BIOLOGY OF AND NOVEL THERAPEUTIC APPROACHES FOR EPITHELIAL CANCERS OF THE AERODIGESTIVE TRACT

Organizers: Jack Roth, Waun Ki Hong and James Mulshine

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Biology of and Novel Therapeutic Approaches for Epithelial Cancers of the Aerodigestive Tract

Genetic Mechanisms (Session sponsored by Abbott Laboratories)

CE 001 ONCOGENES AND DIFFERENTIATION EVENTS IN LUNG CANCER, Stephen B. Baylin, Barry D. Nelkin, Linda F. Barr, Joseph P. Falco and Mack Mabry, Oncology Center and Department of Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21231.
The initial phenotype of each major form of lung cancer, and transitions between these neoplasms during tumor progression, probably reflect interaction of the consistent genetic abnormalities described in lung cancers with normal maturation events in the bronchial epithelium. To learn more about these relationships, our lab group has developed cell culture models for small cell lung cancer (SCLC) which involve insertion of genes known to be altered in various stages and/or types of lung cancer. We have learned that one tumor progression event of potential clinical importance, the transition from the small cell (SCLC) to the non-small cell (NSCLC) lung cancer phenotype, can be obtained by inserting the viral Harvey ras oncogene (v-Ha-ras) into SCLC cells having either endogenous c- or N-myc amplifications, or expressing an exogenous human c-myc gene. These manipulations result in transition from SCLC to a large cell undifferentiated carcinoma phenotype. This change is accompanied by a decrease in gene expression events typical for the neuroendocrine characteristics of SCLC (including an autocrine loop involving gastric releasing peptide and its receptor) and acquisition of growth factor gene expression typical of NSCLC but not SCLC lung tumors (TGF-α, EGF-receptor and PDGF). The complementation events for v-Ha-ras and myc genes in the above transition appear to involve a myc gene induced increase in PKC-ß gene expression. In one particular line of SCLC, we have found that the v-Ha-ras gene actually increases parameters of endocrine differentiation and slows growth. In these cells, insertion of a P53 gene mutation interferes with the cell signals that mediate v-Ha-ras induced neuroendocrine differentiation. From these above data, a working model is presented which depicts how genes known to be altered in lung cancer might guide normal maturation events in bronchial mucosa—and how alterations in these genes may cause specific initiation and tumor progression events for the major forms of lung carcinomas.

CE 002 COMPARATIVE STUDIES OF TUMOR SUPPRESSION BY THE HUMAN RB AND p53 GENES, Phang-Lang Chen, Yumay Chen, Robert Bookstein, Hoang To, Eva Lee, Wen-Hwa Lee, Department of Pathology, University of California at San Diego, La Jolla, CA 92093-0612
Tumor suppressor genes are defined as genes for which loss-of-function mutations are oncogenic. Wild-type alleles of such genes may thus function to prevent or suppress oncogenesis. Both alleles of the retinoblastoma susceptibility gene (RB), the prototype of this class, are mutated in all retinoblastomas and in a subset of osteosarcomas, soft-tissue sarcomas, and carcinomas of lung, breast, bladder and prostate. Mutations of the gene encoding p53, a 53 kD cellular protein, are found frequently in osteosarcomas, rhabdomyosarcomas, and carcinomas of lung, colon, and breast. We have used retrovirus-mediated gene transfer to introduce single copies of wild-type RB into human tumor cells with mutated endogenous RB alleles (RB^- cells). Restoration of wild-type RB expression in retinoblastoma cells and RB^- osteosarcoma, breast carcinoma, and prostate carcinoma cells demonstrably suppressed their tumorigenicity in nude mice, but had no effect on osteosarcoma cells with wild-type endogenous RB. These experiments provided direct evidence for tumor suppression by a single gene. To test whether wild-type p53 could suppress in a similar fashion, we again used retrovirus-mediated gene transfer to introduce single copies of point-mutated and/or wild-type p53 into a human osteosarcoma cell line, Saos-2, that completely lacked endogenous p53. Expression of wild-type p53 suppressed the tumorigenicity of Saos-2 cells, whereas expression of mutated p53 conferred a growth advantage to cells in culture. Saos-2 cells coexpressing both wild-type and mutated p53 behaved identically to those expressing only wild-type p53, i.e., wild-type p53 was phenotypically dominant to mutated p53 at equal gene dosage. These studies suggest that, as with RB, mutation of both p53 alleles is required for an oncogenic effect, and that replacement of either tumor suppressor gene product can signficantly modify the neoplastic phenotype of appropriate tumor cells.

Biology of and Novel Therapeutic Approaches for Epithelial Cancers of the Aerodigestive Tract

CE 003 THE *ras* ONCOGENE FAMILY AND LUNG CANCER.

Sjoerd Rodenhuis, Robert J.C. Slebos, Siegina G. Evers, Otilia Dalesio, Nico van Zandwijk, Wolter J. Mooi, Departments of Experimental Therapy, Clinical Pathology and Biostatistics, The Netherlands Cancer Institute, Plesmanlaan 121, NL-1066 CX Amsterdam, The Netherlands.

The three *ras* genes H-*ras* , K-*ras* and N-*ras* encode 21 kD proteins with GTP binding activity and are presumably involved in signal transduction. Alterations in the protein leading to diminished intrinsic GTPase activity have been shown to induce malignant transformation of cell lines *in vitro* and have been associated with a variety of human neoplasms. Normal *ras* genes become activated oncogenes when they acquire a point mutation in one of the critical codons 12, 13 or 61. We have previously shown that the K-*ras* gene is activated in about 30% of adenocarcinomas of the lung, but rarely in other types of lung cancer (1). Mutation-positive tumors are not microscopically distinguishable from mutation-negative ones, but they are associated with a very poor prognosis in patients who underwent apparently radical resections of their tumors (2). Only few of the patients with lung adenocarcinoma of the lung whose tumors were investigated had never smoked. In none of their tumors were *ras* mutations could be detected, suggesting that (in analogy to animal experiments) a carcinogenic ingredient of tobacco smoke might be directly responsible for the mutation.

In collaboration with Drs. Hruban and Offerhaus (Johns Hopkins Hospital, Baltimore) we have now examined 27 tumors of non-smokers and compared the results with those of 27 tumor samples of patients who did smoke. These data essentially confirm the association between smoking and K-*ras* mutation, although 2 non-smokers with mutations were now identified. *Ras* mutations are found in only a minority of patients with lung cancer. One obvious explanation might be that other genetic alterations have similar effects in malignant transformation. Prime candidates for such a role are the proteins belonging to the superfamily of monomeric GTP-binding proteins. One of these, Krev, has been reported to actually have tumor-suppressor activity in K-*ras* transformed cells. We have investigated 42 lung tumor samples for expression and possible sequence alterations of this gene, employing the polymerase chain reaction and SSCP (Single Strand Conformation Polymorphism) analysis. Krev is transcriptionally active in nearly all lung tumors but no point mutations were detected with this technique. Because of reports linking *ras* oncogenes with resistance against cytotoxic drugs and irradiation, a clinical study was initiated to evaluate the value of *ras* gene mutation assays in response-prediction to chemotherapy. The number of patients enrolled is too low to be evaluated, but at least one excellent response has been noted in a K-*ras* positive adenocarcinoma patient.

1. Rodenhuis et al., New Engl J Med 1987, 317: 929-935.
2. Slebos et al., New Engl J Med 1990, 323: 561-565.

Clinical Aspects of Molecular Genetics
(Session sponsored by Bristol-Meyers Oncology Division)

CE 004 Jun and Fos Interactions in Early Cancer

Michael J. Birrer, Powel H. Brown, Lisa H. Preis, Dennis A. Sanders, Eva Szabo, and Rhoda Alani

Careful analysis of the pathology of human malignancies and data obtained from in vitro and in vivo models of tumorgenesis suggest that many epthelial neoplasms develop through a series of discrete stages. Tumor promotion is certainly one of the earliest and most important events in this neoplastic process. In the aerodigestive tract, tumor promotors have yet to be well defined, but most likely include growth factors such as gastrin releasing peptide and a wide range of substances found in tobacco smoke. Using a variety of growth factors for lung cancer cell lines and known tumor promotors we examined the response patterns of 'early response' genes in both lung cancer cell lines and primary epithelial cells. Multiple different growth factors showed similar patterns of induction of these 'early response genes' in different cells. Of note, the cells most dependent on growth factors showed the greatest induction of these genes. Differences and similarities among cell lines and between malignant and primary cells will be discussed. This data suggests that transcription factors such as Jun and Fos play an important role in mediating cell responses to factors thought to be tumor promotors. Therefore, we have examined the mechanisms by which these genes mediate these biologic effects. We have used an in vitro model system involving the ability of phorbol esters (TPA) to cooperate with an activated ras gene to transform rat embryo cells. This cotransforming activity of TPA can be replaced by deregulated expression of the c-jun gene. Further, deletion analysis of the cJun has shown that this cotransforming activity is depended on regions of the protein required for protein dimerization, DNA binding and transactivation activities. This suggests that c-jun (and probably TPA) cotransforming activity requires the transactivation of AP-1 responsive downstream genes. Deletion mutants have been designed which can block the biologic effects of c-jun in this assay and therefore function as 'dominant negative' mutants. These dominant negative mutants can be used to dissect and ultimately inhibit jun-mediated and phorbol ester pathways. Finally, the application of these assays to the discovery of relevant epithelial tumor promotors and agents able to block their effects (chemopreventive agents) will be discussed.

CE 005 **myc FAMILY DNA AMPLIFICATION IN 107 TUMORS AND TUMOR CELL LINES FROM PATIENTS WITH SMALL CELL LUNG CANCER TREATED WITH DIFFERENT COMBINATION CHEMOTHERAPY REGIMENS**, Bruce E. Johnson, Theresa O'Connor, Robert W. Makuch, Edward Russell, R. Ilona Linnoila, Adi F. Gazdar, Daniel C. Ihde, and John Brennan, National Cancer Institute-Navy Medical Oncology Branch, National Naval Medical Center, Uniformed Services University of the Health Sciences, Bethesda, MD 20889-5105; and the Division of Biostatistics, Yale University, New Haven, CT 06510

We studied 107 specimens (38 tumors and 69 tumor cell lines) from 90 patients with small cell lung cancer to determine the characteristics and clinical situations of patients from whom tumor cell lines could be established and the myc family DNA copy number. The proportion of extensive stage small cell lung cancer patients from whom a tumor cell line could be established prior to the initiation of therapy increased during the 10 years (1977-1986) of the study ($p < 0.001$). This coincided with the introduction in 1983 of an extensive stage small cell lung cancer study which included systematic biopsy of tumors prior to the initiation of chemotherapy treatment. Ten of 119 (8%) untreated patients with extensive stage small cell lung cancer had tumor cell lines established from 1977 to 1982 compared to 24 of 78 (31%) patients after the start of 1983 ($p < 0.001$). Amplification of one of the myc family genes occurred in 3/40 (8%) of the untreated patient specimens compared to 19/67 (28%) of the treated patient specimens ($p = 0.01$). Chemotherapy treated patients whose cell lines had DNA amplification of c-myc lived a shorter time than patients whose cell lines did not ($p = 0.002$). The myc family DNA amplification occurred in 17/54 (31%) of the specimens from patients treated with cyclophosphamide based combinations and 2/13 (15%) of the specimens from patients treated with etoposide and cisplatin ($p = 0.25$). Both tumors and tumor cell lines were obtained from 17 patients with small cell lung cancer and the myc family DNA copy number was similar in 16 of the 17 patients. We conclude that: 1). myc family DNA amplification occurs more commonly in specimens from treated than untreated patients, 2). there are no prominent differences in the frequency of amplification following treatment with different chemotherapy regimens, 3). myc family DNA amplification is similar in tumors and tumor cell lines from the same patients.

CE 006 MOLECULAR APPROACHES TO LUNG CANCER THERAPY, Jack A. Roth, Tapas Mukhopadhyay, Nancy Yen, Elizabeth Putnam, Alan Casson, Departments of Thoracic Surgery and Tumor Biology, M. D. Anderson Cancer Center, Houston, TX 77030. Molecular mechanisms possibly contributing to the development and progression of human non-small cell lung cancer (NSCLC) include expression of cell surface receptor-like tyrosine kinases, autocrine and paracrine growth loops, activation of dominant oncogenes, and loss of tumor suppressor genes. ERBB1 (EGFR) and ERBB2 (neu/HER-2) are highly expressed by NSCLC cell lines and fresh tumors but not SCLC. Fresh NSCLC show high mRNA expression of ERBB2 compared to normal lung. Four cell lines representing the major NSCLC types expressed EGFR by Scatchard and phosphorylation in immune complex kinase assays, expressed TGF-α mRNA, and showed increased soft agar colony formation and [^{3}H]Thd uptake in response to TGF-α suggesting an autocrine loop. Anti-TGF-α moab AB-3 inhibited growth of 2 cell lines at low density. Two cell lines not inhibited by AB-3 were growth inhibited by suramin. TGF-α specifically reversed AB-3 and suramin inhibition suggesting extra-cellular and intracytoplasmic ligand-receptor binding mechansims. Mutations occur in the dominant oncogene K-ras in a poor prognostic subset of adenocarcinomas. Human NSCLC cell line H460a with a homozygous spontaneous K-ras mutation was transfected with a recombinant plasmid synthesizing a 2kb genomic segment of K-ras in antisense (AS) orientation. Expression of K-ras mRNA and p21 was specifically inhibited without changes in H-ras and K-ras expression. AS cells showed inhibition of growth in culture and tumorigenicity in nu/nu mice compared to H460a. This technique may be useful for specfic suppression of dominant oncogene expression. Mutations in the p53 oncogene occur in NSCLC. We detected these using PCR amplified DNA with single strand conformational polymorphism analysis (SSCP). Mobilty shifts of tumor DNA compared to normal lung in 12% polyacrylamide gels correlated well with mutations shown by direct DNA sequence analysis. Sense and AS p53 constructs were transfected into NSCLC cell lines with or without homozygous endogenous p53 mutations. Stable clones could not be rescued from cells with mutant alleles indicating absense of p53 expression is not sufficient for transformation, and the presence of mutant p53 is necessary for tumor cell growth. We identified the presence of p53 mutations in Barrett's epithelium suggesting that p53 may be an important marker of premalignant change in epithelial cancers.

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Growth Factors and Growth Regulation

(Session sponsored by Bristol-Meyers Oncology Division)

CE 007 MECHANISMS OF GROWTH, DIFFERENTIATION AND TOBACCO-RELATED PATHOLOGY IN CULTURED HUMAN ORAL EPITHELIUM, Roland C. Grafström, Kristina Sundqvist, Yun Liu and Prabha Kulkarni, Department of Toxicology, Karolinska Institutet, S-104 01 Stockholm, Sweden.

Human buccal epithelial cell cultures, which have been derived as explant outgrowths or following enzymatic dispersal of tissue, can be grown and transferred in serum-free MCDB 153 medium containing defined mitogens. Cultured cells express keratins and typical structural features, exhibit up to 40% colony-forming efficiency and divide at about 1 population doubling (PD) per day on fibronectin/collagen-coated dishes for about 1 month. Cells commonly undergo 50 PD during 2 months, although certain cells may divide for up to 4 months undergoing more than 65 PD. Modulation of the growth of buccal epithelial cells by various agents indicate that epidermal growth factor, cholera toxin, retinoic acid, and human and bovine pituitary extracts enhance growth, that transforming growth factor β-1 (TGF-β) inhibits growth, whereas fetal bovine serum (FBS), the tumor promoting agent 12-O-tetradecanoylphorbol-13-acetate or an elevated Ca^{2+} concentration (from 0.1 to 1 mM) induces squamous differentiation as indicated by measurements of growth rate, colony forming efficiency, cell surface area, migratory activity, involucrin expression and formation of cross-linked envelopes. Transfection and expression of SV40 T antigen causes an extended life span of buccal epithelial cells, and moreover, on two occasions seem to have immortalized cells that survived the crisis that followed their extended life span in culture. Karyotype analysis shows that normal cells are diploid, whereas SV40 T antigen-transfected cells from both pre- and post-crisis cultures, as well as buccal squamous carcinoma (SqCC/Y1) cells, all show an increase in total number of chromosomes (>60) per metaphase. SqCC/Y1 cells, which regularily grow in serum-dependent conditions, exhibit markedly higher colony forming efficiency and growth rate when transferred to serum-free MCDB medium, and moreover, are resistent to growth inhibition by TGF-β, elevated Ca^{2+} and FBS in these conditions. These agents seem to variably affect growth of different SV40 T antigen-transfected cell populations. The tobacco-specific carcinogen 4-(metylnitrosoamino)-1-(3-pyridyl)-1-butanone is actively converted to electrophilic metabolites in buccal explant and epithelial cell cultures as indicated by microautoradiographic analysis and metabolism studies. Moreover, normal cells are more sensitive to the toxicity of tobacco extracts than transformed cells. The development of defined culture conditions now permit carcinogenesis studies in tissue and both normal and transformed epithelial cells from human buccal mucosa.

