PANC-1 cells (human adenocarcinomas which overexpress c-erbB-2) in immunohistochemistry. The Abs show a range of ability to inhibit the *in vitro* proliferation of LTR-1/erbB-2 and PANC-1 cells, and certain pairs of antibodies appear to act synergistically. Epitope mapping and the *in vivo* effects of these Abs on tumors of c-erbB-2 expressing human adenocarcinoma cells lines in nude mice are currently being evaluated.

This panel of antibodies promises to be useful in the quantitation and recognition of c-erbB-2 expression in human breast and other adenocarcinomas, in the elucidation of the mechanisms of transformation by c-erbB-2, and in the determination of the the macromolecular structure of this receptor.

CC 405 EFFECTS OF SURAMIN ON GROWTH FACTOR-INDUCED SIGNAL TRANSDUCTION PATHWAYS IN HUMAN PROSTATE CARCINOMA CELLS. JONES, J.A., VLAHOS, C.J., STAMM, N., HIRSCH, K.S. DEPARTMENT OF UROLOGY, INDIANA UNIVERSITY SCHOOL OF MEDICINE, INDIANAPOLIS, IN 46202; LILLY RESEARCH LABORATORIES, ELI LILLY AND COMPANY, INDIANAPOLIS, IN, 46285. The capacity of suramin to inhibit the mitogenic effects of polypeptide growth factors (GF) is well documented. The proposed mechanism of action appears to involve the antagonism and/or displacement of GF from their cell surface receptors. This activity has led to the evaluation of suramin in clinical trials involving patients with advanced prostate cancer. In the present study, the effects of suramin were examined on the growth and signal transduction pathways induced by GF using the human prostatic carcinoma cell line DU-145. Basal [^{3}H]-thymidine incorporation was relatively high in unstimulated DU-145 cells. The addition of PDGF, FGF, and EGF produced maximal increases of thymidine incorporation of 11% (20 ng/ml), 60% (25 ng/ml), and 31% (5 ng/ml), respectively. The limited responses to the GF is probably due to the already high rate of DNA synthesis observed in the unstimulated cells. Suramin caused a 35% decrease in the GF-induced DNA synthesis. Phosphatidyl inositol-kinase (PIK) activity was seen in unstimulated cells, and was not significantly increased following exposure to GF. HPLC analysis revealed low levels of PI-3'-P relative to PI-4'-P. Suramin produced only modest reductions in the levels of PIK products. Whereas, PDGF exposure did not induce the phosphorylation of the 175KDa PDGF receptor, it did cause a 2-7 fold increases in the phosphorylation of proteins with molecular weights of 145 (PLC-γ), 123 (GAP), 76, 95, and 60 KDa. FGF resulted in a 3-7 fold increased phosphorylation of the 142 KDa FGF receptor along with proteins of 95 and 75 KDa. Modest increases in the phosphorylation of 125(GAP) and 56KDa proteins were also seen. EGF produced a 75-fold increase in the phosphorylation of the 177KDa EGF receptor along with 3-10 fold increases in the phosphorylation of 205, 96, 85(PI-3'-K), and 78 KDa proteins. Identification of the remaining phosphoproteins is in progress. 316mM Suramin attenuated the GF-induced protein phosphorylation to levels at or below that observed in unstimulated cells. These studies suggest that unstimulated DU-145 cells are rapidly dividing and that PIK is constitutively activated. Exposure of the cells to PDGF, FGF, or EGF resulted in the phosphorylation of a number of proteins and cell proliferation. These GF-induced effects can be inhibited by exposure of the cells to suramin.

CC 406 ROUTINE IMAGING STUDIES FOR THE POST-TREATMENT SURVEILLANCE OF THE ASYMPTOMATIC PATIENT WITH CANCER OF THE BREAST AND PROSTATE, A. Robert Kagan and Richard J. Steckel, Department of Radiation Oncology, Southern California Permanente Medical Group, Los Angeles, CA 90027 and UCLA-Jonsson Comprehensive Cancer Center, Los Angeles, CA 90024.

Recommendations for 2 to 3 month followup visits enriched with multiple tests after treatment for localized adenocarcinoma of the breast and prostate are easily made by most oncologists. Although costly, post-treatment imaging and blood tests in the asymptomatic patient are often unproductive: oncologic emergencies are not prevented, and recurrences are discovered by the patients between visits. False negative tests lead to a sense of false security for the patient and false-positive tests lead to aggressive treatment of nonexistant disease.

The occasions where periodic testing in the asymptomatic patient are indicated are minimal, the detection of a second primary in the contralateral breast being one of them. Other examples will be given.

1) A. R. Kagan, R.J. Steckel, Routine Imaging Studies for the Posttreatment Surveillance of Breast and Colorectal Carcinoma, J. of Clin. Oncol. Vol 9, No. 5 (May), 1991: pp 837-842.

2) R.J. Steckel, A. Robert Kagan, Pitfalls in the Diagnosis of Metastatic Disease or Local Tumor Extension with Modern Imaging Techniques, Invest. Radiol., 1990; 25: 818-823.

