

Molecular Origins of Lung Cancer: Prospects for Personalized Prevention and Therapy

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Abstract: The first Meeting on “Molecular Origins of Lung Cancer – Prospects for Personalized Prevention and Therapy” was held from January 10 to 14, 2010 in San Diego, California. The purpose of the meeting was to discuss important basic, translational, and clinical work aimed at improving lung cancer prevention, detection, and treatment. Topics included drug design, target identification, early detection, cancer stem cells, microRNAs, genome wide approaches to determining risk and outcome, mouse models, and tumor microenvironment. The role of cancer advocates in supporting research was an important component of the meeting. Meeting presentations demonstrated that emerging technologies can molecularly dissect lung cancers that have important relevance for clinical utility. This includes molecular based strategies not only for treatment of established cancers but also individualized and molecularly-based strategies for cancer risk reduction and chemoprevention.

INTRODUCTION

It is now apparent that translational science using genomic and proteomic technologies can define the molecular pathways that drive cancer progression for lung cancer. This knowledge in turn translates into more effective therapies that target these driver pathways in patients. Continued progress, however, is critical given the large incidence and death rates associated with lung cancer. Our understanding of these molecular pathways in lung cancer continues to evolve and new targets have been identified. In addition, clinical trials are keeping pace with laboratory studies. Progress in the understanding of non-small cell lung cancer (NSCLC) biology and advancement of care will depend upon the identification and integration of genetic changes in tumors coupled with interactions with stromal elements within tumor microenvironment. The rapid progress in whole genome sequencing will contribute to this progress as will use of other ‘omic technologies that accurately profile the state of human lung cancers. This meeting was the first partnership between International Association for the Study of Lung Cancer (IASLC) or American Association for Cancer Research (AACR) to create a dedicated meeting focused on the discovery and translation of new pathways important in lung cancer biology.

The keynote address was delivered by Dr. Tyler Jacks who presented an overview of how mouse models of lung cancer can be used to pilot strategies for early detection, chemoprevention, or therapy. A review of previous work with K-Ras mouse models was presented along with models of advanced lung cancer with K-Ras activation crossed with p53 loss. A few vignettes of ongoing work were presented. The first used K-Ras/p53 model to examine cisplatin sensitivity and resistance and found that lung cancers regressed independently of p53 activity. The most interesting finding was that rapid repair of DNA damage mediates resistance to cisplatin chemotherapy. Overall this work suggests that inhibiting DNA repair mechanisms may be one strategy to overcome resistance to cisplatin and possibly other DNA damaging agents. A second line of work revealed how these models can help understand and therapeutically manipulate metastasis. Lung cancers

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developing in the K-Ras/p53 model can metastasize to liver and adrenal sites similar to human tumors. A novel system to activate tumors using lentivirus was developed that allows for molecular tagging of tumors facilitating the ability to track lineages of primary and metastatic tumors. This could ultimately be used to understand biologic differences between primary and metastatic sites as well as differences between primary tumors that do or do not metastasize. Early work was described using gene expression profiling that yields insights into differences between primary tumors that do or do not metastasize. One of the genes that was reported included NKX2-1 or TTF-1. Both *in vitro* and *in vivo* studies suggested that TTF-1 can in some contexts act as a metastasis suppressor in lung cancer. Therefore, through these models, novel and rationale strategies to treat lung cancers can be developed and translated to the clinic.