CE 008 **LUNG COLONIZATION BY METASTATIC TUMOR CELLS IS DETERMINED BY THE PROPERTIES OF UNIQUE TUMOR CELLS AND LUNG-ASSOCIATED CYTOKINES.** Garth L. Nicolson and Philip G. Cavanaugh, Department of Tumor Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

The lung is a common site of metastatic involvement, and this is probably due to nonspecific (mechanical) and specific (nonmechanical) properties of tumor cells. We have been interested in the specific distributions of metastases that are not determined by anatomical or mechanical considerations. The tumor cell properties that appear to be important in the nonrandom spread of cancer are their expression of organ-associated endothelial cell and basement membrane adhesion components, their responses to organ-associated tumor cell motility factors, and their ability to respond to soluble and insoluble organ-associated growth factors and inhibitors. We have found that highly metastatic tumor cells differentially respond to organ-derived, soluble and insoluble (particulate-bound) paracrine growth factors that are present in dissimilar amounts in different organs. One of the most potent of these paracrine growth factors for lung-colonizing tumor cells has been purified to homogeneity from lung tissue-conditioned medium and shown to be a M_r ~66K transferrin-related glycoprotein. This cytokine has been isolated from rodent and porcine lungs and binds to a specific M_r ~180K receptor on lung-metastasizing tumor cells. Lung-metastatic and brain-metastatic tumor cells expressed the highest numbers of the transferrin receptor and responded the best to the lung-derived transferrin-like growth factor. The source of the transferrin-related mitogen was found to be mainly lung microvascular endothelial cells. Other lung-derived host cells also release soluble mitogens that differentially stimulate the growth of lung-metastasizing tumor cells. For example, the conditioned medium from lung-derived fibrobalsts stimulated the growth of lung-metastasizing tumor cells better than conditioned medium from fibroblasts from other tissues. High M_r particulate-bound growth factors can also differentially stimulate metastatic cell growth. We investigated the mitogenic activity for lung-metastasizing tumor cells of detergent-solubilized components from a lung tissue particulate fraction. Rat lungs were homogenized in buffer, filtered, centrifuged, and the pellet was suspended in buffered 0.5 M KCl, recentrifuged, suspended in buffered CHAPS, and centrifuged again. The resulting supernatant contained mitogenic activity for lung-metastasizing rat and human mammary carcinoma cells. The mitogenic activity was eluted from QAE Sepharose with high salt (>1 M NaCl, pH 8.5), and it migrated as a M_r >200,000 component(s) on gel filtration chromatography. Removal of the CHAPS detergent by dialysis resulted in loss of solubility of the mitogenic activity. SDS-PAGE demonstrated 3 major bands of M_r ~200,000, ~115,000 and ~95,000. Differential expression of organ particulate and soluble growth factors may determine, in part, the ability of organ-colonizing tumor cells to proliferate in target organs for metastasis. These and other tumor cell and host properties may eventually be used to predict and explain the unique metastatic distributions of certain malignancies.

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Clinical Studies of Growth Factors

CE 009 SYSTEMATIC DEVELOPMENT OF BOMBESIN/GRP ANTAGONISTS, David H. Coy and Robert T. Jensen, Peptide Research Laboratories, Department of Medicine, Tulane University Medical Center, New Orleans, LA 70112 and Digestive Diseases Branch, National Institutes of Health, Bethesda, MD 20892

Several families of very potent Bn receptor antagonist analogues have recently been developed and their biological potencies evaluated in a number of *in vitro* systems including guinea pig and rat pancreatic acini and Swiss 3T3 cells. These studies showed that analogues can exhibit diverse properties ranging from full antagonists, partial agonists or full agonists depending on the assay system and animal species employed. We have developed 2 classes of more potent, shorter chain antagonists based on [ψCH_2NH(13-14)]Bn(6-14) and desMet^{14}Bn(6-13)NH_2 structures. In binding studies, ψBn(1-14) and Bn(1-13)NH_2 were 21 and 72-fold less potent than Bn. Whereas a D-Phe^6 residue substituted in either structure did not improve inhibitory potencies, in the shortened ψBn(6-14) and Bn(6-13)NH_2 analogues potencies were increased 5 and 30-fold, respectively. Thus, D-Phe$^6\psi$Bn(6-14) was a potent antagonist (Ki 6 nM) in Swiss 3T3 cells and guinea pig acini, but exhibited 10% partial agonist activity and lower binding affinity (Ki 60 nM) in rat acini. The partial agonism could be eliminated by using p-Cl-Phe or D-Phe at the C-terminus and almost eliminated using D-p-Cl-Phe in position 6. With the antagonist D-Phe^{6}Bn(6-13)NH_2 (Ki 96 nM), alkyl substituents on the amide group increased affinity 25-fold with the propylamide being the most potent peptide (Ki 4 nM) in 3T3 cells or guinea pig acini. It did, however, have high 40% partial agonist activity in rat acini. Alkyl esters or hydrazide derivatives were, in contrast, pure antagonists in all systems tested with D-Phe^{6}Bn(6-13)OMe having the highest affinity in all systems and also excellent *in vivo* properties. All of the potent antagonists examined had little affinity for neuromedin B receptors which have entirely new ligand SAR's. The similar relationship between Bn analogue position 6 and 14 side-chains and agonist activity suggested a possible interaction between these positions in the Bn receptor-bound conformation. With this in mind, cyclic analogues were synthesized with covalent bridges between these positions. D-Cys^6,D-Ala^{11},Cys^{14}Bn(6-14) retained significant binding affinity (Ki 64 nM). This appears to confirm that Bn/GRP adopts a folded configuration in its receptor-bound state and there is now an excellent possibility of developing potent conformationally-restricted Bn agonists and antagonists which would be of value in computer modeling and physicochemical studies. The effects of some of these analogues on tumor cell growth *in vitro* and *in vivo* will be discussed.

CE 010 POTENTIAL CLINICAL APPLICATIONS OF ANTI-EGF RECEPTOR MONOCLONAL ANTIBODIES. John Mendelsohn, M.D. Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, NY 10021

The epidermal growth factor receptor (EGFR) is a potential target for antitumor therapy. Recent studies from many laboratories have found that this receptor is expressed in high levels on a variety of human tumor cells. Furthermore, the EGFR has been implicated in autocrine stimulation of cell growth in a number of experimental studies. We have produced anti-EGFR monoclonal antibodies (MAbs) which block the binding of EGF and TGFα, and can prevent ligand-stimulated activation of EGFR tyrosine kinase. These MAbs have been useful in studies of EGFR function. Experiments utilizing the MAbs to block ligand binding have demonstrated that autocrine stimulation of EGFR phosphorylation can occur via an extracellular pathway, involving TGFα-mediated activation of EGFR on the surface of the cell. The capacity of anti-EGFR MAbs to inhibit cell proliferation has provided evidence for an autocrine stimulatory pathway in cultures of malignant human skin, breast, colon and lung cells. Growth of human tumor xenografts can be inhibited in situations where autocrine dependency is demonstrable in cell culture. Imaging studies with anti-EGFR MAb labeled with ^{111}Indium demonstrated selective uptake in xenografts expressing high receptor levels. Based on these observations, a phase I trial was carried out with ^{111}In-labeled anti-EGFR MAb 225 IgG1 in patients with advanced squamous cell lung carcinoma, a tumor which invariably expresses large numbers of EGFR. Escalating doses of 225 IgG1 were administered as a single intravenous injection over one hour. A dose of 120 mg 225 IgG1 could produce saturating antibody levels in the serum for >3 days and successfully imaged primary tumors as well as metastases >1 cm in diameter, without producing toxicity. The tumor uptake determined by area-of-interest scanning was 3.4% of the injected dose, and there was considerable uptake in the liver. This study establishes the principle that anti-EGFR agents which block receptor function can be safely administered to patients and will localize, preferentially, to tumor cells bearing high levels of EGFR. Future studies will explore the potential therapeutic efficacy of repeated doses of anti-EGFR MAbs.

CE 011 PRECLINICAL STUDIES OF LUNG CANCER. Terry W. Moody. Dept. Biochem. & Molecular Biology, George Washington Univ. Med. Ctr., Washington, D.C. 20037

Numerous growth factors have been identified for lung cancer. These include bombesin/gastrin releasing peptide (BN/GRP) for small cell lung cancer (SCLC) and TGFα for non-small cell lung cancer (NSCLC). Here agents which alter the growth of SCLC and NSCLC were investigated. BN receptor antagonists such as ($Psi^{13,14}$, Leu^{14})BN reduced the growth of SCLC by approximately 60% using a clonagenic assay in vitro and in nude mice in vivo. Also, ($Psi^{13,14}$, Leu^{14})BN inhibited ^{125}I-GRP binding to SCLC cell line NCI-H345 with high affinity and the increase in cytosolic Ca^{2+} caused by BN. In addition to BN receptor antagonists, the growth of SCLC was inhibited by somatostatin (SRIF) analogues. The secretion of GRP from SCLC cell lines NCI-H345 and H-209 was increased by vasoactive intestinal polypeptide (VIP) which elevates the cAMP levels. SRIF analogues inhibit the increase in intracellular cAMP caused by VIP, the secretion of GRP caused by VIP and SCLC growth. VIP receptors are also present on NSCLC cells. Thymosin α1 inhibits specific ^{125}I-VIP binding to membranes derived from squamous cell carcinoma cell line EPLC-65H and the growth of NSCLC cells in vitro and in vivo. Also, a monoclonal antibody against the EGF receptor (mAb 108) inhibited ^{125}I-EGF binding to NSCLC cell lines. MAb 108, which bound with high affinity to NSCLC cells and was not internalized, inhibited the growth of NSCLC cells. These data suggest that the proliferation of lung cancer cells can be inhibited by numerous agents which inhibit growth factor secretion or growth factor receptor binding. Supported by NCI grants CA-48071 and CA-53477.

Growth and Differentiation of Normal and Preneoplastic Epithelial Cells
(Session sponsored by Abbott Laboratories)

CE 012 CHANGES IN CARBOHYDRATE ANTIGENS RESULTING FROM DIFFERENTIATION INDUCTION. Sen-itiroh Hakomori, The Biomembrane Institute, Seattle, WA 98119.

Fucosylated or sialylated type 2 chain or type 1/ type 2 chain hybrid expressed in epithelial cells represent typical markers for stages of differentiation. This has been observed in early embryogenesis as well as various stages of tissue differentiation in established organs. Examples will be presented using various monoclonal antibodies directed to specific structures.

Secondly, gangliosides and their degradation products modulate transmembrane signal transduction (1-3). Using these compounds, we successfully induced cellular differentiation and modulation of tumor growth. A few examples will be presented, involving N,N-dimethylsphingosine and anti-carbohydrate antibodies coupled to butyrate-encapsulating liposomes.

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CE 013 MULTI-STAGE PROGRAM OF SQUAMOUS DIFFERENTIATION IN HUMAN EPIDERMAL AND TRACHEOBRONCHIAL EPITHELIAL CELLS, Anton M. Jetten, Clara Nervi, Thomas M. Vollberg and Margaret George, Laboratory of Pulmonary Pathobiology, NIEHS, Research Triangle Park, NC 27709. Squamous differentiation is the normal pathway of differentiation for epidermal keratinocytes (NHEK). In tracheobronchial epithelial (TBE) cells this pathway of differentiation occurs under pathophysiological conditions including vitamin A-deficiency, mechanical or toxic injury, and squamous cell carcinomas. A multi-stage model of squamous cell differentiation has been proposed. In the initial stage, cells become irreversibly growth arrested. This growth-arrest appears to be a requirement for the second stage, the expression of the squamous-differentiated phenotype. This stage of differentiation is accompanied by changes in the expression of several genes including Type I transglutaminase, cholesterol sulfotransferase, involucrin, specific keratins and SQ37, SQ10 and C12 which encode gene products with a still unknown function. The regulation of these genes appears to occur, at least partially, at the transcriptional level. Several factors have been identified that regulate proliferation and differentiation of these cells at each of these stages including retinoids. Retinoids do not inhibit the commitment to irreversible growth arrest but specifically suppress the induction of squamous cell markers. Examination of the structure-activity relationship appears to indicate that this action is mediated by nuclear retinoic acid receptors (RARs). Epidermal keratinocytes and tracheobronchial epithelial cells express RARα and RARγ transcripts. RARβ transcripts are induced after retinoic acid treatment in TBE cells but not in NHEK cells. A role for RARβ in mucous cell differentiation has been proposed. Certain human lung carcinoma cells exhibit an altered expression of RARβ and RARγ indicating a possible role for aberrant RAR expression in lung carcinogenesis.

CE 014 **NEUROENDOCRINE AND PERIPHERAL AIRWAY CELL DIFFERENTIATION IN NORMAL AND NEOPLASTIC RESPIRATORY EPITHELIUM.** R. Ilona Linnoila, Sandy M. Jensen, Jos L. V. Broers, Adi F. Gazdar, John C. Ruckdeschel, and James L. Mulshine, National Cancer Institute-Navy Medical Oncology Branch, Bethesda, MD 20889-5105, and Albany Medical College, Albany, New York 12208.
Respiratory epithelium contains many subpopulations of highly differentiated cells. Neuroendocrine (NE) cells, distributed throughout the airways are characterized by the production of neuropeptides and amines, and express the general NE markers neuron specific enolase, Leu-7, chromogranin A, synaptophysin and adhesion molecule N-CAM shared by all the cells of the diffuse NE system, and the specific NE markers gastrin releasing peptide (GRP), calcitonin and serotonin that are endogenous products of pulmonary NE cells. Using these markers we found hyperplasias and dysplasias of NE cells in nonneoplastic human lungs surrounding resected non-small cell lung carcinomas (NSCLC). The cells were most commonly positive for GRP, a known growth factor in lung. Furthermore, 17% of the 400 NSCLC tumors examined were found to be positive for two or more general NE markers, as determined by immunohistochemistry(IHC). These NSCLC with NE features were initially more responsive to chemotherapy than other NSCLC, resembling the chemosensitive small cell lung carcinoma which is a recognized pulmonary NE tumor. - The progenitors of the peripheral airway cells (PAC) and their tumors include metabolically active Clara cells and type II pneumocytes characterized by their defined products: a specific 10 kD protein (CLR10) and a major surfactant associated protein (SP-A), respectively. By IHC CLR10 was found in normal and hyperplastic bronchial and bronchiolar epithelial cells, and SP-A in normal and hyperplastic type II cells while other pulmonary epithelial cells remained negative. The most intense labelling was detected in the hyperplasias. The results were confirmed by RNA-RNA in situ hybridization. Furthermore, 30% of the 400 NSCLC tumors examined were positive for these PAC markers by IHC. The reactivity was most common in adenocarcinomas with papillolepidic growth pattern. Tumors with PAC markers were associated with younger age and lighter smoking history. We conclude that the cells of respiratory epithelium can differentiate along multiple pathways which have neoplastic correlates with distinct clinicopathologic features.