CC 407 INSULIN-LIKE GROWTH FACTOR MODULATION THROUGH A SOMATOSTATIN ANALOGUE IN PATIENTS WITH HORMONE REFRACTORY PROSTATE CANCER (HRCaP), Philip W. Kantoff and Thomas Lynch, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115

Prostate cancer is the most common malignancy in men in the United States. Standard therapy for patients with metastatic disease consists of androgen deprivation. Once this treatment fails, further therapy is usually inadequate and the clinical course is brief and marked by severe disability. The mechanism by which prostate cancer cells grow during androgen deprivation as is seen in the hormone refractory phase is unknown. The extent to which in vivo growth during this phase is governed by autocrine and/or paracrine factors is unknown. We are currently conducting a phase I study using a somatostatin analogue somatuline in patients with prostate cancer in an effort to establish at a dose capable of suppressing serum IGF levels in patients and determining if by so doing, whether inhibition of prostate cancer will occur. Patients with HRCaP have been entered into this Phase I study in which somatuline has been administered over an 8 week subcutaneous constant infusion beginning at a dose of 6 mg/week. Patients have been monitored for toxicity, IGF and growth hormone levels and tumor response. Results of this study will be presented.

CC 408 EXPRESSION OF ERBB2 AND INT2 IN HYPERPLASIA (BPH) AND NEOPLASIA (PCA) OF THE PROSTATE. Jill A. Macoska[1], Wael Sakr[2], Isaac J. Powell[1], and J. Edson Pontes[1], Departments of Urology[1] and Pathology[2], Wayne State University School of Medicine, Detroit, MI 48021.

Few studies to date have attempted to identify consistent molecular genetic alterations in adenocarcinoma of the prostate that could be used as "markers" indicative of events occurring during neoplastic progression. In contrast, markers with potential prognostic and therapeutic utility have been identified in other adenocarcinomas. Some of these potential markers are growth factor-related genes. In human breast carcinomas, DNA amplification of the **ERRB2** (epidermal growth factor receptor-like) gene occurs in 20-30% of tumors examined and RNA overexpression is observed in a subset of those lesions; DNA amplification of the **INT2** (basic fibroblastic growth factor-related) gene is observed in approximately 20% of tumors analyzed. We have recently examined primary prostate carcinomas and hyperplasias for DNA amplification and RNA overexpression of **ERBB2** and **INT2**. These studies were accomplished using differential polymerase chain reaction (**PCR**) techniques, which allow the simultaneous amplification of two gene transcripts in the same reaction, permitting multiple comparisons of transcript levels between specimens. These investigations did not detect DNA amplification or RNA overexpression of **ERBB2** in 6/6 prostatic hyperplasias and 13/13 neoplasias examined. Although DNA amplification of **INT2** was not detected in 4/4 BPH and 9/9 PCAs examined, varied levels of RNA expression between paired BPHs and PCAs and among PCAs were observed. In paired BPH and PCA specimens from the same patients, 3/4 demonstrated more **INT2** expression in malignant than hyperplastic tissue and 1/4 expressed equivalent levels of **INT2** message. One primary tumor from a metastatic carcinoma demonstrated overexpression of **INT2** message compared to hyperplastic tissue from the same patient or neoplastic tissue from others. Further studies are in progress to determine if the observed differences in levels of **INT2** expression in BPH and PCA correlate with and may potentially serve as genetic markers for stages of tumor progression or for clinical outcome.

CC 409 REGULATION OF THE NORMAL VENTRAL PROSTATE (VP) BY GROWTH FACTORS (GF) AND ANDROGENS. Susan Ruth Marengo and Leland W.K. Chung. Urology Research Laboratory, University of Texas M.D. Anderson Cancer Center, Houston TX, 77030

