drugs and combinations of therapy including lung cancer cell lines, xenografts, including orthotopic (lung) xenografts and xenografts made directly from patient samples without intervening cell culture. For this reason we have developed a very large (>200) panel of lung cancer cell lines of all histologic types and including all of the various genetic changes found in lung cancer. Coupled with this we have developed xenograft models (including orthotopic models) of lung cancer in >50 lung cancers including xenografts made from lung cancer lines and from primary patient materials. We can determine expression profiles in these preclinical models as a step toward developing biomarker signatures that can be tested in the clinic. The use of bioluminescence imaging (BLI) to help follow the growth and response of xenografts to therapy is an important new tool. From all of these we need to know how good or bad such preclinical model systems are in both identifying drug response phenotypes and in developing predictive signatures that will work in patient specimens. For example the whole EGFR TKI story coupled with EGFR TKI domain mutations as well as EGFR TKI resistance was present in the large panel of lung cancer cell lines and xenografts we have developed. Because of this we have been testing several new drugs in development alone and in combination such as a SMAC mimetic and Peloruside A. We have found not only dramatic differences between lung cancer cells in response to the drugs as single agents, but complex patterns of synergy (which are often dramatic) when they are combined with standard drugs. Genome Wide Approaches to Discovery of New Therapeutic Targets Including Synthetic Lethal Screens Large scale DNA sequencing and DNA amplification screens are going on to find oncogenic changes that represent “druggable” targets. However, another approach that is gaining attention are genome wise functional screens for such targets such as genome wide screening of siRNA and shRNA libraries for genes that when knocked down sensitize tumor cells to low doses of chemotherapy agents. This approach has led to the discovery of 87 genes, which when knocked down by siRNAs dramatically sensitize lung cancer cells to paclitaxel. This information could provide “signatures” for predicting paclitaxel response, but also targets that could be addressed in combination with paclitaxel. Some of these (such as a panel of cancer testis antigen genes) are selectively expressed in tumor cells. Of course these siRNA/shRNA screens can be coupled with screening of small molecule libraries. Related to this is miRNA profiling which identified two miRNAs whose expression was inversely correlated with paclitaxel resistance. By re-expressing these two miRNAs, paclitaxel resistant lung cancers were converted to a paclitaxel sensitive phenotype. Thus, such miRNAs can be considered for therapeutic development. Lung Cancer Stem Cells There is considerable evidence mounting that a small subset of lung cancer cells (~1% or less of the total tumor cell population) has the properties consistent to give immortal and metastatic disease and are referred to as “cancer stem cells” or “stem-like” cells. We have developed ways to isolate and study these cells and they have clear differences in gene expression programs for a variety of stem cell proliferation genes as well as increased ability to clone in soft agar, form tumors in xenografts, and give metastatic disease in xenografts. Key issues will be to develop good, easy to use markers (such as monoclonal antibodies) to identify these cells, to see how their response to therapy compares with the bulk of the tumor (since it is likely they may be more resistant to therapy), and whether their presence or amount is of prognostic significance. Of course their identification in preneoplastic stages will be important as new molecular markers for early lung cancer detection. New Model Systems for Studying Lung Cancer Pathogenesis In order to determine which steps are most important we have developed a new system for immortalizing human bronchial epithelial cells (HBECs) in the absence of viral oncoproteins using hTERT (human telomerase providing part of the immortalization pathway) and cdk4 (bypassing the p16/Rb checkpoint) expression vectors. We have established over 30 such strains from persons with and without lung cancer. These expressed stem cell markers, can differentiate in 3 dimensional cultures into ciliated epithelium and are do not expression malignant properties. We have added oncogenic KRAS (found in 30% of non-small cell lung cancers, NSCLC) and knocked out p53 (found in 50% of all lung cancers) alone or together. These (together with hTERT and cdk4) only partially progress the HBECs toward malignancy. We have also made changes in multiple other genes such as CMYC, BCL2, PTEN, and EGFR and find that again these only partially progress HBECs towards malignancy. However, combining KRASV12, p53 knockdown, and CMYC coupled with biologic selection in SCID mice can identify a subset of fully malignant tumor cells. In addition, the addition of these oncogenic changes leads to changes in cytokine secretion patterns that provide new information for early lung cancer detection through screening for these cytokines in blood. These HBECs are providing a new model for systematically testing the importance of additional genetic changes in lung cancer pathogenesis. In developments by several other groups, mouse models of lung cancer have been developed based on genetic abnormalities found in human lung cancer with development of mouse systems for specific development of these abnormalities in lung as a target tissue. These have been developed for non-small cell lung cancer (NSCLC) (based on oncogenic KRAS) and for small cell lung cancer (SCLC) (based on p53 and Rb abnormalities). These models are proving extremely valuable in the preclinical testing of early detection, chemoprevention, and new treatment strategies.