CE 015 TGFβ, C-*MYC*, AND THE RB GENE, Harold L. Moses and Jennifer A. Pietenpol, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, USA.
TGFβ1 is the prototype of a large family of genes involved in growth control, extracellular matrix production, and development. This family consists of several genes more distantly related to TGFβ1, isolated from several species and three closely related genes isolated from mammals (TGFβ1, TGFβ2 and TGFβ3). TGFβ1 has marked stimulatory effects on connective tissue formation. It is chemotactic for fibroblasts, an indirect mitogen for certain mesenchymal cells and a stimulator of extracellular matrix deposition. This latter effect is achieved through stimulation of synthesis and secretion of matrix macromolecules and inhibition of matrix degradation through inhibition of metalloproteinase synthesis and stimulation of protease inhibitor production. The TGFβ's are also potent inhibitors of proliferation of most cell types in culture. *In vivo* studies have demonstrated TGFβ1 growth inhibition of mammary epithelial cells, liver following partial hepatectomy, lymphocytes, and myeloid cells. Studies with the chicken chorioallantoic membrane (CAM) have demonstrated TGFβ1-induction of gross angiogenesis and a hypercellular lesion mediated through chemotactic effects in the face of growth inhibition of all cell types, including epithelial cells, fibroblasts and endothelial cells. Thus, the results from *in vivo* experiments indicate that the predominant effect of TGFβ1 on cell proliferation is inhibition. We have investigated the mechanism of TGFβ1 inhibition of skin keratinocyte proliferation. In quiescent cells stimulated with EGF, TGFβ1 can inhibit DNA synthesis when added at any point in the G1 phase of the cell cycle up to the G1/S boundary. TGFβ1 rapidly reduced c-*myc* mRNA and protein at any point in G1 by inhibiting transcriptional initiation. Like TGFβ1, antisense c-*myc* oligonucleotides inhibited proliferation when added during G1, supporting the hypothesis that TGFβ1 suppression of c-*myc* is important in the mechanism of growth inhibition. DNA tumor virus transformed keratinocytes were shown to be resistant to the growth inhibitory and c-*myc* suppression effects of TGFβ1. Since the DNA tumor viruses have in common the ability to bind and inactivate the retinoblastoma tumor susceptibility gene product (pRB), it was hypothesized that pRB may be an intermediate in the pathway for TGFβ1 suppression of c-*myc* and growth inhibition. This possibility was investigated using expression plasmids for the DNA tumor virus transforming genes. Transient expression of human papilloma virus-16 (HPV-16) E7 gene, adenovirus E1A and SV40 large T antigen blocked TGFβ1 suppression of transcription of c-*myc*/CAT constructs. This effect was not observed with DNA tumor virus transforming proteins mutated in their pRB binding domain. These observations indicate that pRB or another protein that interacts with this binding domain mediates TGFβ1 regulation of c-*myc* gene expression and growth inhibition. Experiments with expression plasmids for RB demonstrated that pRB can suppress c-*myc* transcription as effectively as TGFβ1 directly implicating pRB in this process.

Growth and Differentiation Markers
(Session sponsored by Bristol-Meyers Oncology Division)

CE 016 $\alpha^6\beta_4$ INTEGRIN EXPRESSION AND NEOPLASTIC PROGRESSION IN SQUAMOUS EPITHELIA. Thomas E. Carey, Leena Laurikainen, Head and Neck Oncology Division, Department of Otolaryngology, University of Michigan School of Medicine, Ann Arbor, MI 48109-0506.
Alterations in expression of extracellular matrix proteins or matrix receptors have been linked to malignant behavior in a variety of animal and human cancers. Integrins are integral membrane proteins expressed as alpha and beta heterodimers that function as extracellular matrix receptors in a variety of cell types. The A9 antigen previously described in our laboratory as a marker of neoplastic progression in squamous carcinoma, has immunological and biochemical identity with the newly defined $\alpha^6\beta_4$ integrins. In normal epithelia the A9/$\alpha^6\beta_4$ antigen is expressed only on the basal surface of basal keratinocytes. Squamous carcinomas and basal cell carcinomas exhibit alterations in expression of this integrin. Basal cell carcinomas which do not have the capacity for metastasis have weak expression of this integrin and fail to produce it at the leading edge of tumor nests. Among squamous carinomas there are three patterns of A9/$\alpha^6\beta_4$ expression. Those squamous carcinomas which exhibit the highest level of A9/$\alpha^6\beta_4$ expression are more likely to recur than squamous cancers that have low expression. In vitro studies of primary and recurrent cancers from the same patients show that expression of this integrin is one of the few markers that changes with tumor progression. These findings suggest that overexpression or loss of this gene product is related to the malignant behavior of epithelial tumors. The factors that result in altered A9/$\alpha^6\beta_4$ expression in the genesis and progression of epithelial carcinomas are unknown. The β4 gene has been cloned and sequenced and provides a molecular probe for investigating the genetic basis for altered antigen expression. To elucidate the mechanisms responsible for the patterns of expression that have been observed in benign and malignant epithelial neoplasia we are using Northern and Southern blot analysis to investigate β_4 gene dosage and gene expression in normal, transformed and malignant keratinocytes. A clearer understanding of these mechanisms could lead to the development of novel therapeutic approaches for one of the most common neoplastic diseases of man.

CE 017 PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE (PAM), A BIFUNCTIONAL ENZYME RESPONSIBLE FOR THE α-AMIDATION OF PEPTIDES. Betty A. Eipper, L'Houcine Ouafik, Brian T. Bloomquist, E.Jean Husten, Doris A. Stoffers, Richard E. Mains. Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The production of small bioactive peptides from their inactive precursors is a complex process involving several subcellular compartments and many co- and post-translational processing enzymes. PAM (EC 1.14.17.3) is one of the few enzymes involved in the biosynthesis of peptides to have been identified. Peptide α-amidation is a two-step process. The peptidylglycine substrate is converted into a peptidyl-α-hydroxyglycine intermediate by peptidylglycine α-hydroxylating monooxygenase (PHM) in a copper, molecular oxygen and ascorbate dependent process. At physiological pH, conversion of the α-hydroxylated intermediate into an α-amidated product requires the action of another enzyme, peptidyl-α-hydroxyglycine α-amidating lyase (PAL). The 108 kDa PAM precursor consists of an NH_2-terminal signal peptide followed by the PHM and PAL catalytic domains, a transmembrane domain and a cytoplasmic domain. A single complex gene encodes PAM, and tissue specific and developmentally regulated alternative splicing generates mRNAs encoding many different PAM proteins. Rat PAM-2 differs from rat PAM-1 by the deletion of a 105 amino acid segment separating the PHM and PAL domains. Rat PAM-3 is identical to rat PAM-2 except for the deletion of an 86 amino acid segment that includes the transmembrane domain and yields a soluble PAM protein containing both catalytic domains. Two forms of PAM mRNA encoding only the PHM domain have been identified. The PHM and PAL domains of the PAM precursor form stable functional domains that can be efficiently released from the PAM precursor proteins found in rat atrial membranes by digestion with several endoproteases. The individual catalytic domains have both been expressed by transfection of the appropriate cDNA in mammalian cells. AtT-20 cells are corticotrope tumor cells that produce pro-ACTH/endorphin and its product peptides, including joining peptide, an α-amidated peptide. AtT-20 cells express PAM, and their ability to produce α-amidated joining peptide is limited by the availabiity of ascorbate and copper. Stable AtT-20 corticotrope tumor cell lines transfected with a cDNA encoding PAM exhibit increased PHM and PAL activity, while cell lines transfected with a truncated cDNA encoding only the PHM domain exhibit increased PHM activity with no change in PAL activity. Both cell lines exhibit increased ability to produce α-amidated joining peptide. AtT-20 cells transfected a cDNA encoding an antisense PAM RNA exhibit decreased levels of PHM and PAL activity in vitro and a reduced ability to produce α-amidated joining peptide. PAM may play an important role in the production of peptides that act as autocrine or paracrine growth factors.

CE 018 MODULATION OF GENOMIC AND PROLIFERATION MARKERS DURING TUMORIGENESIS, W.N. Hittelman, J.S. Lee, J.Y. Ro, A. Sahin, N. Cheong, D. Shin, S. Lippman and W.K. Hong, Departments of Medical Oncology and Pathology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030

Tumors of the upper aerodigestive tract are proposed to represent a "field cancerization" process whereby a whole tissue region has experienced carcinogenic insult and is at increased risk for development of multiple lesions. In addition, tumorigenesis appears to be a multistep process involving an accumulation of genetic damage leading to dysregulated proliferation and differentiation. To characterize the genetic events associated with these processes, the technique of premature chromosome condensation has been utilized to directly measure chromosome alterations in the target tissue. In a Syrian hamster cheek pouch model of DMBA-induced tumorigenesis, progression from normal tissue to hyperplasia to dysplasia/metaplasia to carcinoma in-situ was associated with an accumulation of chromosome damage in the target tissue. In a similar fashion, normal lung tissue obtained at the time of human lung tumor resection has been shown by the PCC technique to harbor significant chromosome changes, often parallel in number to that found in the tumor. These findings support the notions of "field cancerization" and multistep tumorigenesis. To determine the phenotypic result of an altered genome, tissue sections obtained from biopsies of human premalignant (i.e. leukoplakia, bronchial dysplasia/metaplasia) and malignant conditions (i.e. head and neck and lung tumors) have been characterized with immunocytochemical and molecular probes for evidence of dysregulation of proliferation and differentiation. For example, using an antibody to proliferating cell nuclear antigen (PCNA), normal bronchial mucosa shows less than 1% cells expressing detectable levels of PCNA, and this fraction becomes progressively higher as the tissue progresses from hyperplasia to metaplasia/dysplasia to carcinoma-in-situ. At the same time there is evidence for spatial dysregulation of proliferation away from the basal layer. The goal of these studies is to determine the specific chromosome changes associated with specific phenotypic alterations in tissue regulation during tumorigenesis.

CE 019 SQUAMOUS DIFFERENTIATION MARKERS IN PRENEOPLASTIC EPITHELIAL CELLS, Robert H. Rice, Department of Environmental Toxicology, University of California, Davis, CA 95616.

Two distinctive markers of squamous differentiation are the cross-linking enzyme keratinocyte transglutaminase and a major substrate, involucrin. These markers are indicative of keratinocyte programming in tissues undergoing squamous metaplasia, a process that can be mimicked in culture. Their expression in keratinocytes is regulated by physiological agents (retinoids, glucocorticoids, calcium) and is often suppressed or disorganized in dysplasias and neoplasias of squamous epithelium. In addition, they serve as markers for elucidating actions of carcinogens on gene regulation in target cells. Unlike the ubiquitous tissue transglutaminase or the blood clotting factor XIII catalytic subunit, the keratinocyte enzyme is membrane-bound by virtue of acylated fatty acid. It also undergoes tetradecanoyl phorbol acetate-stimulated phosphorylation of serine near the membrane anchorage (acylation) site. This enzyme (90 kDa) is sensitive to proteolytic release from the membrane, with a consequent reduction to 80 kDa, the same size as the other two transglutaminases. Examination of the primary structure deduced by molecular cloning has identified likely sites of fatty acid acylation and phosphorylation in the N-terminal 105 amino acid extension distinguishing this form of transglutaminase from the others. The amino acid sequences of the rat and human keratinocyte enzymes are highly conserved (92% identical). In contrast, sequence conservation among involucrins of different species is much lower due to the mechanism of its rapid evolution. The human protein (≈70 kDa) contains 39 repeats of a 10 amino acid motif which accounts for its high content of Glx residues (46%). Involucrin from species beyond the primates have differing numbers of repeats, repeat lengths and repeat sequences. Although these proteins are thus immunochemically distinct and can differ considerably in size, they share unusual solubility properties which facilitate their identification in animal species useful as models of human disease.

Carcinogenesis and Chemoprevention in Animal Models/Modulation of Biomarkers During Carcinogenesis and Chemoprevention

CE 020 MODULATION BY RETINOIDS OF SQUAMOUS CELL DIFFERENTIATION IN HUMAN ORAL PREMALIGNANT LESIONS AND SQUAMOUS CELL CARCINOMAS, Reuben Lotan[1], Anton M. Jetten[2], Jin S. Lee[3], and Waun K. Hong[3], Departments of [1]Tumor Biology and [3]Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030, and Laboratory of Pulmonary Pathobiology, NIEHS, Research Triangle Park, NC 27709

The nonkeratinizing epithelial cells of the oral mucosa can differentiate in vivo along the squamous pathway under adverse conditions including injury, chronic infection, vitamin A deficiency, or exposure to carcinogens or tumor promoters. Vitamin A and some of its metabolites (e.g., retinoic acid) prevent mucosal epithelial cells from undergoing aberrant squamous differentiation. Dysplastic premalignant lesions (e.g., leukoplakia) and head and neck squamous cell carcinomas (HNSCC), also undergo some degree of squamous cell differentiation. Thus, squamous cell differentiation in the lining mucosa of the oral cavity is usually an abnormal one. Since retinoids can reverse squamous metaplasia caused by vitamin A deficiency in normal epithelial tissues in vivo, as well as squamous cell differentiation in cell culture, we investigated the ability of β-all-trans retinoic acid (RA) to modulate the growth and squamous cell differentiation in human head and neck squamous carcinoma cells (HNSCC) in vitro. RA inhibited the proliferation of 7/8 cell lines and suppressed their ability to form colonies in semi-solid medium. The growth of these cells in culture is accompanied by an increase in transglutaminase type I, involucrin, and keratin K1, three established markers of squamous cell differentiation. Higher levels of these differentiation markers were detected in cells cultured in delipidized serum (DLS), from which endogenous retinoids have been extracted, than in cells cultured in fetal bovine serum (FBS), which contains retinoids. Treatment with 1 μM retinoic acid (RA) decreased the levels of the various differentiation markers. These results indicate that some of the malignant HNSCC cells recapitulate the main characteristics of normal squamous cell differentiation in culture and that retinoic acid suppresses this differentiation as it does in normal keratinizing epithelial cells. Nuclear retinoic acid receptors (RARs), which are related to the steroid receptor superfamily act as ligand-inducible trans-acting transcription-modulating factors. mRNAs of RARs were detected in 2 HNSCC by Northern blotting and these receptors might be involved in suppression of the expression of squamous cell differentiation. The oral cavity is an ideal site for chemoprevention trials combined with biomarker evaluation because it is readily accessible by noninvasive approaches and premalignant lesions can be followed and biopsied during chemoprevention trials. We evaluated squamous cell differentiation markers as potential intermediate biomarkers in an ongoing leukoplakia prevention study using 13-cis retinoic acid. Immunohistochemical methods have indicated that a large proportion of the premalignant lesions expressed involucrin and keratin K1, not expressed by the normal oral mucosa. Evaluation of the changes in the expression of these markers in 50 patients who completed 3 months of treatment with 13-cis RA showed no consistent effect of the treatment. These preliminary results demonstrate that the squamous cell differentiation markers that are abnormally expressed in premalignant oral lesions cannot serve as intermediate biomarkers for response to retinoic acid treatment in vivo.

Biology of and Novel Therapeutic Approaches for Epithelial Cancers of the Aerodigestive Tract

CE 021 CHEMOPREVENTION OF EXPERIMENTAL RESPIRATORY CANCER. Richard C. Moon, Kandala V.N. Rao, Carol J. Detrisac and Gary J. Kelloff*, Life Sciences Research, IIT Research Institute, Chicago, IL 60616, and *Chemoprevention Branch, National Cancer Institute, Bethesda, MD 20892.
Of the several models for lung carcinogenesis, two appear appropriate for chemoprevention studies based upon dose response, tumor type and tumor localization. One model utilizes the direct acting carcinogen, methyl-nitrosourea (MNU), and the other utilizes a carcinogen (diethylnitrosamine, DEN) requiring metabolic activation. Tumors appear rapidly in both models (within six months), and the model systems are responsive to modulation by several classes of potential chemopreventive agents. For example, the retinoid N-(4-hydroxyphenyl)retinamide (4-HPR) reduces the incidence of lung adenosquamous carcinoma by 61%. Retinol or ß-carotene are ineffective when administered alone, although concomitant administration of these compounds reduces the incidence of non-neoplastic dysplasias as well as adenosquamous carcinomas of the lung. In the MNU system retinoids have been ineffective, however, both Oltipraz and diallyldisulfide (thiols) decreased the incidence of tracheobronchial squamous cell carcinomas. (Supported by NCI contracts N01-CN-45192 and N01-CN-55448.)