Development, physiology and pathology of the prostate gland is regulated by several mechanisms, including androgen and GF mediated pathways. To determine if epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), insulin-like growth factor-I (IGF-I), platelet derived growth factor-AB (PDGF-AB), and transforming growth factor-β_1 (TGF-β) are able to stimulate the growth of the VP *in vivo*, 0.1 ng of each GF adsorbed on a Matrigel vehicle (GF lobes) was injected into one lobe of the VP of Sprague-Dawley rats. Contralateral lobes were injected with vehicle only (Veh lobes). Rats were sacrificed after 3 wk. EGF (132$\pm$8%; X $\pm$ SEM), bFGF (138$\pm$9%), and IGF-I (120$\pm$6%) increased ($p \leq 0.025$) the wet weight of GF vs Veh lobes. Increased doses (1, 10 ng) of EGF increased ($p \leq 0.05$) the wet weight of GF vs Veh lobes. Injection of 10 ng, but not 1 ng, of bFGF stimulated wet weight of GF vs Veh lobes. There was no difference ($p > 0.05$) among the 3 doses of EGF or bFGF in their ability to stimulate prostate growth. The effects of EGF and bFGF were abolished by neutralizing antibodies. To confirm these results *in vitro*, cell lines derived from the Nb rat VP epithelium (NbE-1.4) and stroma (NbF-1) were cultured for 7 d with 0,0.1,1,10, or 100 ng/ml of each GF. Growth of NbE-1.4 was stimulated ($p \leq 0.05$) by 0.1,1, and 10 ng/ml of EGF and 0.1 and 10 ng/ml of PDGF-AB. None of the doses of bFGF, IGF-I or TGF-β tested affected the growth of NbE-1.4. In contrast, growth of NbF-1 was stimulated by 0.1,1 and 10 ng/ml of EGF, bFGF and by 10 ng/ml of PDGF. IGF-I had no effect on the growth of NbF-1. Although bFGF, PDGF-AB and TGF-β differentially stimulated NbE-1.4 and NbF-1 *in vitro*, there were no detectable changes in the relative ratios of the epithelial and stromal compartments in paraffin slides from GF lobes. Thus, these data suggest a differential autocrine and paracrine mechanism of GF on prostate growth. To determine if the above effects of GF required the presence of androgens to stimulate prostatic growth, rats were castrated at the time of treatment (0.1 ng of GF) and sacrificed 3 wk later. Both IGF-I (160$\pm$22%) and PDGF-AB (161$\pm$16) stimulated ($p \leq 0.05$) wet weight of GF vs Veh lobes, while EGF, bFGF, and TGF-β had no effect. Thus, EGF and bFGF must interact with androgen mediated pathways to stimulate prostatic wet weight, whereas IGF-I's and PDGF-AB's effects appear to be inhibited by androgen mediated pathways. We are presently examining the gene expression in rat VP by RNA blot analysis.

CC 410 GROWTH FACTOR ANALOGUES: A DIFFERENT APPROACH, Neil V.McFerran, Brian Walker, John Nelson, Robert J. Campbell and Clifford Taggart, Division of Biochemistry, School of Biology and Biochemistry, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Rd., Belfast BT9 7BL, N.Ireland, U.K.

The incomplete success of oestrogen analogues such as tamoxifen in the treatment of breast cancer and the finding that expression of the epidermal growth factor receptor and the product of the related oncogene *neu* are both correlated with poor prognosis in this disease, has led to considerable interest in investigating the role of ligands for this receptor, notably EGF and TGF-α, in the disease process.

The fold of EGF and TGF-α show a striking similarity with a clearly defined β-turn-β structure in the B-loop region which has the interesting property of being palindromic both at the primary and secondary structure level. It has been proposed that this loop plays a structural role in the overall fold of EGF.

Analogues of EGF/TGF-α showing high receptor affinity have been exclusively derivatives of the whole molecule, although reports exist of synthetic fragments having mitogenic activity, albeit at considerably higher concentration than the native molecule. Despite extensive investigations at single points in the whole molecule using both site-directed mutagenesis and synthetic approaches no 'core' region has been resolved which governs receptor interaction, rather three key residues have been highlighted by both Campbell and Tam: Tyr/Phe_{13}, Arg_{41} and Leu_{47} (EGF seqence numbering). These are widely dispersed over a 25Åx20Å patch on the surface of the molecule. B-loop fragments showing low level stimulation of cellular proliferation in tissue culture do not fall within this 'patch'.

By the use of cassette mutagenesis we have developed whole molecule analogues which retain the structural elements in the B-loop region to varying degrees: their properties in the context of a structural or functional role for this region will be discussed in this presentation.

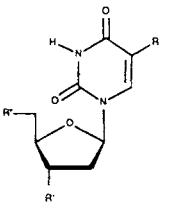
CC 411 INHIBITION OF HUMAN FOETAL THYMIDINE KINASE BY PYRIMIDINE NUCLEOSIDES, Jean-L. Morinière, P. Jouan, Jean-Y. Lacolle, Laboratoires Debat 153, Route de Buzenval 92380 Garches, France.

Two isoenzymes were found in fast growing tissues.

Adult form : TKA and Foetal form : TKF [Stafford AM. et al. Biochem. Biophys.Acta 277 (1972) 439].

TKF is present in all studied breast and prostate cancers [Jouan P. et al, Bull. Cancer 73 (1) (1986) 8].

We determined in vitro inhibition of TKF and TMPK (thymidilate kinase) (from human breast cancer cells MCF7 and prostate cancer cells PC3) by new deoxy-2' uridine derivatives which have the general formula :



R is chosen from an alkyl radical and a radical of formula $NH\text{-}R_1$

in which R_1 denotes an amino acid residue or a peptide residue ; R' and R" are chosen from the same radical in which R_1 has the same meaning as above. We discussed the activity of these new derivatives, which are useful in the treatment of cancers, with an "in vivo" model according to Riffaud J.P. et al, [European Urol. 18 (1) (1990) 282]. In these conditions the most active compounds are the 3'-substituted derivatives.