M10-02 Emerging Field of Lung Cancer Research, Tue, Sept 4, 10:30 - 12:00

Inflammation in lung carcinogenesis: the complicity of host cellular networks in lung tumorigenesis

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The pulmonary environment presents a unique milieu in which lung carcinogenesis proceeds in complicity with the host cellular network. The pulmonary diseases that are associated with the greatest risk for lung cancer are characterized by abundant and deregulated inflammation. Pulmonary disorders such as chronic obstructive pulmonary disease (COPD)/emphysema and pulmonary fibrosis are characterized by profound abnormalities in inflammatory-fibrotic pathways. For example, among the cytokines and growth factors aberrantly produced in these lung diseases and the developing tumor microenvironment (TME), IL-1β, PGE2, and TGF-β have been found to have deleterious properties that simultaneously pave the way for both epithelial mesenchymal transition (EMT) as well as destruction of specific host cell-mediated immune responses. Loss of E-cadherin expression by epithelial cells is a characteristic of EMT and is associated with progression of lung cancer. Our recent work and that of others implicates E-cadherin expression as a marker of sensitivity to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) in NSCLC. In fact a biological signature that would allow for the detection of EGFR TKI-sensitive NSCLC tumor cells with an activated EGFR pathway has recently been identified, independent from the mechanism leading to activation. A gene signature indicative of an epithelial
versus mesenchymal phenotype appears to be predictive of EGFR TKI-mediated growth inhibition in NSCLC tumor cell lines. These findings also suggest that EMT features may serve as potential biomarkers in predicting EGFR TKI clinical activity in NSCLC patients. Our recent studies have shown that inflammatory mediators are potent regulators of E-cadherin expression in lung cancer: IL-1β and PGE2 contribute to the downregulation of E-cadherin expression in NSCLC via distinct pathways that include induction of the transcriptional repressors ZEB-1, Snail1 and Twist, and the PGE2 pathway upregulates the E-cadherin ubiquitin ligase, Hakai. Here we report on new studies that define the cellular elements within the TME that contribute these inflammatory mediators affecting expression and maintenance of E-cadherin, as well as other markers of EMT. These relationships are being assessed in organotypic model systems. Thus, TME-mediated inflammation provides a pathway to both EMT and resistance to targeted therapies in NSCLC. Full definition of these pathways will afford the opportunity to intervene in specific inflammatory events mediating lung tumorigenesis and resistance to therapy.

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Mouse models of lung cancer

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In the post-genome era mouse is becoming an increasingly important tool of lung cancer research. The application of innovative technologies to generate accurate mouse models is likely to accelerate the discovery of new molecular targets and imaging biomarkers for the early detection of lung cancer (1,2). It remains to be seen which models will be most suitable for preclinical tests (3).

Comparative oncology of lung tumors

While mice and humans share many similarities in the molecular pathology of lung tumorigenesis, there are also differences. Much of the earlier work focused on chemical carcinogenesis establishing parallel roles of many toxic chemicals as well as genetic and epigenetic alterations in lung carcinogenesis of mice and humans (4). The incidence of spontaneous (and induced) lung tumors among the commonly used inbred strains varies markedly from high 61% in A/J mice to very low 6% in C57Bl6 for males at 2 years. Unlike humans with four distinct lung cancer types that readily disseminate and reveal complex molecular genetics, mice are prone to pulmonary adenomas which are rare in humans or, infrequently, to adenocarcinomas, which seldom metastasize. Both of these mouse tumors are predominantly associated with Kras mutations. Premalignant lesions that are well characterized in the human airway epithelium and squamous cell carcinoma have been surprisingly difficult to replicate in mice.