CE 022 AN ANIMAL MODEL FOR THE STUDY OF CANCER OF THE ESOPHAGUS, Michael J. Wargovich, W. Ki Hong, Department of Medical Oncology, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030
Cancer of the esophagus, even in the US, is distinguished from other aerodigestive cancers by its high mortality which in 1991 nearly matches the incidence of the new disease. In parts of China where the prevalence of esophageal cancer is high, a carcinogenic nitrosamine has been isolated that induces esophageal cancer in the rat. Nitrosomethylbenyzlamine (NMBA) esophageal cancer in the Sprague-Dawley rat bears striking parallels to human esophageal cancer, thus affording an animal model targeted for chemopreventive approaches. We have studied NMBA-induced esophageal cancer in our laboratory and have conducted pilot chemoprevention studies with diallyl sulfide (DAS), a thioether from alliums. Also we performed studies with retinyl acetate since Vitamin A and its anlogues have been suggested as possible intervention agents for squamous cell cancers. DAS was found to be a very potent inhibitor of esophageal cancer and the effect was dose-related. Interestingly, when DAS was tested for post-initiation effects no protection was observed. Retinyl acetate, however, failed to prevent esophageal cancer, rather, the effect observed was enhancement of carcinogenesis. This finding is consistent with other studies of retinoids in animal models. The rat model has allowed for development of intermediate markers for cancer based upon the cell biology of the squamous epithelium in transition from normal cellular patterns to carcinoma. Among the markers we have studied are the proliferation markers, BuDR and PCNA. Early studies of TGF α expression in tumors of the esophagus indicate a relationship with the progression of esophageal neoplasms. NMBA carcinogenesis provides an attractive model for the study of chemoprevention and cell biology of esophageal cancer. Newer studies using molecular biological approaches may provide an insight into ways in which to intervene in the progression of this deadly aerodigestive tract cancer. Supported by AICR Grant 89B25 and the Wakunaga Pharmaceutical Company, Osaka, Japan.

Biology of and Novel Therapeutic Approaches for Epithelial Cancers of the Aerodigestive Tract

Chemopreventive Approaches
(Session sponsored by Bristol-Meyers Oncology Division)

CE 023 STUDIES IN BARRETT'S ESOPHAGUS, Harinder S. Garewal, Richard E. Sampliner, M. Brian Fennerty, Department of Medicine, Tucson VA Medical Center and University of Arizona Health Sciences Center, Tucson, Arizona 85724

Barrett's esophagus is a premalignant lesion in which the lower esophagus is lined with metaplastic columnar epithelium rather than the normal stratified squamous epithelium. It is a precursor lesion for adenocarcinoma of the esophagus. We are studying Barrett's esophagus as a model premalignant lesion for adenocarcinoma from the standpoint of identifying biologic markers of increased cancer risk as well as therapeutic strategies for eradicating the lesion. Our studies on the polyamine pathway have shown that ODC activity in Barrett's mucosa is greater than in normal, adjacent mucosa from the same patient. Furthermore, ODC appeared to be higher in dysplastic specimens than in the absence of dysplasia. However, polyamine content was not significantly altered suggesting dysregulation of the polyamine pathway. Our studies of aneuploidy and its significance in a premalignant lesion continue to accrue patients. Initial results have demonstrated that aneuploidy and dysplasia can be discordant. Studies using short-term epithelial cultures established from endoscopic biopsies of the lesion have demonstrated the presence of clonal karyotypic abnormalities. The clinical significance of aneuploidy, however, remains to be proven. Chemopreventive intervention trials have included a study using 13-cis-retinoic acid. Considerable toxicity was encountered and the lesion showed no change in extent in 11 evaluable patients. A follow-up clinical trial with a biologic endpoint used DFMO, an irreversible inhibitor of ODC, to test whether a low dose could produce changes in polyamine content in GI mucosa. Significant changes have been observed in polyamine content after 6 weeks of DFMO treatment at .5g/m ^{2} TID suggesting that this clinically tolerable dose can result in measurable effects on the polyamine pathway. Additional strategies to be tested for lesion response include use of acid reflux suppression combined with "differentiating" agents.

CE 024 THERAPEUTIC STRATEGIES FOR PREVENTION OF SECOND PRIMARY TUMORS
W.K. Hong and S.M. Lippman, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030.

Formation of epithelial cancer from the aerodigestive tract is a multistep tumorigenic process. The entire epithelium at risk is exposed to repeated carcinogen exposure, such as tobacco, and manifests multiple independent premalignant and malignant foci. This concept of field carcinogenesis postulates a basic mechanism which links primary tumor and second cancer development in the aerodigestive epithelium. Overall, head and neck cancer patients develop second primary tumors at a constant rate of 3-4% per year. The development of primary and second primary tumors depends on the nature of the interactions between the inherent tissue susceptibility of the individual and the degree (intensity and duration) of their carcinogenic exposure. Recently, the genetic susceptibility for developing this type of tumor has come to be appreciated.

Vitamin A and retinoids are important agents for control of cell differentiation and proliferation in aerodigestive epithelial tissue. Retinoids are well known to suppress carcinogenesis in several epithelial tissues including skin, bladder, oral mucosa, and tracheobronchial epithelium in both animals and humans. Our group first reported a randomized placebo-controlled clinical trial in oral premalignancy which showed that retinoic acid has a profound effect on the reversal of premalignant oral lesions (N Engl J Med 323:1501-1505, 1986). More recently, our group again demonstrated the prevention of second primary tumors with retinoic acid in patients with squamous cell cancer of the head and neck (N Engl J Med 323:795-801, 1990). Aerodigestive epithelial cancer is a significant public health problem and is a unique model from a clinical and biological perspective to test the hypothesis of chemoprevention (as an adjunct to smoking cessation) to prevent primary and second primary tumors. Future studies for chemoprevention with retinoids and other related agents in individuals at high risk for tobacco-related epithelial cancers will be presented and discussed.

CE 025 RETINOID AND CAROTENOID CHEMOPREVENTION TRIALS IN ORAL PREMALIGNANCY, Lippman SM, Toth BB, Wargovich MJ, Martin JW, Lee JS, Batsakis JG, Hong WK; Department of Head, Neck and Thoracic Medical Oncology, UT M.D. Anderson Cancer Center, Houston, Texas 77030

Retinoids and carotenoids are promising agents for upper aerodigestive tract cancer chemoprevention. Preclinical data and an ideal toxicity profile led to β-carotene study in oral leukoplakia. Response data from three independent single-arm trials have been mixed, with reported overall response rates ranging from <30% (60mg/d x 6-months) to >70% (30mg/d x 3-months). Only the retinoid 13cis-retinoic acid (13cRA), however, has withstood the rigor of randomized study. Our phase III chemoprevention trial established significant activity of high-dose 13cRA (1-2mg/kg/d x 3-months) in oral premalignancy (Hong et al. NEJM 1986). Problems included significant toxicity and high relapse rates after stopping therapy. Based on these findings, our current study includes a 3-month high-dose 13cRA induction phase (1.5mg/kg/d) followed by a 9-month randomized maintenance phase--low dose 13cRA (0.5mg/kg/d) vs β-carotene (30mg/d) (NCI CA 46303). After the 3-month induction phase the clinical response was 66% (32/48) and histologic response was 35% (17/48); progressive disease occurred in 10% (5/48). This trial (the only randomized oral premalignancy trial which includes β-carotene) indicates that low-dose 13cRA is more effective maintenance therapy than β-carotene as monitored by clinical and histological outcome, and by micronuclei (MN) frequency (a marker of acute genotoxicity).

	Standard Clinical/Histological Outcome			Increased
	Progression	Further Response	Stable	MN Frequency
Low dose 13cRA	10% (2/20)*	40% (8/20)	50% (10/20)	25% (4/16)
β-carotene	48% (11/23)*	17% (4/23)	35% (8/23)	43% (9/21)

*2-sided P-value < 0.01

Differentially expressed biomarkers (K1, EGF-R and blood group antigens) are under investigation as potential intermediate study endpoints. Critical issues of oral premalignancy trials will be presented including: study design and interpretation, intermediate endpoint biomarkers, and future directions of this field.

CE 026 BIOMARKERS (BM) AS INTERMEDIATE ENDPOINTS IN CHEMOPREVENTION (CP) TRIALS OF HUMAN CANCER (C). Frank L. Meyskens, Jr. Clinical Cancer Center, University of California Irvine, Orange, California 92668

Detection of C is the definitive endpoint in the conduct of CP trials. There are however several reasons why C as the endpoint may not be feasible and/or ethical: (1) the usage of patients with easily followable preneoplasias may preclude the development of C, (2) the time to a C event may be long and/or the incidence uncommon, even in individuals at high risk (R) for a common C. Two major types of BM should be considered: (a) those that identify individuals at high R and (b) those that serve as a surrogate for C. Various epidemiologic features, including family history, have been used to estimate relative R. This approach however only slightly decreases the size of populations needed for CP trials and only little addresses the question of individual R. Advances in understanding the genetic basis for C will lead to the development of probes that will help assess R in many Cs. It may well be that either inherent or acquired individual heterogeneity in the primary structure of DNA in critical may allow identification of individuals at higher R. A variety of scenarios can be envisioned; two representative examples include retinoblastoma (gene deletion) and "normal" colon tissue with a $5q^-$ and ras activation. Advances in understanding of gene changes predicting R for C will lead to economic design of CP trials. Surrogate BM for C are different than tumor markers in several different ways: (a) BM may be indirectly related to the process of C formation, e.g. detectable changes in the non-involved tissue or host cells, (b) BM may not represent tumorous alterations per se. There are innumberable BM that can be identified as associated with C formation, including genetic, epigenetic, and histological features. The challenge is not in identifying potential BM, but in showing that they are relevant. Carcinogenesis has been shown to be carcinogen, inhibitor, dose, tissue, and species specific; it is likely that relevant BM will need to be identified, studied, and verified in human models. The upper aerodigestive (UAD) system should be a rich source for BM studies as tissue is readily available, the carcinogenic process can be monitored, and there are currently available reasonable compounds to use in BM modulation and CP trials. This human model should be used for extensive in vitro characterization of the BM/CP concept. I will also report on a CP model we have established for cutaneous melanoma. Many of the difficulties faced in the establishment of this model and their resolution are pertinent to improving the broader approach to BM and CP, including UAD C.

Biology of and Novel Therapeutic Approaches for Epithelial Cancers of the Aerodigestive Tract

Drug Resistance

CE 027 THE ROLE OF DNA TOPOISOMERASES IN MULTIDRUG RESISTANCE. William T. Beck, Mary K. Danks, and D. Parker Suttle, St. Jude Children's Research Hospital, Memphis, TN 38101

"Natural product" multidrug resistance (MDR) can take several forms. We and others have described one form in which tumor cells display a broad cross-resistance to many anticancer drugs that interact with DNA topoisomerase II (topo II), but are not altered in drug accumulation, do not overexpress P-glycoprotein (Pgp), and are unaffected by Pgp modulators such as verapamil (1-3). This form of MDR is associated with alterations in topo II activity or amount (at-MDR). As a model, we selected human leukemic CEM cells for resistance to VM-26 (teniposide) and cross-resistance to VP-16-213 (etoposide), a drug used in the treatment of both leukemia and small cell lung cancer (SCLC). Drug-stabilized DNA-protein complex formation and catalytic activity of topo II were decreased in the nuclear extracts (4) and catalytic activity and amount of enzyme were also decreased in the nuclear matrix (5) from our at-MDR cells, compared to those from drug-sensitive cells. The nuclear matrix result appeared to be due to differences in salt extractability of the resistant enzyme, suggesting that the resistant enzyme is physically altered. We also showed that the ATP requirement of topo II is increased in the at-MDR cells, most likely because the enzyme binds ATP less well than its counterpart from sensitive cells (6). Together, these observations suggested the existence of a mutation in the gene encoding the enzyme in the resistant cells. To test this hypothesis, we analyzed certain regions in the topo II cDNA from the resistant cells in order to determine whether nucleotide alterations exist. By sequencing PCR products from cDNA, we identified a single base mutation resulting in an amino acid change of Arg to Gln at position 449 (7). We have confirmed the presence of this alteration in the drug-resistant cells by hybridization of allele-specific oligonucleotides to cDNA and by chemical mismatch cleavage analysis. We do not yet know whether expression of a topo II-Gln_{449} protein confers at-MDR or if another mechanism has altered the expression of the enzyme. It is clear from these studies, however, that MDR can be due to more than one mechanism, and postulate that the form associated with alterations in topo II may have clinical consequences. Present studies are directed toward understanding the molecular basis for the alteration in topo II, evaluating whether it occurs in other cell lines as well as in tumors from patients, and determining its consequences for drug resistance. In this regard, we have developed a functional assay using intact cells that may allow us to identify in clinical specimens those cells that express either Pgp-MDR or at-MDR. Results of these studies will be reported, as will studies of at-MDR expression in SCLC cell lines. (Supported in part by research grants CA-30103, CA-40570, and CA-47941, CORE grant CA-21765, all from NCI, Bethesda, MD, and in part by ALSAC)

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CE 028 CLINICAL DRUG RESISTANCE. Kenneth H. Cowan, Medicine Branch, National Cancer Institute, Bethesda, MD 20892.

The development of resistance to antineoplastic agents is a principle problem in clinical oncology. Tumors such as small cell lung cancer, breast cancer, ovarian cancer, lymphomas, and leukemias which are initially quite sensitive to cancer chemotherapy are less responsive to either the same chemotherapy or to secondary salvage regimens. In contrast, tumors such as colon cancer, non-small cell lung cancer, and melanoma are refractory to even initial chemotherapy. One of the principle goals in cancer chemotherapy is to understand the mechanisms responsible for inherent (or de novo) and acquired clinical drug resistance.

Cell lines selected for in vitro resistance to anticancer drugs have been useful in to identifying mechanisms associated with the development of resistance which include: decreased drug uptake, enhanced drug efflux, decreased expression of drug activating enzymes, enhanced expression of drug metabolizing or scavenging enzymes, increased levels of target enzymes, altered sensitivity of target enzymes, and altered levels of specific cofactors. The genetic mechanisms involved in these changes include alterations in gene expression, gene mutation, and gene amplification. Some of these mechanisms are specific for a single class of anticancer agents (particularly antimetabolites) while others are associated with broad spectrum resistance to several diverse classes of antineoplastic agents.

The development of multidrug resistance in which cells become resistant to a wide variety of structurally diverse anticancer drugs including anthracyclines, vinca alkaloids, and epipodophylotoxins is an example of the latter. In vitro studies have identified a putative drug efflux pump (P-glycoprotein) encoded by the *mdr*1 gene that apparently transports anticancer drugs resulting in drug resistance. The identification of noncytotoxic agents that can interfere with this drug efflux pump and reverse drug resistance has resulted in clinical trials with these modifiers. Other studies have indicated that other mechanisms including alterations in topoisomerases, changes in intracellular glutathione or enzymes requiring gluthatione, and other possible drug efflux pumps may also result in resistance to multiple classes of antineoplastic agents. Understanding the mechanisms involved in drug resistance and their prevalence in different diseases (inherent versus acquired resistance) should result in more rationale therapy for drug resistant tumors.