CC 412 A TYROSINE KINASE INHIBITOR ANTAGONIZES IN VITRO AND IN VIVO GROWTH OF HUMAN BREAST CANCER CELLS, C. Kent Osborne, Kaladhar B. Reddy, Gina Mangold, Daniel R. Ciocca, Asher Zilberstein, Department of Medicine, University of Texas Health Science Center, San Antonio, TX 78284, and Rohne-Poulenc Rorer, King of Prussia, PA 19406.
Human breast cancer cell proliferation is regulated by growth factors that bind to receptors with tyrosine kinase (TK) activity that is crucial for signal transduction. To determine if inhibition of receptor TK activity inhibits tumor growth, we studied the effects of RG-13022, a TK inhibitor with some selectivity in vitro for the EGF receptor (EGF-R). Autophosphorylation of the EGF-R by EGF was inhibited by this inhibitor in human breast cancer cells. RG-13022 also inhibited growth of a panel of cell lines in culture. The effect was rapid and reversible at doses < 10 μM. RG-13022 not only inhibited EGF-induced growth, but also growth stimulated by serum, insulin, IGF-I and II, and TGFα. Interestingly, the inhibitor also blocked estrogen-induced autophosphorylation of the EGF-R (presumably mediated via TGFα secretion) and blocked estrogen-induced growth, suggesting that the EGF-R and/or other functioning TK pathways are required for estrogen action. When RG-13022 is administered to nude mice at doses ≥ 200 μg i.p. daily, tumorigenesis of the MDA-231 breast cancer cells is significantly inhibited in a dose-dependent fashion. Doses as high as 600 μg/day for several weeks are non-toxic to mice. Tumor growth inhibition in vivo is accompanied by focal tumor necrosis and by a marked reduction in tumor cell tyrosine phosphorylation determined by immunohistochemical staining with antiphosphotyrosine antibodies. Tyrosine kinase inhibitors have the potential of providing a new strategy for the "endocrine therapy" of breast cancer.

CC 413 THE ROLE OF PROTO-ONCOGENE/INITIATION FACTOR 4E IN CELLULAR PROLIFERATION AND TRANSFORMATION Robert E. Rhoads, Carrie W. Rinker-Schaeffer, Jeremy Graff, and Stephen Zimmer, Departments of Biochemistry and Microbiology and Immunology, University of Kentucky, Lexington, KY 40536.
The rate of cell proliferation is closely linked to the overall rate of protein synthesis as well as the specific translation of certain translationally repressed mRNAs. Control of the translation rate is exerted primarily at the level of initiation, and particularly at the step of mRNA binding to the 40S ribosomal subunit (RSU). The eIF-4 group of initiation factors is responsible for the melting of secondary structure at the 5'-UTR of mRNA and its transfer to the 40S RSU. The least abundant of these factors, eIF-4E, binds the mRNA cap and accompanies the transfer of mRNA to the 40S RSU. eIF-4E is a phosphoprotein whose phosphorylation correlates with rate of cell growth. Stimulation of quiescent cells by a variety of mitogens leads to an increase in eIF-4E phosphorylation.

Previous studies have shown that overexpression of eIF-4E results in cellular transformation. In order to identify the role of eIF-4E in transformation, the regulation of its activity was examined in *ras*-transformed continuous rat embryo fibroblasts (CREF). We found that the rate of eIF-4E phosphate turnover, and therefore activity, is greatly increased in *ras*-transformed CREF. This implied a functional role for eIF-4E in *ras*-transformation. To test this we decreased the level of eIF-4E in *ras*-transformed CREF by antisense RNA expression. This resulted in reversion of the transformed phenotype both *in vitro* and *in vivo*. These results provide strong evidence for the role of translational regulation, via eIF-4E level or activity, in cellular transformation. We are presently screening tumor tissues for altered levels of eIF-4E. Preliminary evidence suggests that there are differences in eIF-4E levels in the tissues examined. These studies provide a system for the examination of the role of eIF-4E in malignancy and tumor progression. (Supported by grants GM20818 from the NIGMS and 3076 from the Council for Tobacco Research - U.S.A., Inc.)

CC 414 **PRESENCE OF p185^{erbB-2} KINASE IN BREAST AND PROSTATE CANCER CELLS AND INHIBITION BY TYRPHOSTIN.** J. Sarup, P. Juniewicz, S. Hoekstra, W. Xie, M. Wentland, and J. Rake. Departments of Oncopharmacology and Medicinal Chemistry, Sterling Research Group, Rensselaer, NY 12144.

The erbB-2 gene product (p185^{erbB-2}), is overexpressed in approximately 30% of breast, ovarian, and other cancers and its overexpression correlates with poor prognosis. In this study, we determined p185^{erbB-2} autophosphorylating activity in membranes of SK-BR-3 cells, which overexpress p185^{erbB-2}, and tested its inhibition by (3,4,5-(OH)$_3$ benzylidene)malononitrile tyrphostin. Parallel studies were done with membranes of A431 cells (for epidermal growth factor receptor or EGF-R kinase) and BALB/c 3T3 cells (for platelet derived growth factor receptor or PDGF-R kinase). All three, p185^{erbB-2}, EGF-R, and PDGF-R, exhibited autophosphorylation activity, in their respective cell membranes, on tyrosine residues as demonstrated by immunoprecipitation with anti-phosphotyrosine and receptor specific monoclonal antibodies. The tyrphostin inhibited autophosphorylation of p185^{erbB-2}, EGF-R, and PDGF-R, with IC_{50} of 25, 70, and 100 μM, respectively. Studies were also conducted to examine expression of EGF-R and p185^{erbB-2} in prostate cancer cells. Relative to A431 cells, high levels of EGF-R expression were observed in the androgen-insensitive and androgen-receptor negative DU-145 and PC-3 cell lines, while moderate expression occurred in the LNCaP line, which is androgen-sensitive and androgen-receptor positive. Relative to SK-BR-3 cells, weak p185^{erbB-2} expression was observed in both DU-145 and LNCaP cells, with no expression observed in PC-3 cells.