Tyrosine Kinase Signaling and Targeted Therapy

This session focused on an established clinical target, epidermal growth factor receptor (EGFR), two new clinical targets: EML4-ALK and the insulin-like growth factor receptor 1 (IGF1R). The presence of an activating kinase domain EGFR mutation ("mutant EGFR") predicts clinical response to EGFR tyrosine kinase inhibitors (TKIs). Three studies published in 2009 demonstrate that EGFR TKIs are superior to chemotherapy in EGFR mutant patients (1–3). These studies also demonstrate that patients with wild type (wt) EGFR rarely have partial responses to EGFR TKIs. Although EGFR mutations predict sensitivity to TKIs, all patients develop resistance. There are two defined categories for NSCLC tumors to develop resistance to EGFR TKIs: by developing a second mutation that allows EGFR activation despite the presence of an inhibitor (T790M) or by enhancing alternative signaling pathways (i.e., c-Met or IGF-1R) that allow for the activation of downstream signaling pathways (e.g. PI3K/AKT) despite EGFR inhibition. The T790M mutation is found in about half of mutant EGFR NSCLC tumors in which EGFR TKI resistance develops. Zhou, et. al. have developed EGFR inhibitors that inhibit T790M EGFR more effectively than wt EGFR (7). These agents are effective *in vitro* and in mice and hold promise for treating patients who develop resistance to EGFR TKIs. c-Met amplification is found in about 10 % of patients with acquired resistance to EGFR TKIs. In mouse models of acquired resistance, the combination of EGFR and c-Met inhibition obliterates such tumors (8).

A key clinical question is whether or not one can predict the mechanism of EGFR resistance prospectively. Transient exposure to the c-Met ligand hepatocyte growth factor (HGF) results in resistance to EGFR inhibition via activation of the c-Met pathway. One would predict that removal of HGF would restore EGFR TKI sensitivity and indeed this happens in most cell lines. However, in HCC827 cells a transient exposure to HGF led to c-Met amplification and resistance to EGFR TKIs. c-Met amplification was present in 0.01 % of HCC827 cells. This was not due to contamination because re-isolation of cells derived from single HCC827 cells also developed the same 0.01 % of cells

with c-Met amplification. Incubation with HGF simply selected for the pre-existing population. The key questions are if this phenomenon exists in human tumors and, if so, can one predict the resistance mechanism before it emerges by identifying this population? Both EGFR and c-Met are receptor tyrosine kinases that, when activated by their ligands, can lead to the activation of downstream signaling pathways. However, there is also evidence for lateral signaling between c-Met and EGFR. One study suggested that EGFR activates c-Met in a c-Src-dependent, ligand-independent manner (11).

Another key question is whether inhibition of downstream pathways, such as PI3K or AKT, can overcome resistance? A single agent PI3K inhibitor was ineffective in NSCLC (with T790M-L858R EGFR) *in vitro*. However a combination of MAPK and PIK inhibition was effective. This is distinct from HER2-amplified breast cancer cells where PI3K inhibition alone is effective (9). In the NSCLC cells, the inhibition of PI3K led to a decrease in Mcl-1 and the inhibition of MAPK led to an increase in BIM – both were required to achieve apoptosis.

In addition to EGFR, novel fusion genes between the echinoderm microtubule-associated protein-like 4 and anaplastic lymphoma kinase (EML4-ALK) are potent drivers of lung cancer growth and survival (4). Laboratory and clinical studies demonstrate the transforming power of EML4-ALK and the critical role of ALK kinase in this process. Expression of EML4-ALK in transgenic mice leads to the development of multiple lung adenocarcinomas (5). The formation of adenocarcinomas is inhibited by ALK kinase inhibition. Likewise, treatment of patients with NSCLC tumors that possess EML4-ALK with an ALK / c-Met inhibitor (PF-02341066) led to a partial response in 17 of 29 (59 %) evaluable patients and stable disease in 7 of 29 (24 %) - many patients were heavily pre-treated (6). Testing for the EML4-ALK fusion protein is technically challenging. EML4-ALK can be fused at different sites – 12 have been identified so far, making standard polymerase chain reaction (PCR) assays problematic. Standard immunohistochemistry (IHC) is difficult because protein expression is relatively low. Fluorescence in situ hybridization (FISH) assays have been used but are also technically challenging because the EML4 and ALK genes are both located on the short arm of chromosome 2 and the inversion is small.