CE 029 THE ASSOCIATION BETWEEN DRUG RESISTANCE OF LUNG CANCER CELL LINES AND NEUROENDOCRINE DIFFERENTIATION AND ONCOGENE ACTIVATION. Adi F. Gazdar, Giuseppe Giaccone, and Tetsuya Mitsudomi, NCI-Navy Medical Oncology Branch, National Cancer Institute and Naval Hospital, Bethesda, MD 20889

Lung cancer is usually divided into small cell (SCLC) and non-small cell (NSCLC) lung cancer types. SCLC is a neuroendocrine (NE) tumor and is usually sensitive to cytotoxic therapy at diagnosis. While NSCLC usually lack NE markers, about 15% of tumors (termed NSCLC-NE) express the entire program. Most NSCLC tumors demonstrate *de novo* resistance to cytotoxic therapy. Other types of related NE tumors include bronchial carcinoids, and small cell tumors arising at extra-pulmonary sites (ExPuSC). We performed drug sensitivity testing (DST) of a comprehensive panel of 50 lung cancer cell lines using 5 cytotoxic drugs and confirmed the clinical findings that most SCLC and carcinoid lines were relatively sensitive while most NSCLC lines were relatively resistant. However, 5/6 NSCLC-NE lines were relatively sensitive. Thus, NE differentiation in NSCLC may be associated with a therapeutically responsive subgroup. DST patterns of lung cancer lines were not characteristic of that associated with over-expression of the multidrug resistance associated MDR1 gene, as all drugs were tightly correlated with each other. Expression of the MDR1 gene in normal lung, lung tumors and cell lines, and was not associated with cell type, therapy status or *in vitro* drug sensitivity. Surprisingly, MDR1 expression in chemosensitive NSCLC-NE cell lines was relatively high. Also, there was no clear association with the glutathione redox pathway and the enzymes of glutathione metabolism. We examined the state and expression of topoisomerase I and II genes in lung cancers, and found a mutation rate of approximately 10%. While there was no association between DST and expression of the topoisomerase I gene, there was a strong correlation with expression of topoisomerase II gene (with the exception of a NSCLC-NE cell line). In experimental models, mutations of *ras* genes are associated with specific carcinogens, exposure to cytotoxic agents, a metastatic phenotype and with resistance to cytotoxic therapy. Mutations of *ras* genes, especially K-*ras*, codon 12, are frequent in NSCLC and are a negative prognostic factor. We screened a large panel of lung cancer cell lines and found an incidence of 35% in NSCLC. In marked contrast, none were present in 42 SCLC lines. In NSCLC, *ras* mutations occurred independently of histology, tumor extent or history of prior drug therapy. *ras* mutations were an independent negative factor for survival irrespective of tumor extent. However, there were no significant differences in the DST patterns of cell lines with and without *ras* mutations. We conclude that drug resistance in lung cancer is complex and may be associated with histological type, NE differentiation and expression of the topoisomerase II gene.

CE 030 RAPID SELECTION OF STABLE MULTIDRUG RESISTANCE IN A SMALL CELL LUNG CANCER (SCLC) LINE AND DIFFERENTIAL CHEMOSENSITIZATION WITH CYCLOSPORIN A (CSA) Glisson B.S. and Alpeter M. Department of Medical Oncology U.T.M.D. Anderson Cancer Center, Houston, TX 77030

H69 parent cells, established from a treated patient with SCLC (provided by Dr. Adi Gazdar, NCI-Navy Oncology Branch, Bethesda, MD) were exposed to etoposide 20 μM (peak serum concentration in adults given 100 mg/M^2 IV dose) for 1 h three days in succession every 21-28 days. Resistance to etoposide emerged after the third treatment (approximately 60 days) and has been stable in the absence of drug exposure for eighteen months. The relatively rapid selection of this line with a clinically relevant drug exposure schema and the stability of the resistant phenotype suggest these cells may have been a steady subpopulation of the parent line through years of serial passage in vitro. Thus, this cell line may possess particular clinical relevance as regards the therapy of small cell lung cancer. The H69/VPR-2 line exhibits cross resistance to Adriamycin and vincristine, both of which are commonly used in the treatment of this disease. Immunoblot analysis of membrane vesicle preparations demonstrated increased expression of P-glycoprotein in H69/VPR-2 cells relative to the parental line. However, steady state concentrations of etoposide and daunomycin are reduced minimally, 1.5 and 3-fold respectively, in H69-VPR-2 cells, only partially explaining a 15-20 fold level of resistance to both agents. Due to retention of a five-fold level of resistance to etoposide-induced strand breaks in isolated nuclei from H69/VPR-2 cells, we have studied topoisomerase II catalytic and etoposide-stimulated DNA cleavage activity in nuclear extracts from both lines, but identified no differences. CSA and verapamil, both of which bind to P-glycoprotein, can enhance accumulation of etoposide in H69/VPR-2, yet only CSA is effective in differentially enhancing etoposide cytostasis in H69/VPR-2 relative to H69.

Chemosensitization Ratios With Etoposide		
Cell Line	CSA 2 μg/ml	Verapamil 10μm
H69	1.2	2.1
H69/VPR-2	7.4	3.2

Notably neither verapamil nor CSA significantly enhance cytostatic effects of Adriamycin in either line despite enhanced drug accumulation. The chemosensitizing activity of CSA requires its presence during etoposide exposure, and is dose-dependent over a range of 0.5-2 μg/ml. CSA enhances etoposide-induced DNA single strand break frequency 9-fold in H69/VPR-2 cells, but has no effect in isolated nuclei. These data suggest that correction of altered cytoplasmic drug distribution may be involved in chemosensitization of H69/VPR-2 cells by CSA. Studies are underway to model multidrug resistant SCLC using intrabronchial and intrasplenic implantation of H69/VPR-2 cells in athymic nude mice.

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Strategies for Early Diagnosis and Therapy
(Session sponsored by Bristol-Meyers Oncology Division)

CE 031 RATIONAL MARKERS FOR EARLY LUNG CANCER DETECTION, James L. Mulshine, Frank Cuttitta, Anthony M. Treston, Frank M. Scott, Inga Avis, Prabodh K. Gupta, and Melvyn S. Tockman, Biotherapy Section, NCI-Navy Med Oncology, Natl Naval Med Ctr, Bethesda, MD 20889, Dept of Pathology and Laboratory Medicine, Univ of Pennsylvania, Philadelphia, PA 19104, and Johns Hopkins Medical Institutions, Baltimore, MD 21205

To effect meaningful early lung cancer detection, a phase of lung cancer still confined to the bronchial epithelium has to be the desired end point, since routinely effective treatments for disseminated lung cancer are not available. Initial experience in developing an immunologically-based assay to identify antigens expressed on shed bronchial epithelial cells resulted in a statistical significant correlation of immunostaining with the eventual development of lung cancer 2-4 years prior to routine clinical detection (Tockman et al. J Clin Oncol 6:1685, 1988). Attempts to improve this approach require an understanding of the basis for its success. The sputum immunostaining assay involved the use of two tumor-associated monoclonal antibodies of which one of the two antibodies (624H12) which is principally expressed on small cell lung cancer, was later found to react with the carbohydrate structure, difucosylneolactonorhexaosylceramide (Kyogashima et al. Arch Biochem, Biophys 275:309, 1989). Based on the work of Hakomori and co-workers this difucoslyated Lewis X structure would be a likely marker of carcinogenic transformation of the bronchial epithelium. Other carbohydrate structures would also be reasonable markers to evaluate for early detection application, based on the known pattern of expression of these structures in fetal, dysplastic and neoplastic lung tissue. The second antibody used for sputum immunostaining is not a known member of a likely class of early detection targets. The antibody 703D4 recognizes a 31 kD protein structure that is expressed by the majority of non small cell lung cancers. The reported cases of lung cancer missed by the immunostaining approach included principally adenocarcinoma of the lung, suggesting that the addition of marker(s) of that type of morphologic differentiation should be considered. Adenocarcinoma of the lung is a complex range of neoplasms sharing related histogenesis. Markers to dissect these histogenic relationships are being characterized (e.g. Gazdar et al. Cancer Res 50:5481,1990 and see the Abstract of Linnoila et al.) and these specific markers are available for evaluation in early detection applications.

Since tumor promotion events dominate the phase of lung carcinogenesis in which the cancer is still confined to the bronchial mucosa, then prospectively analyzing aspects of that known biology, such as the expression of promotion factors, provides another class of targets for early detection consideration. The goal of achieving clinically meaningful early lung cancer detection may require complementary analyses with markers which evaluate different aspects of the field carcinogenesis of the bronchial epithelium.

CE 032 IMMUNOLOGIC EFFECTOR CELLS IN HEAD AND NECK CANCER, Theresa L. Whiteside, Dept. of Pathology, University of Pittsburgh School of Medicine, and Pittsburgh Cancer Institute, Pittsburgh, PA 15213

Human head and neck cancers (HNC) are generally well infiltrated with mononuclear cells. The presence of tumor-infiltrating lymphocytes (TIL) in HNC has been considered a good prognostic factor, and the intensity of infiltration in situ seems to correlate with prognosis and disease-free survival. T lymphocytes, many of which are activated ($CD3^+CD25^+HLA\text{-}DR^+$), are the major component of these infiltrates, with $CD8^+$ cells accumulating preferentially in the tumor parenchyma and $CD4^+$ cells in the stroma. These observations indicate that TIL play a role in the host's ability to control tumor growth. Interactions between TIL or lymph node lymphocytes (LNL) and tumor cells influence the loco-regional immune responses, and a better understanding of these interactions is essential for devising novel approaches to therapy of HNC. Functional status in situ of TIL and LNL was investigated using cDNA probes for cytokine mRNA. In addition, cytokine production and antitumor cytotoxicity as well as proliferative assays were performed with freshly-isolated TIL and LNL. Freshly-isolated TIL or LNL showed low or undetectable levels of antitumor cytotoxicity and low proliferation in response to rIL2 or mitogens in comparison to peripheral blood lymphocytes (PBL). After in vitro activation with rIL2, lymphokine activated killer (LAK) cell activity was significantly lower in TIL or LNL than in autologous PBL. TIL and LNL in tumor-involved LN contained mRNA for IL2 in situ. These cells produced IL2 but were unable to produce and release IL1 or TNFα and produced low levels of IFNτ either spontaneously or after in vitro activation. This inadequate immune competence of TIL and LNL in patients with HNC was not due to the presence of lymphoid suppressor cells, as measured in co-culture experiments with autologous PBL responding to specific or non-specific activators, but appeared to be related to in vivo exposure to tumor-derived immunoinhibitory factors. Exogenously supplied cytokines reversed this immunologic unresponsiveness of LNL or TIL and induced generation of antitumor effector cells in culture. Positive selection on antibody-coated devices of $CD3^+CD8^+$ or $CD3^+CD8^+$ T cells prior to culture in the presence of cytokines showed that LNL are a good source of broadly-reactive, non-MHC-restricted antitumor effector cells in patients with HNC.

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CE 033 BIOLOGIC STAGING AND THERAPY SELECTION IN PATIENTS WITH EARLY SQUAMOUS CARCINOMA OF THE HEAD AND NECK, G.T. Wolf, T.E. Carey, J.M. Truelson, J. Poore, L. Glaser, and C. VanWaes, Department of Otolaryngology - Head and Neck Surgery, University of Michigan and VA Medical Center, Ann Arbor, MI 48109. Conventional treatment strategies for patients with early squamous carcinomas of the head and neck consists of single modality surgical resection or radiation therapy. Despite the effectiveness of surgery or radiation for small cancers, 10-30% of patients will experience tumor recurrences or metastases and 20-40% will develop second primary tumors. Current clinical staging parameters have not been adequate to define high risk individuals with early disease who are unlikely to be cured with conventional therapy. Recent studies of tumor cell expression of α^6B_4 integrin and ABH blood group antigens in 82 patients indicates that these markers are useful in identifying patients likely to suffer early tumor relapse and death. 58% of patients with tumors showing high expression of the integrin marker and 78% of patients with tumors that had loss of normal blood group expression suffered early tumor relapse. Additional studies of a homogenous group of 88 patients with laryngeal cancer indicate that analysis of tumor cell DNA content also predicts prognosis better than conventional clinical prognostic factors. The use of these biologic staging parameters in the initial assessment of patients with early cancers should allow selection of more aggressive primary treatment strategies for individual patients.

CE 034 IMMUNOMODULATORY THERAPY OF LUNG CANCER. Stephen C. Yang, Elizabeth A. Grimm, and Jack A. Roth, Departments of Thoracic Surgery and Tumor Biology, M. D. Anderson Cancer Center, Houston, TX 77030. The treatment of advanced primary non-small cell lung cancer is refractory to IL-2 based immunotherapy as shown in early trials. The purpose of this study was to use combinations of biologic agents for the induction <u>in vivo</u> of lymphokine activated killer activity (LAK). Phase I trials were initiated to test the toxicities, anti-tumor and immunobiologic effects of combining low dose OKT3, IL-2 and TNF in patients with Stage IIIb and IV NSCLC. 16 pts (Group A) received a continous daily IV infusion of IL-2 ($6x10^6$ IU/m^2/day) and a simultaneous IM dose of TNF (25-100 $\mu g/m^2$/day) for 5 days. 15 pts received a bolus IV infusion of OKT3 (27-108 $\mu g/m^2$/day) for 2 days followed by the same IL-2 and TNF regimen as Group A. These doses of each biologic agent were ineffective in previous studies. Therapy was given at 3 wk intervals. Side effects (<grade 3) experienced by patients in Group A included fever, local skin reaction at the TNF injection site, pancytopenia and general malaise. In Group B, toxicities associated with OKT3 alone consisted of fevers, chills and headaches. All these reactions were reversible within 48 hr after cessation of therapy. Of the 12 patients in Group A evaluable for response, 1 PR and 3 MR occurred. Seven patients had radiographic stabilization of their disease, a median of 12 wks (range 4-16 wks) before progression. In Group B, 2 MR in 8 evaluable patients have been observed. OKT3 alone generated significant levels of LAK activity compared to baseline values (7-42 LU, $p<0.05$). LAK induction by OKT3 alone was dose related, with the highest level generated using 108 $\mu g/m^2$ (42.0 ± 10.5 LU). Lytic activity after IL-2 with TNF was synergistic with a dose of 54 $\mu g/m^2$ of OKT3 (72.6 ± 10.9 LU) compared to that without OKT3 (29.6 ± 5.5 LU, $p<0.05$). OKT3 alone induced significant production of endogenous IL-2 and TNF (mean increases of 225 IU/ml and 750 pg/ml, respectively) with higher levels noted following completion of IL-2+TNF. Serum soluble IL-2 receptors increased following OKT3 alone (mean 265 U/ml), with further increases of 2762 U/ml following completion of IL-2+TNF. Phenotypic analysis revealed increases in TAC expression after OKT3 alone and following IL-2+TNF (mean 18 and 28%, respectively). These results support the combination use of T-cell activating antibodies with biologic agents to enhance and prolong endogenous anti-tumor responses.

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Role of Neuropeptides in Regulation of Tumor Growth

CE 035 MOLECULAR CLONING OF THE BOMBESIN\GRP RECEPTOR FROM SWISS 3T3 CELLS

James F. Battey[1], James M. Way[1], Martha H. Corjay[1], Hagit Shapira[1], Kiyoshi Kusano[1], Richard Harkins[2], James M. Wu[2], Timothy Slatery[2], Elaina Mann[2], and Richard I. Feldman[2], [1]Laboratory of Neurochemistry, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892, and [2]Triton Biosciences Inc., 1501 Harbor Bay Parkway, Alameda, CA 94501

The mammalian bombesin-like peptides, gastrin-releasing peptide (GRP) and neuromedin B (NMB), regulate numerous and varied important cell physiologic processes in different cell types, and have also been implicated as autocrine growth factors influencing the pathogenesis and progression of human small cell lung carcinomas. We report here the first molecular characterization of the bombesin/GRP receptor. Structural analysis of cDNA clones isolated from Swiss 3T3 murine embryonal fibroblasts show that the GRP receptor is a member of the G-protein coupled receptor superfamily with seven predicted hydrophobic transmembrane domains. In vitro transcripts from cloned cDNA templates encompassing the predicted protein coding domain, when injected into Xenopus oocytes, resulted in expression of functional GRP receptors. The predicted amino acid sequence of the open reading frame in cDNA clones matches the amino terminal sequence, as well as the sequence of four tryptic fragments isolated from the purified protein. Expression of the GRP receptor cDNA in model systems potentially provides a powerful assay for the development of subtype-specific receptor antagonists which may prove to be of therapeutic importance in human small cell lung carcinoma.