CC 415 p53 GENE ANALYSIS OF A BREAST CANCER FAMILY INVOLVING BOTH MALE AND FEMALE MEMBERS, A. Seth[1], R. Metcalf[2], S. D. Kottaridis[3], A. Panayiotakis[1], H. Li[1], D. Thompson[1], P. Kosmidis[4], C. Harris[2] and T. S. Papas[1], [1]Laboratory of Molecular Oncology, National Cancer Institute, Frederick, MD 21702-1201; [2]Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD 20892; [3]Hellenic Anticancer Institute, Athens, Greece; [4]Metaxa Hospital, Athens, Greece.
Deletions or point mutations in the p53 tumor suppressor gene are currently the most common known genetic defects associated with human cancers. Loss of heterozygosity at 17p, the location of the p53 gene, have been estimated to be as high as 70%, whereas the frequency of point mutations in the highly-conserved exons 5,6,7 and 8 is approximately 25%.

Li-Fraumini syndrome consists of families with an inherited susceptibility for multiple cancers, the most common of which is breast cancer. All of the families examined thus far have an inherited p53 mutation. Breast cancer occurs in 8% of women during their lifetime; of these, it has been estimated that 31% are familial.

We have examined a family of breast cancers occurring in both male and female first-degree relatives. The members so far studied have been found to be negative for p53 mutations (in exons 5,6,7 and 8), for loss of heterozygosity, and for overexpression of the p53 protein as analyzed by immunohisto-chemistry. This finding is in contrast to the Li-Fraumini syndrome where breast carcinomas are the most common malignancies and where all of these families exhibit a germline p53 mutation. Thus, the inherited genetic defect in this distinct breast cancer family appears to be different from the p53 mutations associated with the inherited breast cancer in the Li-Fraumini family. We have recently examined several breast tumor cell lines and found that one-fourth of the samples contained mutations at codon 157 in exon 5; currently, we are sequencing exons other than 5,6,7 and 8.

CC 416 CELL PROLIFERATION PATTERNS IN MAMMARY AND UTERINE EPITHELIUM OF SUB-ADULT AND ADULT MICE, Suzanne M. Snedeker, Lillian D. Weng, and Richard P. DiAugustine, Hormones and Cancer Workgroup, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

The cell proliferation stimulated by ovarian steroids may be an important determinant of cancer risk in hormonally responsive tissues, since there is an increased risk of genetic errors occurring during the process of cell division. DNA labeling index (DNA LI) was determined in mammary glands of female 35-day old (n=9) and adult cycling (3 to 8 months old, n=29) CD-1 mice, and was contrasted with each animal's uterine lumenal and glandular epithelium labeling pattern. Vaginal smears were obtained to determine stage and regularity of the estrous cycle. Animals were injected (i.p.) with 5-bromo 2'-deoxyuridine (BrDU) at 50 mg/kg to label nuclei in S-phase, and 2 hours later the fourth mammary gland, a mid-section of the uterine horn and a piece of the duodenum were removed, fixed in ice-cold 4% paraformaldehyde overnight, dehydrated, embedded in paraffin and sectioned. Labeled nuclei were detected immunohistochemically with a rat anti-BrDU monoclonal antibody (1:400, Accurate Laboratories) using the avidin biotin peroxidase method. DNA LI was expressed as the percent of labeled epithelial nuclei. Despite a variable pattern in the labeling of uterine epithelium in the 35-day old animals, all mammary glands examined displayed extensive labeling in the terminal end buds (TEBs) (Mean $\pm$ SEM, 13.1 $\pm$ 0.3). Ductal labeling was comparatively low (1.3 $\pm$ 0.2) and did not appear to be related to the pattern of uterine epithelial labeling in these sub-adult mice. In contrast, mammary ductal labeling in adult animals was negatively correlated with the combined uterine lumenal and glandular epithelial DNA LI ($p<0.05$, $r=-0.426$). Animals (n=7) with the highest uterine DNA LI (40.2 to 70.8) had low mammary ductal DNA LI (< 1.0), while the 4 animals with the highest mammary ductal DNA LI (4.4 to 10.9) had a uterine DNA LI of < 0.5. Several animals with high mammary labeling had thin uteri, and were at the end of estrus or in metestrus. The asynchronous patterns of DNA LI in the uterus and mammary gland suggest that the hormonal control of epithelial proliferation in the mammary gland may be different than that of the uterus in the normal, adult, cycling animal.