A second novel target is the insulin-like growth factor receptor 1 (IGF-1R). An understanding of the normal physiology of the insulin and insulin-like growth factor (IGF) signaling pathways is important in order to understand the toxic effects of drugs targeting IGF-1R and the design of biomarkers. Components of the network include the ligands insulin, IGF1 and IGF2; IGF binding proteins that affect ligand availability; 2 IGF receptors: IGF-1R and IGF-2R, and 2 insulin receptors (A and B). There are at least 24 drugs in development that target IGF-1R and 12 in clinical trial. These fall into 2 broad categories: antibodies that target IGF-1R and small molecule TKIs (10). Antibodies are more specific and were designed to avoid affecting the insulin receptor. Most of the TKIs also have significant activity against the insulin receptor. This difference in specificity could affect efficacy

and toxicity. One would expect that IGF-1R TKIs would cause profound diabetes due to the inhibition of the insulin receptors. However, significant metabolic disturbances have not been observed likely due to poor drug accumulation in the muscle. A phase II study of the anti-IGF1R monoclonal antibody CP-751,871 in combination with paclitaxel and carboplatin demonstrated that the combination was safe in patients with NSCLC. Response rates for the combination were high – especially in those with squamous carcinoma. However, in December 2009, the phase III study of the combination of chemotherapy and CP-751,871 for front line treatment of NSCLC was terminated because an interim analysis suggested that the addition of CP-751,871 to chemotherapy was unlikely to improve overall survival. Possible next steps to increase the tolerability and efficacy of anti-IGF-1R therapy include: exclusion of diabetics; combination with metformin, which may have anti-cancer properties of its own via reduction in circulating insulin levels; combinations with non-chemo regimens; combinations with regimens that do not require steroids; and an exploration of biomarkers to define host-tumor interactions that may predict tolerability and efficacy. Possible biomarkers include circulating levels of IGF-BPs and tumor amplification of IGF-1R.

Early Detection for Premalignant Lesions and Field Cancerization

Assuming that lung cancer progresses sequentially through stages I-IV, detecting more early stage cancers suitable for surgical or radiotherapeutic intervention offers the greatest chance of increasing the cure rate among those affected by the disease. Defining who to screen and how to screen for early stage lung cancers continues to be discussed. Although previous radiographic screening studies have raised the possibility that radiographically detected early stage lung cancers could represent a different (milder) clinical condition, rather than the initial event in a sequential stage progression, the National Lung Screening Trial (NLST) is set to answer this question definitively. Beginning in August 2002, the NLST randomizing 53,476 high risk men and women (greater than or equal to 30 pack years, and if a former smoker having quit more than or equal to 15 years previously) to either 3 annual chest x-rays or 3 annual low dose helical CT scans of the chest. The study was sized to detect a 20% difference in lung cancer specific mortality with 90% power. Secondary endpoints included all course mortality, lung cancer prevalence and incidence and the stage distribution of any detected cancers. Biospecimen collected during the NLST include plasma, buffy coat, sputum, urine and any subsequently resected tumors, that could be used to validate biomarkers that may help distinguish benign from malignant nodules, or assist in detecting tumors in the first place are now available for analysis. ACRIN will organize applications for research on these specimens from March 2010.

Detecting premalignant lesions or premalignant field changes offers two possible advantages – firstly to redefine a high risk cohort to apply other screening tests to. Secondly, assuming that, like stage, carcinogenesis also progresses sequentially, should effective chemopreventatives be developed, the detection of premalignant disease could trigger

specific interventions designed to reverse these changes. Affymetrix chips have been used to identify the most commonly altered genes in the bronchial epithelium of smokers compared to non-smokers. Of these, several genes persist among those who have quit smoking, suggesting that these may be more associated with long term carcinogenic risk. Using an 80 gene airway signature as an adjunct to standard cytology/biopsies at bronchoscopy the combination had a positive predictive value of 84% and a negative predictive value of 95%. This combination is now being explored prospectively in 800 subjects undergoing bronchoscopy internationally. Additional research directions include exploring the potential for applying the same gene signatures to cells derived from the nasal or buccal mucosa; whether microRNAs can be used instead of microarrays; and whether whole RNA sequencing technologies (RNA-Seq) which can address both mRNAs and miRNAs will add additional useful information.