CE 036 RESPONSIVENESS OF LUNG CANCER CELL LINES TO A SERIES OF NEUROPEPTIDES, Bunn, P.A., Jr., Chan, D., Dienhart, D., Tagawa, M., Jewett, P., Division of Medical Oncology, University of Colorado Cancer Center, Denver, CO, 80262. We evaluated the effect of seven classes of neuropeptides [bradykinin (BK), cholecystokinin 26-33 (CCK), neurotensin (NT), arginine-8 vasopressin (AVP), tyr-4 bombesin (BN), somatostatin (SO), and motilin (MO)] on 16 human lung cancer and 4 human breast cancer cell lines to determine the pattern of responses. Flow cytometric analysis of Indo-1AM loaded cells was used to quantitate the intracellular calcium response of individual cells produced by these peptides alone, or in simultaneous or sequential combinations. The number of cell lines responding to each peptide (100 nM) is shown in the table:

	Number of Cell Lines Responding to Each Peptide						
Cell Line	BK	CCK	NT	AVP	BN	SO	MO
SCLC							
Classic	4/5	5/5	5/5	4/5	4/5	2/5	3/5
Variant	4/6	2/6	2/6	1/6	2/6	4/6	3/6
NSCLC	5/5	2/5	3/5	2/5	2/5	3/6	3/6
Breast	0/4	0/4	0/4	1/4	0/4	1/4	1/4

All 16 lung cancer cell lines responded to one or more peptide classes with classic small cell lines displaying the greatest responsiveness followed by variant small cell lines and non-small cell lung cancer cell lines. Breast cancer cell lines demonstrated little or no response to any peptide. There was great variability in the magnitude of response and pattern of response in individual cell lines and between cell lines. Bradykinin was the most potent peptide and produced responses in the largest number (13/16) of lung cancer cell lines. Simultaneous administration of active peptides produced greater intracellular calcium release than single peptides, though in a less than additive manner. Response to each peptide was followed by a refractory period lasting several hours. The refractoriness was peptide-specific implying that each peptide has a distinct pathway, at least at the receptor level. Bradykinin antagonists could abrogate the calcium response to bradykinin but not other peptides. Similarly, specific peptide antagonists for CCK, GRP, and AVP blocked the response for only their specific agonist. The Substance P derivative [D-Arg^{1}, D-Phe^{5}, D-Trp^{7,9}, Leu^{11}]-Substance P inhibited responses to GRP and to a lesser extent AVP and CCK. The clinical anti-tumor utility of these antagonists alone or in combination with other neuropeptide antagonists is under investigation.

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CE 037 **NOVEL BIOACTIVE PEPTIDES IDENTIFIED WITHIN THE PRECURSOR MOLECULES OF KNOWN GROWTH FACTORS,** Frank Cuttitta, Jill Siegfried, Anthony M. Treston, Frank M. Scott, Kathryn A. Quinn, Philip G. Kasprzyk, Ingalill L. Avis and James L. Mulshine, Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814; Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261; Molecular Oncology Inc., Gaithersburg, MD 20878; Biotherapy Section, NCI-Navy Medical Oncology Branch, National Naval Hospital, Bethesda, MD 20889

It is now a widely accepted phenomenon that human tumor cells have the ability to produce their own growth factors. This aspect of malignant disease was originally conceptualized by Sporn and Todaro as the autocrine growth hypothesis (N. Engl. J. Med 303: 878-880, 1980). Though major scientific emphasis has been placed on the transcriptional and translational events leading to growth factor expression, it is in fact the enzymatic modifications that occur following precursor protein generation which convey biologic activity. Most peptide hormones and growth factors are initially expressed as a larger, functionally impotent, proform. The precursor protein undergoes a series of post-translational processing steps, ultimately giving rise to a bioactive end product. Of all the post-translational modifications that can occur during internal cellular processing, amidation is the best studied with regards to its biological significance. Peptide amidation involves an enzymatically mediated alteration of the C-terminal amino acid resulting in the substitution of a amide (-$CONH_2$) for the acid (-COOH) terminus. More than half of the known peptide hormones of man are amidated. In fact many biochemists use amidation as *de facto* proof that a newly identified peptide will have biological activity in the cell system from which it was isolated. Mechanistically, there are at least four distinct enzymatic steps routinely involved in peptide amide formation. These include; trypsin-like cleavage event occuring at basic amino acid residues (lys or arg), carboxypeptidase activity which removes the basic residue and results in the formation of a glycine extended intermediate, and two additional genetically linked enzymatic steps (see abstract of Treston *et al.*) to form the bioactive peptide amide end product.

Recently, molecular genetic analysis of many human peptide hormones and growth factors has been accomplished and as a result of cDNA cloning, predicted amino acid sequences of precursor proteins determined. There appear to be distinct amino acid consensus sequences or motifs which signal peptide amidation. Using these target sequences, we were able to identify at least two previously unknown amidated peptides in the "E" region of the insulin-like growth factor IB (IGF-IB) precursor molecule. These peptides were capable of mediating trophic effects on both normal and malignant human cell lines and tissues. In addition, we have demonstrated that these peptide amides initiate their physiological effects on respective target cells through an alternative receptor system to the type I receptor of IGF-I. Our approach in identifying cryptic bioactive peptides within the precursor molecules of known growth factors offers an alternative avenue of investigative study for defining growth regulatory mechanisms of normal and malignant cell systems.

CE 038 **HUMAN NEUROENDOCRINE-LIKE CELL LINES CONTAIN PEPTIDE α-AMIDATING ACTIVITY** Anthony M. Treston, Frank M. Scott, Kathryn A. Quinn, Ingalill L. Avis, Betty A. Eipper, Frank Cuttitta and James L. Mulshine. Biotherapy Section, NCI-Navy Medical Oncology Branch, Naval Hospital, Bethesda, MD, 20889 USA; Neuropeptide Laboratory, Johns Hopkins University Medical School, Baltimore, MD 21205 USA; and the Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814 USA.

The single most fatal malignancy in Western societies is lung cancer, and the most clinically aggressive form of human lung cancer is small cell (SCLC). As a consequence of the neuroendocrine nature of SCLC, tumors constitutively produce many peptides, and at least some of these peptides can function as autocrine growth factors. The mitogenic effect of autocrine neuropeptides may account for the propensity to early metastatic dissemination in patients with SCLC. The autocrine growth hypothesis, that tumor cell populations can produce factors which stimulate their own growth, is a well-accepted tenet of tumor cell biology. However some specific aspects of autocrine stimulation have not been tested, particularly the presence in tumor cells of the enzymatic machinery necessary for production of the fully processed bioactive form of proposed growth-stimulating factor/s. One of the most important of these post-translational modifications is α-carboxyamidation. This modification is required for complete bioactivity in ca. 50% of known gastro-intestinal and neuroendocrine peptide hormones, including many autocrine growth factors. In all species studied thus far, only one activity which catalyzes the final step in the biosynthesis of an α-amidated peptide has been identified. Originally a single enzyme was proposed to carry out the conversion, but this activity is now believed to be due to two sequential enzymatic steps. Both enzymes involved in α-carboxyamidation are coded for by a single gene.

We report here that a variety of human tumor cell lines express both enzymes (a peptidyl-glycine:peptidyl-α-hydroxyglycine monooxygenase, PHM, and a peptidyl-α-hydroxyglycine:peptidyl-α-carboxyamide lyase, PAL) required for the conversion of a glycine-extended propeptide into its C-terminal α-amide analog. We demonstrate here that, as with all other reported peptidyl-α-amide forming enzymes, the human monooxygenase is Cu^{2+}- and ascorbate- dependent, while the lyase appears to have no co-factor requirements. The cell lines studied show parallel levels of expression of the two activities, and in neuro-endocrine cells the activities are associated with a subcellular fraction containing neurosecretory granules.

PHM, in common with many propeptide post-translational processing enzymes, has precise co-factor requirements. In the absence of these co-factors, bioactive peptide hormones are not produced. The level of one of the co-factors of PHM (ascorbate) has been reported to be maintained in neuro-secretory granules by specific cellular processes. As the requirement for co-factors seems to be a general phenomenon of post-translational processing enzymes, the steps of propeptide modification may be unrecognized control points for cellular homeostasis and function, and may present a target for novel anti-tumor therapeutic interventions.

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Oncogenes, Suppressor Genes and Growth Factors

CE 100 SCRUTINY OF THE HUMAN K-ras-2 GENE FOR MUTATIONS USING DENATURING GRADIENT GEL ELECTROPHORESIS, Ezra S. Abrams and Leonard Lerman, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139. Denaturing Gradient Gel Electrophoresis (dgge) can be used to detect single base changes in DNA fragments of up to several hundred bp in length. We have used the PCR to amplify the five coding exons of the K-ras-2 gene, so that mutations in each exon can be detected. However, maximal sensitivity is obtained only by careful choice of PCR oligonucleotides, and by addition of a GC clamp to each fragment. Computer programs which predict the melting properties of any DNA sequence were used to select PCR amplimers for each of the coding exons of the K-ras-2 gene. Up to three sets of PCR amplimers can be used simultaneously for both the PCR and subsequent analysis on a DGG. We have found 3 regions of the K-ras-2 gene which are polymorphic in the population; these DGG polymorphisms are two or three allele systems, and appear to be distinct from the known RFLPs in this gene. We are currently screening DNA from individuals with different tumors; DNA from both tumor and non-tumor tissue is analyzed to distinguish mutations from polymorphisms.

CE 101 "DOMINANT-NEGATIVE" MUTANTS BLOCK THE TRANSFORMING EFFECT OF C-JUN Brown, P.H., Sanders, D.A., Alani, R., and Birrer, M.J. NCI-Navy Medical Oncology Branch, NCI, and Uniformed Services University of the Health Sciences, Bethesda, MD.

The proto-oncogene c-jun codes for a nuclear protein which is a major component of the AP-1 transcriptional regulatory complex, and is expressed in various epithelial tissues, including adult and embryonic skin and lung tissues. The AP-1 complex appears to mediate the downstream effects of the well known tumor promoter, 12-0-tetradecanoyl-phorbol-13-acetate (TPA). We have previously shown that c-jun can substitute for TPA in cooperating with ras to transform primary rat embryo cells. In an attempt to block the transforming effects of c-jun, we have constructed a panel of mutant jun genes. These mutant jun genes code for proteins which are defective in transcriptional activation and cellular transformation. In addition, these defective proteins can inhibit the functions of the wild-type jun protein, therefore fulfilling the definition of a "dominant-negative" mutant. These dominant-negative mutants inhibit jun-induced transactivation and transformation of primary rat embryo cells. The different molecular mechanisms by which these dominant-negative mutants function, and their possible role as chemopreventative agents in blocking tumor promotion, will be discussed.

CE 102 POINT-MUTATION OF p53, BUT NOT ras, DETECTED IN BARRETT'S EPITHELIUM, SQUAMOUS CELL AND ADENOCARCINOMA OF THE ESOPHAGUS, Alan G. Casson, Tapas Mukhopadhyay, Karen R. Cleary, Jae Y. Ro, and Jack A. Roth, Departments of Thoracic Surgery and Pathology, M. D. Anderson Cancer Center, Houston, Tx 77030

To investigate the molecular genetic events contributing to the development of esophageal cancer, 24 human esophageal tumors were screened for ras and p53 oncogene mutations. Genomic DNA was extracted from archival pathology specimens comprising 10 esophageal squamous cell, 14 adenocarcinomas (7 with adjacent Barrett's epithelium), and normal esophagus from the resection margin. Target exons were amplified by the polymerase chain reaction (PCR). To screen ras (H-K- & N-), batteries of normal and mutated oligonuclide probes were selectively hybridized to PCR amplified DNA. Normal probes hybridized to each tumor, and for selected tumors, no hybridization was seen to any mutated probe. Single-strand conformation polymorphism (SSCP) analysis was used to screen p53 mutations by directly labelling (P-32) the PCR reaction during the final 10 cycles of amplification. Paired tumor and normal samples were electrophoresed across non-denaturing 12% polyacrylamide, 10% glycerol gels at 30W for 6-8 hours. Mutations were detected by relative mobility shifts between tumor and normal samples, and were localized to exons 5 (1 squamous tumor; 4/7 Barrett's) and 8 (1 adenocarcinoma). Sequence analysis confirmed mutations at codons 245 (GGC▸TGC) and 273 (CAT ▸CGT) for squamous and adenocarcinomas respectively. In summary, 1) no ras mutations were detected in esophageal cancer, 2) this is the first report of p53 mutations in esophageal squamous and adenocarcinomas, 3) p53 mutations have been found in Barrett's epithelium (pre-malignant) adjacent to adenocarcinoma, implicating this oncogene in tumorigenesis.

CE 103 FUNCTIONAL ANALYSIS OF RB1 MUTANTS FROM SMALL CELL LUNG CANCER.
Kratzke, R.A.[1], Otterson, G.A.[1], Lin, A.Y.[1], Horowitz, J.M.[2] and Kaye, F.J.[1] [1]NCI-Navy Oncology Branch, National Cancer Institute, Bethesda, MD 20814 and [2]Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710.

We have previously identified several small cell lung cancer (SCLC) specimens that exclusively produce mutant, underphosphorylated RB1 proteins (*PNAS* 87, 1990:2775; *PNAS* 87, 1990:6922). We have further characterized the previously reported inactivation of RB1 due to a single amino acid substitution (cys706->phe) by generating a series of *in vitro* point mutants of this critical domain and assaying for oncoprotein binding. These experiments define a peptide sequence within exon 21 whose conformation appears important for normal RB1 function. In addition, to determine if the naturally occurring mutant RB1 proteins might actively participate in the transformation pathway, we scored foci formation in rat embryo cells (REC) after transfection of mutated *RB1* in the presence or absence of activated *ras*. We did not observe foci formation with three independent, *in vivo RB1* mutants (deletion of *RB1* exon 21, deletion of exon 22, and a cys706 substitution). This is in contrast to experiments using mutated p53. In addition, whereas wild type p53 can suppress *myc/ras* foci formation, we did not observe foci suppression of *myc/ras* or SV40 large T/*ras* with increasing doses of wild type *RB1*, although continued expression of transfected wild type *RB1* mRNA was seen in transformed cells after serial passage. These experiments suggest the tumor suppressive functions of RB1 and p53 involve distinct cellular mechanisms.

CE 104 EXPRESSION AND REGULATION OF WILD TYPE p53 GENE (wtp53) IN HUMAN NON-SMALL CELL LUNG CANCER (NSCLC) CELL LINES CARRYING NORMAL OR MUTATED p53 GENE.
Tapas Mukhopadhyay, Adriana C. Cavender, Cynthia D. Branch, Jack A. Roth. M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas, 77030. NSCLC cell lines expressing genes encoding wild type or mutated p53 were transfected with a plasmid expression vector containing complete wtp53 cDNA in either sense or antisense orientation with the transcription of the insert DNA under control of an actin promoter. The vector contains a dominant neo gene marker for selection of stable transfectants. Stably transfected G418 resistant single colonies were isolated and analyzed separately. Results indicated that H322a and H596 cell lines with endogenous mutated p53 gene express high levels of mutant protein and RNA. These cells show a significant growth inhibition after transfection with wtp53 cDNA. No stable transfectant expressing antisense p53 DNA was obtained. A characteristic low level of wtp53 gene expression was detected in parental H460a and H226b cells carrying endogenous wtp53. Northern and Western blot analysis indicated overexpression of the wtp53 in all transfectants. ^{3}H-TdR incorporation and growth curve analysis data indicated that increased level of wtp53 gene expression in these cell lines did not have any significant effect on their growth kinetics. The H460a parental cell line is highly tumorigenic in nu/nu mice but overexpression of wtp53 in H460a transfectants did not change their growth and tumorigenicity. Flow cytometric analysis of cell cycle indicated that H460a and H226b transfectants overexpressing wtp53 have a much longer G_2 phase which led to a delayed entry of the cells to mitosis. Failure to obtain stable cell lines expressing antisense p53 RNA in cells carrying homozygous mutant alleles indicates that absence of wild type p53 expression is not sufficient for transformation. Mutant p53 genes are essential for the survival of these cells.