CC 417 MUTANT ANALYSIS OF THE STEROID-REGULATED MALIGNANT PHENOTYPE OF THE S115 MOUSE MAMMARY TUMOR CELLS, Eeva M. Valve, Johanna K. Laine and Pirkko L. Härkönen, Institute of Biomedicine, Department of Anatomy, University of Turku, Finland

The S115 mouse mammary tumor cells express a transformed-like phenotype in terms of morphology, rate of proliferation, serum dependence and anchorage independence in the presence of androgens or glucocorticoids. These hormones also induce the expression of mouse mammary tumor virus (MMTV)-related sequences in the S115 cells in which, unlike in other cells known to express MMTV, the major species of RNA is 1.7 kb in size and contains predominantly sequences from the long terminal repeat (LTR) known to contain an open reading frame. Genetic variants of the S115 cells produced by mutation and selection of the mutants for adhesion and anchorage independent growth (Härkönen et al., 1990, Exptl Cell Res 186, 288) were used for the studies on the molecular mechanisms of the steroid regulation of the malignant phenotype. The variants show different hormone responsiveness and the level of MMTV expression. They also differ in morphology and in the organization of the cell-to-cell contacts as well as in their ability to adhere and attach to different matrices, to grow in suspension, to transfrom NIH3T3 cells, to stimulate bone resorption and to promote tumors in nude mice. Two of the mutants are able to invade and metastasize to the lungs in nude mice. In S115 cells, most effects of androgens such as increased rate of cell proliferation, transition to fibroblastic morphology, poor attachment to the matrix and downregulation of syndecan, a putative matrix receptor, can be mimicked by addition of bFGF and reversed by anti bFGF antibody or suramin. The variants show different responsiveness to bFGF and other growth factors. These variants of S115 cells should prove useful for the in vitro and in vivo studies on the mechanisms by which steroid hormones regulate the expression of MMTV and the transformed phenotype in breast cancer cells.

Late Abstracts

INTERACTIONS OF BREAST FIBROBLASTS AND MCF-7 BREAST CARCINOMA CELLS CO-CULTIVATED IN LONG-TERM THREE DIMENSIONAL CULTURE.

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Cell suspensions of MG (mammary gland) fibroblasts and MCF-7 breast carcinoma cells were mixed in different ratios (1 : 10 ; 5 : 10 and 10 : 10), centrifuged and the pellet was cultivated in three dimensional organotypic culture on a semi-solid agar medium. The nodules obtained were cultivated up to 50 days. Immunohistochemical analyses were performed on nodule sections by using monoclonal antibodies to cytokeratin and vimentin specific for epithelial and fibroblastic cells respectively and a specific stain for collagen. At a ratio of 1 fibroblast and 10 carcinoma cells a disperse localization of the fibroblasts was seen within the nodules.This situation did not change at the 7th day of cultivation. Small amounts of collagen are present. At a ratio of 5 : 10, fibroblasts were in the form of clusters disseminated within the nodules at 24 hours while 7 days later they were mainly seen in the center of the nodules surrounded by a large crown of carcinoma cells. The same situation was found when an equal amounts of fibroblasts and carcinoma cells were used. Large amounts of collagen were produced indicating that the fibroblasts remained functionnal. A glandular differentiation of the epithelial cells in the mixed nodules is developping also, especially after long term culture (50 day). These results show that functionnal normal fibroblasts can induce a higher architectural differentiation in tumor nodules.

IMMORTALIZATION AND TRANSFORMATION OF NORMAL BREAST EPITHELIAL CELLS BY SV40 : PHENOTYPIC, KARYOTYPIC AND GENOTYPIC CHARACTERIZATION. A. DEGEORGES, C. GAUVILLE, G. GOUBIN, * B. DUTRILLAUX, PH. BERTHON, F. CALVO. INSTITUT GENETIQUE MOLECULAIRE, PARIS, FRANCE.* INSTITUT CURIE, PARIS, FRANCE.

Normal human mammary epithelial cell (HMEC) cultures originating from 2 mammoplasty reduction surgical samples were transfected with replication-defective simian virus 40 DNA. Two independent cell lines designated as S2T2 and SI3, selected for their increased proliferation potential and lifespan, were propagated for > 22 months in culture. They maintened a near-diploid karyotype with few chromosomal markers such as trisomy 1q (S1T3) and trisomy 8q (S2T2), which are most common in breast cancer *in vivo*. Immortalized S1T3 cells were not tumorigenic, whereas S2T2 cells produced slowly growing tumors in nude mice. One tumor was propagated *in vitro* and the transformed NS2T2 cell line subsequently raised 100% large tumors in the nude mouse. Rearrangement of the SV 40 genome was observed in NS2T2 cells, which was not associated with increased expression of large T antigen, S1T3, S2T2 and transformed NS2T2 cell lines expressed cytokeratins CK18, CK19, the mammary-specific antigen DF3, and functionnal EGF receptors. The most prominent modification observed in the cell lines was an increase of RNA messenger of EGF Rc and TGFα parallel to their increasing tumorigenic potential involving the S2T2 cell line and all its tumorigenic clones. Single step immortalization and malignant transformation of human breast epithelial cells can thus occur upon transfection with SV 40 large T oncogene and therefore can offer a model for the study of breast tumor progression *in vitro*.