Other early detection strategies include exploring other early markers of epigenetic change potentially leading to carcinogenesis, such as covalent histone modifications, that have been described in several established cancers. The least invasive techniques include exploring volatile organic compounds in exhaled breath, which appear to distinguish between non-small cell and small cell lung cancer cell lines when the air above tissue culture dishes are analysed. However, since most of these compounds are simple metabolites, how well they would distinguish a cancer in vivo in the presence of multiple normal cells remains to be seen. Blood derived assays include bead-based multiplex immunoassays looking at multiple different pro-angiogenic, inflammatory and pro-tumorigenic cytokines. When the blood profiles of 40 such cytokines were compared between 28 stage I lung cancer patients and 58 high risk smokers, logistic regression identified a 3 marker distinguishing classifier (IL-2, IL-3 and macrophage derived chemokine (MDC)) that will now be explored prospectively to evaluate its utility in more accurately defining indeterminate pulmonary nodules pre-procedure.

Detection and Prevention: From Mouse to Man

With advances in our understanding of the molecular basis of lung cancer tumorigenesis, investigators are designing and conducting trials which target this biology for chemoprevention. The initial step looks to define molecular pathways which are integral to the process of lung carcinogenesis and identify potential effective agents to target groups at risk. Metastatic lung cancer models that delineate the process of tumor progression have guided researchers to evaluate the tumor microenvironment and the role of epithelial to mesenchymal transition (EMT) in the metastatic potential of lung tumors and pre-malignant lesions. The zinc-finger transcription factor, Snail, is a transcriptional repressor of E-cadherin and its overexpression in lung cancer and pre-malignant lesions can lead to EMT, angiogenesis and tumor growth (12). A murine model that develops adenocarcinoma due to expression of mutant *K-ras* and *p53* demonstrated the ability for tumor cells to transition between epithelial and mesenchymal

states(13). MicroRNA (miR)-200 expression led to inhibition of EMT, which could be reversed by signaling through the Notch pathway. Furthermore, evaluating cells that demonstrate EMT potential has led to the identification of lung cancer self-renewing cells with elevated CD133 and aldehyde dehydrogenase (ALDH) levels, which may be targeted for therapy and prevention. In a murine model, iloprost inhibition of prostacyclin synthase led to a decrease in tumor formation (14). Extending this therapy in a randomized trial demonstrated a reduction in bronchial dysplasia, which was evident in former smokers, but did not benefit current smokers. In addition to targeting signaling pathways for chemoprevention, a novel method to inhibit tumor growth through epigenetic modulation was discussed. Methylation of the promoter region of four genes has been shown to be associated with early recurrence in early stage lung cancer (15), and this data has been used to initiate a trial combining methylation inhibition and histone deacetylase inhibition to potentially inhibit the progression of self-renewing cancer cells.

Other Thoracic Cancers

As molecular testing paired with molecular targeted therapies is starting to change treatment paradigms in non-small cell lung cancer, the same principles are also starting to be explored in other, rarer cancers of the thorax. Comparative Genomic Hybridization (CGH) arrays applied to small cell lung cancers suggest gene copy number alterations relating to the FAK and neuroendocrine pathways commonly occur. Following on from this lead, phosphorylated FAK has been detected in small cell lung cancer cell lines independent of cell adhesion. Given that FAK inhibitors are in clinical development and interfere with small cell lung cancer cell line morphology in vitro, the possibility of exploring FAK inhibitors in small cell lung cancer in a clinical trial is being considered.

In mesothelioma, pemetrexed with cisplatin produces far higher responses rates than cisplatin does alone. One of the major molecular targets of pemetrexed is thymidylate synthase (TS). High levels of TS have been seen in squamous cancer of the lung in association with a lower response to pemetrexed. Using a bespoke IHC scoring system for TS, 40 mesotheliomas were assessed and showed a range of different levels (35% high, 47.5% low, 17.5% negative). However, correlation with clinical outcomes following pemetrexed treatment is awaited. Also in mesothelioma, cell lines vary by whether they are sensitive to Vesicular Stomatitis Virus (VSV), an oncolytic virus, and whether beta-IFN protects the cells from the virus (as it does in most non-malignant cells). Markers associated with the IFN pathway can be assessed by IHC and demonstrate heterogeneity on mesothelioma tissue microarrays raising the possibility that such markers, notably P48, could be explored to assess which mesotheliomas may respond to IFN, and which may be resistant to IFN but susceptible to oncolytic viruses such as VSV. Again, clinical validation of these hypotheses is awaited. In a recent Italian series, ERCC1 expression by IHC did not correlate with response to platinum containing therapy in mesotheliomas. MTOR was also noted to be variably expressed in mesotheliomas, raising the possibility of using MTOR inhibitors in this disease. No activating mutations in

PI3Kinase were noted among the mesotheliomas to explain the MTOR over-expression, and no other mutations were found in EGFR, KRAS, BRAF or MET.