CE 105 HARVEY RAS GENES IN HUMAN AND EXPERIMENTAL ORAL CARCINOGENESIS
L.K. Torrance, W.A.Yeudall, S.S. Prime. Centre for the Study of Oral Disease, Dept. of Oral Medicine, Surgery and Pathology, University of Bristol, U.K.

This study examined the DNA of human oral squamous cell carcinoma (SCC) cell lines (1) and rat oral epithelial cells treated *in vitro* (2) and *in vivo* (3) with the carcinogen 4-nitroquinoline-N-oxide (4NQO), for point mutations in exons 1 and 2 of the Harvey *ras* gene (Ha-*ras*) using the polymerase chain reaction (PCR) and direct nucleotide sequencing.

Rat oral epithelial cells treated *in vivo* with 4NQO showed normal nucleotide sequence over exon 2. Three of 15 cell lines were heterozygous at codon 12 with a consistent GGA to GAA transition; the remaining 12 cell lines showed a normal nucleotide sequence. The presence of a point mutation did not reflect the anchorage independence or tumorigenicity of these cell lines.

Rat oral epithelial cells treated *in vitro* with 4NQO showed normal nucleotide sequence over exons 1 and 2, irrespective of carcinogen dose and despite a range of cellular phenotypes from immortal to overtly malignant. Control cultures of untreated rat oral keratinocytes were also shown to have a normal Ha-*ras* sequence.

Of the 7 human SCC cell line DNAs thus far examined, one sample has been shown to harbour a heterozygous GGT to AGT transition at codon 13. No other point mutations were detected.

The results suggest that there is a low incidence of Ha-*ras* point mutation in both human and experimental oral epithelial carcinogenesis.

(1). J. Pathol. *160*, 259-269 (1990). (2). Carcinogenesis *11*, 965-973 (1990). (3). Carcinogenesis *7*, 1723-1727 (1986).
L.K.T. is funded by the MRC.

Biology of and Novel Therapeutic Approaches for Epithelial Cancers of the Aerodigestive Tract

Epithelial Cell Growth and Differentiation/Carcinogenesis

CE 200 INDUCTION OF DIFFUSE MESOTHELIOMA IN CHICKENS BY INTRAPERITONEAL INOCULATION OF v-src DNA,

James M. England[1], Michael J. Panella[1], Donald L. Ewert[2], and Michael S. Halpern[2], [1]Department of Pathology and Laboratory Medicine, Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, PA 19129; [2]Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104

The presence of peritoneum-based, cytokeratin positive tumor cells, as detected under conditions of intravenous inoculation of chickens with a v-src positive DNA fragment, raised the possibility that such DNA may be inductive for diffuse mesothelioma. To investigate this possibility, chickens were inoculated intraperitoneally with v-src DNA and the resultant peritoneum-based tumor tissue was subjected to histopathological analysis. On the basis of i) morphology (biphasicity, wherein epithelioid and fibroblastoid tumor cells are in close association), ii) immunohistochemistry (cytokeratin-positivity of tumor cells)/histochemistry (hyaluronic acid production by the tumor cells), and iii) ultrastructure (tumor cell-associated microvilli), we concluded that this tissue was analogous to the diffuse mesothelioma of humans.

CE 201 DT-DIAPHORASE ACTIVITY IN NEOPLASTIC MOUSE AND HUMAN LUNG CELLS, Alvin M. Malkinson, David Siegel, David Ross, Larry G. Thaete, Daniel C. Chan, Paul A. Bunn, Herbert K. Oie,* and Adi F. Gazdar.* Molecular Toxicology Program and Colorado Cancer Center, School of Pharmacy and Department of Medical Oncology, University of Colorado, Boulder, CO 80309 and Denver, CO 80262, *NCI-Navy Medical Oncology Branch, NCI, Bethesda, MD 20814.

The flavoprotein, DT-diaphorase (DTD) is generally considered a detoxification enzyme but can also activate certain aziridinyl antitumor agents. In mouse lung DTD was localized to alveolar type II cells by histochemistry, using the NADH-dependent, dicumarol-inhibitable reduction of the dye, 2,6-dichlorophenolindophenol as a detection system. Significant DTD activity was also detected in broken cell extracts from uninvolved lung and type II cell lines. DTD activity was negligible, however, in chemically-induced primary tumors, cell lines derived from these tumors, and spontaneous transformants of non-tumorigenic type II cell lines. DTD is therefore useful in distinguishing normal from neoplastic mouse type II cells, and may exert a role in the postulated differential responsiveness to oxidant injury of normal cells vs. cells at early stages of tumor progression. Forty cell lines derived from primary and metastatic human lung cancer were also assayed. DTD activity was similar (median = 10 nmol/min/mg protein; range = 0 - 271) in 14 small cell lung carcinoma (SCLC) lines to that in normal human lung. In contrast, DTD activity was significantly ($p < .0001$) higher (median = 585; range = 3 - 2690) in 26 non-small cell lung cancer (NSCLC) lines. A highly significant difference ($p < .001$) was also noted when the low DTD activities (median = 39; range = 0 - 1590) in cell lines containing neuroendocrine (NE) markers (14 SCLC lines, 6 carcinoid, and 5 other NSCLC lines) were compared with the higher activities of 15 NSCLC lines lacking NE markers (median = 585; range = 10 - 2690). Increased DTD activity in NE-negative NSCLC cells may provide a useful in vitro marker and encourage more appropriate design of chemotherapeutic regimens based on DTD activity. (Supported by USPHS grants CA 33497 and CA 51210 and ACS grant RD-312).

CE 202 IMMORTALIZATION OF HUMAN ORAL KERATINOCYTES WITH TYPE 16 HUMAN PAPILLOMA-VIRUS. No-Hee Park, Byung-Moo Min, Min Jung Huang, Sheng Lin Li, and Henry M. Cherrick, University of California, Los Angeles, School of Dentistry, Los Angeles, CA 90024.

Human papillomavirus (HPV) is associated with human malignancies. Of more than 60 genotypes of HPV, HPV types 16 (HPV-16) and 18 (HPV-18) are most frequently associated with malignant lesions. To investigate the biological role of HPV in oral carcinogenesis, we transformed normal human oral keratinocytes (NHOK) with cloned HPV-16 DNA and characterized the transformed cells. Seventy percent confluent primary human oral keratinocytes were transfected with pMHPV-16d, a head-to-tail dimer of HPV-16 DNA inserted into the Bam HI site of plasmid pdMMT$_{neo}$, by using liposome. After the transfection, two G418-resistant cells were cloned, subcultured, and named Human Oral Keratinocytes-16A and -16B (HOK-16A and -16B) lines. The proliferation patter, morphology, physical state of HPV-16 DNA, viral expression, and c-*myc* gene expression of these cell lines were investigated. While NHOK or NHOK transfected with vector only showed limited life-span, the HOK-16A and -16B lines showed an immortality and different morphology from the normal counterpart. Approximately 20-40 viral DNA copies per cell were shown to be stably integrated into cellular genome of these cell lines. The Northern blot hybridization analysis showed an overexpression of c-*myc* proto-oncogene and an expression of several viral specific genes including E6/E7 genes from these cell lines. However, these cell lines failed to produce tumors in nude mice, indicating that they are partially transformed cells and are not tumorigenic. The HOK-16A and -16B lines should, therefore, prove useful for investigating the multistep event of oral carcinogenesis. (In part supported by a grant from the STRC Inc., No. 0231)

CE 203 MODULATION OF GENE EXPRESSION BY TUMOR PROMOTERS,Neeta Singh, Department of Biochemistry,All India Institute of Medical Sciences New Delhi 110029,INDIA

HL-60 cells were exposed to tumor promoters phorbol-12-myristate-13-acetate (PMA),benzoyl peroxide (BP) & mezerein (M) & their effects studied on poly ADP-ribosylation,which is a post translational modification of chromatin proteins catalysed by the enzyme poly ADPR Transferase using NAD as the substrate.Poly ADP-ribosylation has been shown to be involved in several aspects of chromatin structure & function.All the tumor promoters studied stimulated poly ADPRT activity by 1.4 to 1.6 fold.This was accompanied by a concomitant drop in NAD levels.An increase in calcium levels of 1.6 to 2 fold was also observed.PMA has been shown to stimulate protein kinase C.The role of this enzyme with respect to BP & M will be discussed.The above results may be important events in tumor promotion & chemical carcinogenesis.

CE 204 MORPHOLOGICAL TRANSFORMATION OF HUMAN PAPILLOMAVIRUS IMMORTALIZED HUMAN BRONCHIAL EPITHELIAL CELLS FOLLOWING EXPOSURE TO IONIZING RADIATION, James C. Willey, David Maillie, and Ellen Miles, Environmental Health Sciences Center and Department of Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, N.Y., 14642

Uranium miners have an increased risk for bronchogenic carcinoma that is directly correlated with levels of radon in the mine. In order to investigate mechanisms for this increased risk, we are evaluating the effects of ionizing radiation on human papillomavirus (HPV) immortalized human bronchial epithelial cells. It was recently reported that irradiation of SV40 immortalized human keratinocytes transforms these cells so that they form colonies in soft agar and form tumors in immunosuppressed mice (Thraves et al, PNAS, 1990). For this study, we exposed the HPV16 or HPV18 immortalized cell lines (BEP2D or BEP3D respectively) to radiation from a ^{137}Cs source and evaluated effects on colony forming efficiency (CFE), morphology, and growth in soft agar following exposure to 2, 4, 6, 8, and 10 Gy. We have determined that the D_0 for CFE is approximately 2 Gy for BEP2D cells. For transformation assays T75 flasks were inoculated with 5×10^5 cells. Cytotoxicity became apparent 24-48 hours following irradiation with an increase in cell size and decrease in mitotic figures, followed by cell sloughing over several days. In the flasks irradiated with 8 or 10 Gy, because most of the cells received a lethal dose, individual colonies from surviving cells became apparent four to five days following exposure. After the first exposure, the morphology of cells within these colonies was unchanged. After the second exposure, some of the colonies of BEP2D cells developing in flasks receiving 8 or 10 Gy appeared morphologically transformed, while no transformants were observed in the BEP3D cells. The transformed cells are smaller, have more mitotic figures and have an increased tendency to pile up. Morphologically transformed colonies have been induced in the BEP2D cells in two experiments and multiple flasks receiving 8 or 10 Gy. Twice irradiated BEP2D cells were evaluated for ability to form colonies in soft agar. While no colonies formed in the non-irradiated cells, increasing numbers of colonies formed in the 8 and 10 Gy irradiated cells. This work was supported by NIEHS grant ES01247.

CE 205 A MOUSE MODEL FOR HUMAN HEAD AND NECK CANCER USING 4-NITROQUINOLONE-N-OXIDE (4NQO), B. Wright, B. Hawkins, S. Martinez, F. Hendler, Departments of Surgery, Medicine, and Biochemistry, U. of Louisville and Louisville VA Medical Center, J. G. Brown Cancer Center, Louisville, KY 40292.

Premalignant and malignant oropharyngeal lesions were produced in CBA mice following topical application of 4NQO to the palate. One group was treated three times per wk for 16 wks to observe the progression of neoplasia. These mice were then sacrificed at 4 wk intervals. The following neoplastic changes were observed: 1) mild atypia at 16-24 wks; 2) moderate atypia and dysplasia at 24-28 wks; 3) severe dysplasia and carcinoma in situ at 33 wks; 4) invasive squamous cell carcinoma of the tongue and palate at 33 wks. All mice had at least 1 squamous cell carcinoma of the oropharynx >2mm at 49 wks. Other mice were treated from 4 to 16 wks and sacrificed at 49 wks to determine the duration of exposure required to produce carcinomas. All mice treated greater than 12 wks developed invasive carcinomas. Lesions from CIS to invasive carcinoma were seen in mice treated 4 to 8 wks. The 4 and 8 wk groups also had mice with no noted lesions at 49 wks. Neoplastic transformation was present in all mice treated $\geq$12 wks and there is clearly a field defect. However, the changes observed with 4NQO are distinctly different from DMBA treated rodents. DMBA induced premalignant and neoplastic changes occur throughout the entire exposed area and develop while the carcinogen is still being applied. However, with 4NQO treated mice, mild atypia is the most severe lesion that occurred after the last application of 4NQO at 16 wks. With 4NQO, morphologically normal mucosa predominates in treated areas. Thus, this model more closely parallels the progression of human head and neck cancer.

Biology of and Novel Therapeutic Approaches for Epithelial Cancers of the Aerodigestive Tract

Chemoprevention

CE 300 COMPLEX REGULATION OF TGF-ß EXPRESSION BY RETINOIC ACID IN THE VITAMIN A-DEFICIENT RAT, Adam B. Glick, Bryan K. McCune, Nariman Abdulkarem, Kathleen C. Flanders, Jeanne A. Lumadue*, Joseph M. Smith and Michael B. Sporn. National Cancer Institute, Bethesda, MD 20892 and *Department of Pathology, The Johns Hopkins School of Medicine, Baltimore, MD 21205.

We report the results of a histochemical study, using polyclonal antipeptide antibodies to the different TGF-β isoforms, which demonstrates that retinoic acid regulates the expression of TGF-β in the vitamin A-deficient rat. Basal expression of TGF-β2 diminished under conditions of vitamin A deficiency. Treatment with retinoic acid caused a rapid and transient induction of TGF-β2 and TGF-β3 in the epidermis, tracheobronchial and alveolar epithelium, and intestinal mucosa. Induction of TGF-β1 expression was also observed in the epidermis. In contrast to these epithelia, expression of the three TGF-β isoforms increased in vaginal epithelium during vitamin A deficiency, and decreased following systemic administration of retinoic acid. Our results show for the first time the widespread regulation of TGF-β expression by retinoic acid in vivo, and suggest a possible mechanism by which retinoids regulate the functions of both normal and pre-neoplastic epithelia.

CE 301 EFFECT OF RETINOIC ACID ON GROWTH OF NON-SMALL CELL LUNG CANCER CELL LINES AND EXPRESSION OF THE RETINOBLASTOMA PROTEIN. Maxwell SA, Mukhopadhyay T, Cavender A, Johnson M, and Roth JA. Department of Thoracic Surgery, M. D. Anderson Cancer Center, Houston, Texas 77030.

Non-small cell lung cancer lines exhibit a varied response in growth properties to retinoic acid. The growth rate in culture of H460 cells is increased approximately two-fold, but growth in soft agar is inhibited, after 3 days of incubation with 5 uM retinoic acid. In contrast, the growth properties of the H226b and H322a cell lines are unaffected by 5 uM retinoic acid. The Rb protein is expressed predominantly as a super-phosphorylated form in the H460, H322a, and H226b cells. After 3 days exposure of H460 cells to 5 uM retinoic acid, the level of super- phosphorylated Rb protein was found to increase to three-fold over that expressed in untreated cells. Prolonged exposure (9-20 days) to retinoic acid resulted in the conversion of about one-half of super-phosphorylated Rb to a dephosphorylated form. In contrast to H460 cells, the phosphorylation of Rb remained unchanged in retinoic acid-resistant H322a and H226b cells. In addition to changes in Rb expression and phosphorylation, a 75K Mr Rb-related protein is induced in H460 cells, but not in H322 cells, by retinoic acid. Preliminary studies have ruled out in vitro-generated proteolytic degradation product of intact 105K Mr Rb protein suggesting that the Rb-related 75K Mr protein is induced in H460 cells in situ by retinoic acid.

CE 302 ABNORMALITIES IN RETINOIC ACID RECEPTOR BETA (RAR-β) ARE COMMON IN HUMAN LUNG CANCER, Benjamin G. Neel[1], Johannes F. Gebert[1], Nadeem Moghul[1], John V. Frangioni[1], and David Sugarbaker[2] [1]Molecular Medicine Unit, Beth Israel Hospital, Boston, MA. and [2]Dept. of Thoracic Surgery, Brigham and Women's Hospital, Boston, MA.