Does smoking cause lung cancer in mice?

For decades, the credibility of mouse models suffered because of the lack of evidence that tobacco smoke, the main cause of lung cancers in humans, would lead to lung tumors in mice. Finally, lung tumors were induced reproducibly in A/J mice by exposing them to cigarette smoke for 5 months followed by a critically important 4 month recovery period (5). In a recent study, exposure of strain B6C3F1 female mice to life-time cigarette smoke (30 months) lead to 48% incidence of benign and malignant lung tumors through distinct genetic and epigenetic pathways (6). In comparison, 9.5% of control animals got tumors. In contrast, cigarette smoke carcinogens such as the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo[a]pyrene (B[a]P) readily cause lung tumors in mice and have been widely used in studies on pathogenesis, chemoprevention and novel therapies (4).

Directing gene expression and cancer to lung

The development of methods for manipulating the mouse genome, combined with the sequencing of the mouse genome, has advanced our ability to develop a plethora of new, genetically engineered mouse (GEM) models for lung cancer. In many cases the genetic modifications are targeted to specific subsets of lung epithelial cells. The surfactant protein-C promoter directs the expression mainly into alveolar type II cells in the parenchyma, while CC10/CCSP promoters primarily target the nonciliated secretory (Clara) cells along the airways. The first GEM lung cancer models involved the constitutive expression of SV40 Tag using these promoters, which resulted in aggressive adenocarcinomas without metastases (2). Many other transgenic mice followed using the same strategy, providing good models for observed amplification and epigenetic modification of the oncogenes. Same promoters are also used in many conditional mouse models.

Conditional bitransgenic mouse models for lung adenocarcinoma, oncogene dependency and stem cells

Most oncogenic activating mutations in lung cancer are somatic, which prompted the generation of conditional models, where activation of genetic events can be regulated both temporally and spatially (1). Mutations in Ras oncogenes are commonly associated with both human and mouse adenocarcinomas. A tetracycline inducible K-RasG12D lung tumor model developed epithelial cell hyperplasia, adenomas and adenocarcinomas after administration of doxycyclin. Interestingly, tumors completely regressed after withdrawal of doxycyclin demonstrating oncogene dependency. In a sporadic tumor model using Cre/loxP system a mutation can be directed to occur strictly in a set of differentiated cells while the genome of the adjacent cells remains unaltered. As a proof of principle, adenoc-Cre-mediated expression of mutant alleles of oncogenic K-RasG12D or K-RasG12V in the lung produced multiple adenomas and adenocarcinomas, revealing the precise role of Ras oncogenes in human lung carcinogenesis. Same approach helped to identify potential stem cells, BASCs in the peripheral lung.

Model for Small Cell Lung Cancer (SCLC)

Almost all human SCLCs have sustained mutations of p53 and Rb1 which rarely occur in mice. Somatic application of the Cre-loxP system was used to obtain primarily airway epithelium specific deletion of Rb1 and p53, which resulted in multiple tumors closely resembling human SCLCs (2). It is notable that the tumors demonstrated neuroendocrine features and had a marked capacity to metastasize to liver, brain, adrenal gland, bone and ovaries which mirrors the behavior of human SCLCs. Cell lines obtained from the mouse tumors also revealed characteristics of SCLC including amplification of L-myc. Other lung cancer models such as CC10-SV40Tag/hASH1 bitransgenic or p18/Men1 deficient mice have also been associated with pulmonary neuroendocrine tumors, but lack the typical histology and metastatic pattern of SCLC (7,8).

Squamous cell carcinogenesis in mouse lung

Normally neither human nor mouse lung contains squamous epithelium. However, the classic sequence of premalignant changes in human