Tumors of the thymus represent a diverse collection of rare thoracic malignancies. The current WHO histological classification is based on assuming different cell types of origin, suggesting that these are likely to represent distinct diseases. Types A, AB, and B1 cluster together because of their good prognosis. Types B2, B3 and C, the last two of which represent well and poorly differentiated squamous carcinomas, respectively, have poorer prognoses. Recent molecular profiling of 45 thymic tumors from Memorial Sloan Kettering showed that 1 of 38 thymomas and 1 of 7 thymic carcinomas were KRAS mutant; 2 of 7 thymic carcinomas were cKIT mutant (with in vitro evidence of imatinib and sunitinib sensitivity); but no EGFR mutations were detected. Some smaller series have detected rare exon 21 and exon 18 mutations in thymomas but never in thymic carcinomas. By IHC, IGF1R appears to be commonly expressed in both thymomas and thymic carcinomas, raising additional possibilities to explore with targeted therapies in the clinic.

Targeting the JAK/STAT Pathway

The potential importance of the janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway was discussed. JAK/STAT signaling can be a common pathway activated by diverse upstream signaling proteins, including growth factor receptors, cytokines, and non-receptor tyrosine kinases such as SRC and ABL. STAT proteins, in particular STAT3, are oncogenic in part by activating a gene transcription program that affects multiple cancer hallmarks. This includes cell proliferation (cyclin D, Myc), anti-apoptotic signaling (Mcl-1, Bcl-xL), angiogenesis (VEGF), and immune evasion (16). While non-tumor cells have robust systems to allow for only transient activation of this pathway, tumor cells acquire persistent pathway activation through various mechanisms. Targeting strategies such as small molecule inhibitors, natural products, RNA interference, and tyrosine kinase inhibitors are potential strategies to target STAT3 signaling in cancer. A JAK kinase inhibitor, AZD1480, was shown to have selective activity against Jak2 that suppressed tyrosine phosphorylation, DNA binding, and nuclear translocation of STAT3 (17). Activity of this agent was shown in mouse tumor models. Other approaches include developing compounds that target the STAT3 molecule itself, including agents that inhibit STAT3 dimerization (necessary for gene transcription) and DNA binding. Studies with animal tumor models suggest that dual STAT3 and tyrosine kinase inhibitors may be a rationale strategy to induce tumor cell death. As opposed to structure-based development of STAT3 inhibitors, another approach is using chemical libraries of compounds to identify STAT3 inhibitors in a large scale screen. Preliminary compounds have been identified that inhibit STAT3 transcriptional activity and further studies are investigating both mechanisms as well as translational strategies to use these compounds in early phase clinical trials. It was pointed out the importance of incorporating pharmacodynamic studies in early phase trials to assess STAT3 target inhibition. Finally, increasing evidence suggests that STAT3

activation may be resistance mechanism in lung cancer cells facing tyrosine kinase inhibitors such as SRC kinase inhibitors (18). A combination of in vitro, in vivo, and human tumor studies suggests that dual SRC and STAT3 inhibitor studies should be considered for early phase trials. Overall the results of this session suggest near term deployment of STAT3 inhibitors in clinical trials and suggest dual tyrosine kinase inhibitor plus STAT3 inhibitor strategies are worth further consideration.

Advocacy, Regulatory, and Funding Issues

An important component of this meeting was the session on Advocacy. This included presentations by Laurie Monroe, a lung cancer survivor and patient advocate, and Dr. Joan Schiller, head of the National Lung Cancer Partnership. Amongst the topics discussed were efforts at patient education, advocacy for research funds for lung cancer, and identifying mechanisms for promoting cooperation between patient advocates and lung cancer researchers.