Most evidence suggests that a tumor suppressor gene(s) is located in the 3p21-24 region, and is deleted in nearly all lung cancers. Since retinoids modulate epithelial cell growth and differentiation, and vitamin A deficiency causes bronchial squamous metaplasia, we studied RAR-β expression in twenty-four lung cancer cell lines and nine primary tumors. In normal tracheobronchial epithelium and in normal bronchial epithelial cells maintained in short term culture, there are two RAR-β mRNAs, 3.2 and 2.8 kb. About 25% of lung cancer cell lines lacked detectable RAR-β expression, one had trace amounts of a truncated transcript, ten to fifteen percent showed selective loss of the lower mRNA and the remainder showed the normal pattern. In some cell lines, expression of one or both transcripts could be induced by treatment with retinoic acid, but in others, the RAR-β gene was completely silent. Absent or significantly decreased RAR-β RNA was also found in 3 primary tumors and another tumor showed selective loss of the lower transcript. Three of 14 cell lines analyzed by Southern blotting had gross DNA rearrangements in at least one RAR-β allele. We suggest that abnormalities in the structure and expression of RAR-β play an important role in the pathogenesis of human lung cancer. RAR-β may be a new type of tumor suppressor gene, which maintains normal cytodifferentiation.

CE 303 **INTERMEDIATE BIOMARKERS FOR EPITHELIAL CELL TRANSFORMATION: RELEVANCE TO CANCER CHEMOPREVENTION** *Nitin T. Telang, H. Leon Bradlow and Michael P. Osborne. Breast Cancer Research Laboratory, Memorial Sloan-Kettering Cancer Center, and Institute for Hormone Research New York.* Intermediate biomarkers provide a quantitative basis to predict the target tissue susceptibility for carcinogenic insult at molecular, metabolic and cellular levels. This comparative study has utilized mammary explant cultures from murine and human tissue to examine i.) alteration in DNA repair synthesis, perturbation in ras oncogene expression (molecular biomarkers), and alteration in estradiol metabolism (endocrine biomarker) after in vitro exposure to prototype chemical carcinogens, and ii.) ability of selected dietary fatty acids to modulate the carcinogenic response. Treatment of the mammary tissues with chemical carcinogens resulted in 7-22 fold increase ($P=0.001$) in hydroxyurea insensitive ^{3}H-thymidine uptake in the cellular DNA (DNA repair). The same carcinogens induced 2-5 fold increase in [α-^{32}P] GTP binding to cellular ras p21 ($P=0.004$), and 3-16 fold increase ($P=0.007$) in the conversion of 17β estradiol to 16α-hydroxestrone, a presumptive promoter of mammary cell transformation. Treatment of the cultures prior to and during carcinogen exposure with the tumor promoting linoleic acid substantially enhanced, while treatment with the tumor suppressing eicosapentaenoic acid significantly suppressed ($P<0.005$) the carcinogen-induced perturbation of these intermediate biomarkers. The perturbation of same biomarkers in mouse and human tissues during initiation of transformation and effective modulation of these end points by the fatty acids suggest that the molecular and endocrine biomarkers may provide clinically relevant endpoints for evaluation of potential human carcinogens and for efficacious chemopreventive interventions. (Supported by NIH grant #R29 CA44741 to NTT and by the Iris & B. Gerald Cantor Fund and the Wanda Jablonski Fund to MPO).

Biology of and Novel Therapeutic Approaches for Epithelial Cancers of the Aerodigestive Tract

Growth Factors

CE 400 THE ROLE OF GROWTH FACTOR PRODUCTION IN HUMAN AND RAT EPITHELIAL CARCINOGENESIS

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This study examined EGF/TGF-α and TGF-ß production in two models of epithelial tumour progression using human ras-transfected keratinocytes - HaCaT (1) and cell lines derived from 4NQO-mutated rat oral epithelial tissues (2). Growth factors were separated from conditioned media by ion exchange chromatography and quantified in competitive inhibition assays. Receptor profiles of individual cell lines were examined using radioligand binding assays.

HaCaT cells expressed high levels of EGF receptors but produced no detectable EGF/TGF-α. ras-transfected clones had fewer EGF receptors than HaCaT cells but tumour progression was associated with a progressive increase in EGF receptor expression and EGF/TGF-α production. There was a decrease in TGF-ß production associated with tumour progression and a marked loss of TGF-ß receptors in ras - transfected clones.

Tumour progression in 4NQO rat oral keratinocytes was characterised by decreased EGF/TGF-α production and increased EGF receptor expression. Normal controls produced little EGF/TGF-α but expressed the greatest number of receptors. TGF-ß production was unrelated to receptor expression and malignancy.

The results suggest that the transfection of human keratinocytes by Ha-ras as a model of tumour progression is associated with decreased TGF-ß and increased EGF/TGF-α production. By contrast, tumour progression in rat oral epithelial cells was associated with a decrease in EGF/TGF-α production and appeared unrelated to TGF-ß secretion.

1. Cancer Research 50: 2840-2847 (1990)
2. Carcinogenesis 11: 965-973 (1990)

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CE 401 ALTERATION OF PREPROGRP GENE EXPRESSION IN SMALL CELL LUNG CANCER CELLS BY PHORBOL ESTERS. M. Draoui, T. Moody, Z. Fathi and J. Battey. Dept. Biochem. & Mol. Biology, George Washington Univ. Med. Ctr., Washington, D.C. 20037 and Lab. Neurochem., NINDS, NIH, Bethesda, MD 20892.

GRP is an autocrine growth factor for some SCLC cells. The preproGRP gene, which has 2 introns and is localized to human chromosone 18q, has been cloned from SCLC cells. G-protein coupled receptors, such as VIP and GRP, regulate the production of SCLC second messengers such as cAMP and diacylglycerol. Here we investigated if these second messengers, which activate protein kinases, alter GRP gene expression in SCLC cells. By Northern analysis, cell lines NCI-H345 and H209, had appreciable levels of GRP mRNA (0.9 kilobases). TPA increased the level of GRP mRNA relative to an actin control in a concentration and time dependent manner. One μM TPA increased the GRP mRNA approximately 3-fold after 8 hours. The effect was not observed using inactive phorbol esters. Because TPA did not appear to alter GRP mRNA metabolism, protein kinase C may alter GRP gene expression. Also, 8-bromo-cAMP or forskolin increased GRP mRNA levels. These data suggest that there are protein kinase A and C sensitive components in SCLC cells. In this regard, nucleotide sequencing reveals a cAMP-responsive element (TGACGTCA) upstream from the GRP gene. Supported in part by NCI grant CA-53477.

CE 402 MACROPHAGE-COLONY STIMULATING FACTOR (CSF-1) ENHANCES INVASIVENESS IN CSF-1 RECEPTOR (CSF-1)-POSITIVE LUNG CANCER CELL LINES, Filderman AE, Bruckner A, Kacinski B, Remold H, Pulmonary Section, New England Deaconess Hospital, and Harvard Medical School, Boston, MA; Yale University School of Medicine, New Haven, CT.

Exposure of CSF-1R-positive cells to CSF-1 enhances the ability of the cell to invade normal tissues. Because of this action of CSF-1 on normal cell types, we examined whether CSF-1 had a similar effect on lung cancer cells found to contain CSF-1R. Cell lines known to be positive (BT-20) or negative (MCF-7) for CSF-1R expression were used as controls. Two lung cancer cell lines- A549 and Calu-1- both positive for CSF-1R expression, were starved for 24 hours in serum-free medium (to upregulate CSF-1R expression), cultured with varying concentrations of CSF-1 (10 to 750 ng/ml) for 1 to 48 hours, labeled with IUDR, and invasiveness was assessed using an amnion invasion assay. CSF-1 exposure induced cellular invasion up to 4-fold in both lung cancer cell lines at an optimal concentration of 250 ng/ml. Invasiveness was increased maximally in cells cultured with CSF-1 for 24 to 48 hours. CSF-1 had a similar effect on invasiveness of BT-20 cells induced by glucocorticoids to express high levels of the CSF-1R. In contrast, CSF-1 had no effect on invasiveness of the CSF-1R-negative MCF-7 cell line. There was also no effect of other factors including GM-CSF, G-CSF, gamma-interferon, or TNF on invasiveness of the lung cancer lines. Experiments using anti-urokinase antibodies (MPW5UK) and plasminogen-activator inhibitors (PA-I, PAI-2) significantly diminished invasiveness of the CSF-1-stimulated A549 and Calu-1 cell lines. Thus, CSF-1 induces invasiveness in CSF-1R-positive lung cancer cell lines, and this effect on invasiveness may be mediated through the urokinase system.

CE 403 EGF RECEPTOR (EGF-R) EXPRESSION PREDICTS PROGNOSIS IN SQUAMOUS CELL CARCINOMAS (SCC) OF THE RESPIRATORY TRACT. F. Hendler, A. Shum-Siu, B. Hawkins, M. Oechsli, S. Martinez, B. Ozanne. University of Louisville, Louisville VA Med Ctr, Louisville KY 40292 and Beatson Institute, Glasgow, Scotland, UK.
The EGF-R is consistently overexpressed in SCC of the respiratory tract. The receptor content of tumors was determined by the binding of [^{125}I]-EGF-R1, a monoclonal antibody, to cryopreserved tissue sections in a competitive direct binding assay. Binding was quantitated by computerized grain counting of emulsion autoradiography. In 107 respiratory tract tumors studied, 69 of 72 SCC had increased EGF-R greater than that detected in normal skin (range 1.3 to 20X). Two of the 3 tumors with low EGF-R expression were associated with overexpression of the EGF receptor in the adjacent normal epithelium. Receptor overexpression is associated with gene amplification (>5X) in 30% of tumors. All tumors with $\geq$3 fold overexpression of receptor have significant gene amplification. These tumors are more undifferentiated than those with more modest overexpression ($p \leq .04$). Nodal disease, keratin formation, vascular invasion were not associated with the level of EGF receptor expression. Overexpression is associated with poor survival. If the EGF-R is $\geq$3 fold normal skin, median survival from diagnosis is 9 months; if <3 fold, median survival is 26 months ($p \leq .001$). Those patients whose tumors have less than 3 fold overexpression of EGF-R can be subdivided into 2 groups: those that do not respond to therapy and survive similarly to those with high EGF-R levels and those that do well having a median survival of 57+ months. Therefore, the EGF-R appears to be segregating respiratory tract tumors into biologically different populations which predict prognosis.

CE 404 ANTI EGF-RECEPTOR ANTIBODY-DRUG IMMUNOCONJUGATES, David A. Johnson, William L. Scott, Cynthia L. Nichols, Magda C. Gutowski and Daniel V. Fix. Cancer Research MC7R1, Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285.

The EGF receptor reactive antibody of Mendelsohn et al, 225 IgG1, has good reactivity with human lung squamous carcinoma (Sobol et al JNCI 79:403, 1987). This antibody was previously used by us to produce *vinca* alkaloid immunoconjugates with activity against human lung squamous carcinoma xenograft targets (Gutowski et al, Antibody Immunoconj. Radiopharm. 3:66, 1990). These investigations have been expanded to include evaluation of methotrexate based conjugates with the 225 IgG1 antibody. Methotrexate gamma hydrazide was linked to the antibody via lysine (Scott et al, Antibody Immunoconj. Radiopharm. 3:64, 1990). Resultant immunoconjugates retained good antigen binding capacity when evaluated in *in vitro* live cell binding assays. Drug activity was maintained as demonstrated in *in vitro* cytotoxicity assays. A second lung carcinoma reactive antibody, L/1C2, was conjugated to methotrexate with similar *in vitro* results. When tested *in vivo* using human tumor xenograft models, MTXHYD immunoconjugates showed significant anti tumor activity against established human lung carcinoma xenografts. This activity was clearly superior to that obtained with free antibody or with equivalent amounts of unconjugated methotrexate or methotrexate gamma hydrazide. These results indicate that methotrexate based immunoconjugates are viable candidates for evaluation as therapeutic agents.

CE 405 A SOLID PHASE ASSAY FOR PEPTIDE α-AMIDATING ACTIVITY

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Small cell lung cancer cell lines produce neuropeptides and about half of all neuropeptides are carboxyamidated. In almost all cases this feature is required for full biological activity. Enzymatic activities which catalyze the conversion of glycine-extended precursor peptides to amidated peptides have been characterized in several mammalian species. We have developed a rapid, specific assay for peptide amidating activity. The solid phase assay uses a monoclonal antibody, 2A11, to capture the amidated form of human gastrin releasing peptide (GRP), the product of the amidation reaction. A variety of cancer cell line extracts have been assayed:

		Amidating activity:	Average	Range
Neuroendocrine cell lines	N=6	(pmole/mg/hour)	15.6	3.3-53
Non-neuroendocrine cell lines	N=3		2.1	1.7-2.6

The level of peptide amidating activity as measured by this assay may indicate the proliferative potential of a tissue of interest.

CE 406 CYTOGENETIC ABNORMALITIES AND OVEREXPRESSION OF RECEPTORS FOR GROWTH FACTORS IN NORMAL BRONCHIAL EPITHELIUM AND TUMOR SAMPLES OF LUNG CANCER PATIENTS, Gabriella Sozzi, Monica Miozzo, Elda Tagliabue, Claudia T. Cariani, Luciano Lombardi, Silvana Pilotti, Ugo Pastorino, Marco A. Pierotti and Giuseppe Della Porta, Istituto Nazionale Tumori, Via G. Venezian 1, 20133 Milano, Italy

A cytogenetic analysis was performed on direct preparations and short-term cell cultures of lung tumor and normal bronchial epithelium of 21 patients carrying either a first or a second primary lung cancer. In 11 tumors (8 squamous cell carcinomas, 1 adenocarcinoma, 1 mucoepidermoid carcinoma and one small cell lung carcinoma) successfully analyzed, pseudodiploid and hyperdiploid karyotypes were observed with a heterogeneous pattern of chromosome abnormalities but with a consistent involvement (5 cases) of the short and the long arm of chromosome 3. The normal bronchial epithelial cells had a normal diploid chromosome number in 11 patients whereas in 8 cases structural clonal and non-clonal chromosomal abnormalities were observed. The chromosome changes were different but chromosome 7 was involved in 4 cases. In addition, overexpression of the growth factor receptors EGFR and/or HER-2/NEU was found in 11 out of 20 tumors and in 7 of 15 bronchial epithelium samples. These findings suggest that early genetic lesions could be present in the normal bronchial epithelial cells that are the target of further complex and multiple genetic changes occurring during the pathogenesis of lung cancer.

CE 407 GROWTH INHIBITION OF HUMAN STOMACHIC CANCER CELL LINES WITH ANTI-TRANSFORMING GROWTH FACTOR β MONOCLONAL ANTIBODIES, Zhao Minshun, Chinese Academy of Medical Sciences, Beijing, China

It is well known that the proliferation of tumor cells in culture is controlled by various growth factors, and a similar control mechanism is postulated for the control of tumor cell growth in vivo. Therefore, antibody against growth factor may provide useful therapeutic agents. A biologically active transforming growth factor β (TGF-β) was isolated and purified from human placental villus tissue by gel permeation chromatography followed by acid/ethanol extraction. The molecular weight of TGF-β was estimated to be 25,000-dalton by gel filtration on Bio-Gel p-30. Aliquots were assayed for colony-forming activity in the soft agar assay: in the presence of 2ng/ml EGF, TGF-β was able to induce the phenotype transformation of normal cells and to confer on them the ability to form progressively growing colonies in soft agar. Monoclonal antibodies were prepared against TGF-β . The addition of MoAbs to culture medium inhibits the proliferation of human stomachic cancer cells in culture. TGF-β is known to stimulate proliferation of various types of cells in culture, but when MoAbs added to the culture medium of tumor cell, it results in growth inhibition. This suggests that binding of MoAbs to TGF-β and blocking of TGF-β from binding to surface of tumor cells, is the essential process leading to suppression of tumor cell growth in culture.