IMMUNE RESPONSES IN THE TUMOUR DRAINING AXILLARY NODES OF BREAST CANCER PATIENTS

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Depts of Biochemistry and Surgery (Western Infirmary), University of Glasgow ,G12 8QQ,U.K.

The lymphocyte subsets and activation markers in the axillary draining nodes of breast cancer patients were analysed by two colour flow cytometry with respect to (i) Tumour stage (i.e. the presence of tumour cells in the node) and (2) Distance of the node from the tumour. In general there was a similarity between Stage 1 patients and the uninvaded nodes of Stage 2 patients. However, the comparison of invaded with uninvaded nodes from the same patient showed a change in T cell phenotype with the percentage CD8+ cells increasing in invaded nodes (p=0.002) while the percentage of of CD4+ T cells decreased (p=0.003). In addition, the proportion of mature sIgG+ B cells was increased in invaded nodes(p=0.009).
When analysed by distance from the primary tumour the nodes from Stage 1 patients showed no statistically relevant trends. In contrast, the low axillary nodes (near the tumour) of Stage 2 patients showed several differences from the high axillary nodes (more distant). These differences included elevated levels of several activation markers such as HLA DR on CD4+ cells (p=0.037), CD25 on CD4+ cells (p=0.004), CD25 on CD8+ cells (p=0.009) and sIgG expression on B cells (p=0.023).
These differences with respect to invasion status and distance from the tumour of Stage 2 patients indicate that both B and T cell compartments of the immune response are involved in the control of tumour metastasis.

HUMAN ADULT NORMAL PROSTATIC EPITHELIAL CELLS CAN BE IMMORTALIZED BY LARGE T-SV 40 GENE : CHARACTERIZATION OF THREE DIFFERENT CELL LINES.

O. CUSSENOT, A. DEGEORGES, I. MOWSZOWICZ, F. DESGRANDCHAMPS, A. LE DUC and F. CALVO. Depatment of Urology and Department of Pharmacology, Institute of molecular genetics, Paris, France.

Simian Virus SV 40 has been widely used to immortalize epithelial cells of mammalian origin. We report here the immortalization of normal adult prostatic epithelial cells in culture by transfection of a plasmid containing SV40 genome with a defective replication origin (SV40 ori-) encapsulated into liposomes. Three cell lines were generated from two different normal cultures, PN1A, PNT1B from normal specimen 1 and PNT2 from normal specimen 2. They express large T protein, present the phenotype of differentiated luminal prostatic cells (positive with antibodies to cytokeratin 18, 19, weakly positive for prostatic acid phosphatase and porstatic specific antigen, negative with anticytokeratin 14 and KL2 antibody). They all contain high affinity receptors for dihydrotestosterone and 5 alpha reductase activity is significantly conserved in the 3 cell lines. None of the cell lines were able to raise tumors when injected subcutaneously into the nude mouse. Preliminary experiments showed that: 1°)PNT1A and PNT1B have a C-MYC gene amplification, 2°) the three cell lines have a c-ERB-B2/NEU gene amplification, 3°) in the PNT1B, 5 to10 gene copies of the EGF receptor gene were present as compared to normal cells. These cells thus provide a useful tool to study the biology and the pathology of adult prostatic epithelial cells, specially to understand the steps leading to prostatic transformation.

P120 EXPRESSION IN BREAST CANCER: RELATIONSHIP TO PROGNOSIS AND CELL CYCLE KINETICS

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Monoclonal antibodies have been developed and cDNAs isolated to a nucleolar antigen (P120), that is detected in rapidly proliferating cells, but not in normal resting cells nor in many benign and slow-growing malignant tumors. In breast cancer, P120 expression was associated with a poor patient prognosis (p=0.0001); a combination of positive nodes and P120 expression was a better predictor of prognosis than either parameter alone. Node negative patients whose tumors express P120 had a much poorer prognosis than node negative patients whose tumors were negative for P120 (67% vs. 90%, 5 yr. survival). A correlation was found between P120 immunoreactivity and increasing tumor grade. The regulation of P120 expression was examined in four mammary cell lines: 1 normal cell line (HS578N) and 3 malignant cell lines (HS578T, MCF-7/6, MCF-7 (AZ). P120 expression was not correlated with cell growth rates. P120 protein level was highest in the relatively slow growing cell line, MCF-7 (AZ); however, this cell line was highly invasive in a transwell Matrigel assay. P120 protein was 3 to 8 fold less in the rapidly growing HS578T and MCF-7/6 cells which were non-invasive. Only a trace amount of P120 protein was detected in the HS578 normal mammary cell line. In synchronized cell cultures, P120 protein expression was low in early G_1 and a dramatic increase of P120 protein was observed at S-phase; P120 was not detected in serum deprived (G_0) fibroblasts. Cell cycle progression from G_1 to S-phase was blocked in cells transfected with P120 antisense oligonucleotides. These studies suggest that P120 may be a prognostic marker in breast cancer. While P120 protein level is not related to growth rate; it is related to cell cycle phase and may be associated with other biological properties such as tumor invasiveness.