Cancer Stem Cells

There is growing evidence that a rare subpopulation of undifferentiated cells which exhibit self-renewal, tumor initiation and propagation properties exists for lung cancer. Although the markers to identify these subsets are not fully known, these cells have been shown to express CD133, ALDH, Oct-4 and c-kit, in addition to other markers. Within the tumor and its microenvironment, signaling events through pathways such as Notch are associated with accumulation of these cells, and blocking this pathway can lead to a decrease in ALDH+ cells. Moreover, inherited and acquired genetic or epigenetic alterations may modulate groups of genes leading to propagation of these self-renewing cells. Although cytotoxic agents can kill cancer cells, cancer stem cells likely remain behind to repopulate and migrate, and novel approaches for inhibiting cancer stem cells are needed. Reversing the process of hypermethylation and histone acetylation through pharmacologic agents such as 5-aza-2'-deoxycytidine and histone deacetylase inhibitors has the potential to inhibit these stem cell populations from proliferating and spreading. In addition, targeting cytokine pathways such as stem cell factor-c-kit signaling can inhibit lung cancer stem cells and may improve the efficacy of standard chemotherapeutic agents (19). Self-renewing cancer cells may result from asymmetric cell division resulting in a heterogeneous population of cells within the tumor, and this process has been described in lung cancer (20). During asymmetric cell division, stem cell markers such as CD133 segregated with template DNA while differentiation markers segregated to the opposite cell. Modulation of this process could lead to novel therapies to inhibit the replication of self-renewing cells. Additional insight has been gained through the evaluation of drug-resistant side population cells which demonstrate increased migration and invasive behavior and have elevated Rac1-GTP activity. Cell migration and lung colonization can be blocked by inhibiting Rac1-GTP signaling. Furthermore, these side-population cells appear to be a distinct population of self-renewing cells that do not express CD133, leading to

the hypothesis that multiple populations of self-renewing cells may exist.

Genome-wide Approaches to Risk

Common cancers tend to cluster in families through inherited effects. Some are well recognized and strong predisposing genetic mutations (ie: BRCA1 and 2) however, most are due to individually small but combined effects of different (common or rare) variants (polygenic model of cancer susceptibility) yet unexplained. Another small amount of common variations (6% in breast cancer and 18% in prostate cancer) have been identified by genome-wide association studies (GWAS). Practical applications to the identification of genetic variations include the discovery of new targets for intervention and the creation, in combination with other (clinical) risk models, of profiles that allow for population risk stratifications. This is useful to individuals, public health officials and researchers for personalized therapy, screening programs or prospective interventional studies respectively. GWAS have identified factors that contribute to nicotine dependence and smoking quantity and to the risk of lung cancer and results from these studies converge on the same chromosome 15, where the most compelling evidence of risk lies in the region of the a5-a3-b4 nicotine receptor gene. Two distinct genetic variants have been implicated in the a5 receptor subunit; rs 16969968 (causes an amino acid change resulting in a differential response) and rs588765 (contributes to different expression of a5 in the brain). This implicates the nicotine receptor a5 subunit as a major genetic contributor to both disorders. Additional genetic variations associated with lung cancer risk have been identified in chromosomes 5 (rs401681) and 6 (rs3117582) and large cohort studies are ongoing trying to identify common genetic variants affecting lung cancer and COPD risk (COPDGene).

Lung Cancer Profiling with microRNAs

MicroRNAs (miRNAs) are small, endogenously expressed non-coding RNAs sequentially processed from primary transcripts, which regulate protein-coding gene expression through either translational suppression or RNA degradation (bind messenger RNA at complementary sites in the 3' untranslated region). They function either as tumor suppressors or oncogenes and thus, play an important role in carcinogenesis. Furthermore, through miRNA expression profiling, prognostic and predictive biomarkers can be identified. Several miRNAs have been implicated in the pathogenesis of lung cancer. The let-7 miRNA family inversely regulates KRAS and is frequently downregulated in lung cancer (low let-7 upregulates KRAS). Low levels of let-7 predict for poor outcome (shortened postoperative survival), likely due to KRAS upregulation. Similarly, rs61764370, a germline SNP in a let-7 miRNA, present in 7% of the general population but in 20% of NSCLC patients, predicts for increased risk of NSCLC and is associated with increased KRAS expression and decreased let-7 levels. Also, miR-125a-3p is suppressed in KRAS mutated HBECs and NSCLC cell lines, but when overexpressed it blocks proliferation of KRAS mutated HBECs. Conversely, the miR-17-92 cluster is frequently overexpressed in SCLC. It cancels the genera-