TRANSCRIPTIONAL CONTROL OF c-*erb*B-2 IN HUMAN BREAST CARCINOMA CELL LINES

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The *erb*B-2 oncogene is overexpressed in 30% of human breast adenocarcinomas. Overexpression has been correlated with a poor prognosis and a poor response to therapy. Several mechanisms may contribute to the increase in *erb*B-2 levels as overexpression is observed in tumours that contain either a single copy of the *erb*B-2 gene or an amplified *erb*B-2 gene.
In this study *erb*B-2 mRNA levels and gene copy number have been examined by Northern Hybridisation, RNA slot blotting, quantitation of *erb*B-2 mRNA half-life and Southern Hybridisation in a series of immortalised mammary epithelial cell lines and human breast carcinoma cell lines. Malignant cell lines which exhibit 4-8 fold *erb*B-2 mRNA overexpression from a single copy gene have been identified. There is no clear difference in the *erb*B-2 mRNA half-life observed in the cell lines suggesting that the rate of transcriptional initiation is important. Consequently, the level of *erb*B-2 transcription was examined by nuclear run-on assays. Comparison of a non-tumourigenic cell line with baseline *erb*B-2 expression and a malignant cell line with *erb*B-2 overexpression has suggested a 3-fold increase in RNA polymerase density on the *erb*B-2 gene in the malignant cell type. Characterisation of the functional elements within the *erb*B-2 promoter in both types of cell line has been performed by short-term transfection of a series of *erb*B-2 promoter-CAT reporter gene constructs. Results from the CAT assays are presently being correlated with DNaseI footprinting studies performed with nuclear extracts from the same breast cell lines. Our initial results suggest the presence of distinct *erb*B-2 promoter binding sites in the malignant cell lines.

Expression of colony-stimulating-factor-1 (CSF-1) and its receptor (*c-fms*) in invasive breast tumour cells. S.M.Scholl,R.Tang,F.Beuvon,C.Palud,R.Lidereau,P.PouillartLaboratoire de Biologie des Tumeurs Mammaires.Institut Curie,Paris. France 75231

Previous results have shown the presence of CSF-1 in breast tumour samples not only at the level of monocytes/macrophages, but also demonstrated a marked positivity on stromally invasive breast tumour cells (1). In contrast, absence of,or very low specific binding of the CSF-1 antibodies to pre-invasive (intraductal) carcinoma was observed. In-situ hybridisation experiments confirm the presence of CSF-1 specific mRNA's at the level of invasive breast tumour cells. CSF-1-receptor (*c-fms*) transcripts in breast tumour homogenates and cell lines have been previously describes (2). Our results, both by immunohistochemical and in-situ hybridisation techniques confirm the expression of *c-fms* at the level of breast tumor cells; this expression is however quantitatively less important in tumour cells as compared to infiltrating monocytes and plasmocytes. We feel that the prognostic value of high levels of *c-fms* transcripts in glandular epithelial carcinomas (3) may be linked to the presence of large amounts of fms-positive inflammatory cells, themselves a reflection of the quantity of CSF-1 produced by the tumour.
An autocrine or intracrine mechanism of CSF-1 in relation to tumour cell invasion will be discussed.

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ASSOCIATION BETWEEN GENETIC ALTERATIONS AND TUMOR HISTOLOGY IN HUMAN BREAST CANCER. Marc J. van de Vijver, Johannes L. Peterse', Ed Schuuring, Peter Devilee and Cees Cornelisse.
Dept. of Pathology, State University of Leiden, Postbus 9603, 2300 RC Leiden; ' Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Several gene alterations have been found in primary breast carcinomas: amplification of the neu (also known as c-erbB-2 or HER-2), c-myc and int-2 genes; and inactivation of several loci, including the Rb and p53 genes. The purpose of our work is to find a model for the development of breast cancer from a normal epithelial cell and to identify the genetic alterations and the order in which they occur. It is likely that there are several different types of breast cancer, each having specific precursor lesions. We have therefore analysed invasive and intraductal carcinomas for amplification of the neu, c-myc and int-2 genes; and for loss of heterozygosity (loh) of several alleles on each chromosome, looking for association with histologic type. In ductal carcinoma in situ (DCIS), tumors with large, pleomorphic tumor cell nuclei have overexpression of the neu protein in the majority of cases. Neu over-expression was the result of neu gene amplfication in 6 cases studied. Neu protein overexpression was never found in DCIS with small, monomorphic nuclei. In invasive carcinomas, neu overexpression was associated with a large component of large cell DCIS. Amplification of int-2 was not found in DCIS; in invasive carcinomas there was association with estrogen receptor positivity. There was no clear association of int-2 amplification with histologic type. Amplification of c-myc was not found in DCIS and was not associated with histologic type in invasive carcinomas. Work on association of loh with tumor histology is still in progress.