tion of reactive oxygen species induced by Rb inactivation and presumably plays a role in SCLC development. Together, these strongly suggest the role of tumor suppressor-type and oncogene-type miRNA alterations in the molecular pathogenesis of human lung cancers. Applications of miRNA profiling include signatures to predict prognosis (worse with miR17-92 overexpression in resected stage I adenocarcinomas), histology (miR-205 for adenocarcinoma vs. squamous cell carcinoma and miR-301, 183 and 126 for NSCLC vs. SCLC) and therapy resistance (miR92a2*, miR-147 and miR-574-5p in SCLC and miR-424 for vandetanib and erlotinib and miR-21 for sunitinib in NSCLC).

Mouse Models of Lung Cancer

In the mouse models of lung cancer section, multiple speakers highlighted the strengths of genetically engineered mouse (GEM) lung cancer models to genetically dissect the roles of different oncogenes and tumor suppressor in lung cancer initiation and progression. More importantly these GEM lung cancer models were shown to be great platforms for testing of novel therapeutics that targeted specifically the oncogenic drivers of the specific models or its immediate downstream pathways. Examples of mouse clinical trials with GEM models and novel targeted therapeutics as well as conventional chemotherapy were demonstrated in presentations of Dr. Kwok-Kin Wong and Dr. Leisa Johnson. In addition, Dr. Minh To presented his elegant mouse genetic work which dissected the role of the various Kras isoforms in lung cancer initiation and progression. Lastly, Dr. Yifeng Xia presented his technology platform of employing lenti-viral gene delivery to generate de-novo lung cancer.

Other Biomarkers

In this session, a range of topics was addressed covering a variety of biomarkers and discovery platforms. Proteomics biomarkers were discussed by Drs. David Carbone and Rachel Ostroff. Dr. Carbone and colleagues used a variety of mass spectrometry-based platforms to uncover candidate biomarkers for risk assessment, early diagnosis, and prediction of response to therapy. One of these, the serum MALDI-TOF assay predictive of benefit from EGFR-targeted therapies in lung cancer is now a commercial product, Veristat. Data were presented that not only is this test useful in identifying advanced lung cancer patients who benefit from erlotinib and gefitinib, but also the combination of erlotinib and bevacizumab. Perhaps surprisingly, good classification was also observed for lung, head and neck, and colon cancer patients treated with these drugs, as well as cetuximab. Dr. Ostroff discussed exciting data using their aptamer platform to uncover a serum diagnostic for lung cancer. A pattern of proteins was found that was highly accurate in distinguishing patients from cancer from those without. Dr. Gower presented his data extending the data of Dr. Spira evaluating the gene expression pattern in histologically normal bronchial epithelium as a biomarker for the presence or absence of lung cancer. He described a new method, dubbed openSESAME, that allows the cross-experiment and cross-disease type analysis of data from GEO to ask or test signature hypotheses. He

showed data that this was useful in the analysis of patterns from lung, bladder, and head and neck cancer. Dr. Hirsch discussed his data on IGF1R expression and amplification in relation to benefit from targeted inhibitors, and Dr. Tsao presented his exciting data from NCIC randomized clinical trials defining and testing his microarray signature of benefit from adjuvant chemotherapy.

In summary, this session demonstrated the wide range of platforms and biospecimen sources being used to define potentially clinically useful biomarkers, and presented some exciting new specific candidate signatures for lung cancer.

Angiogenesis and the Tumor Microenvironment

Topics for this session included an overview of anti-angiogenesis agents and their corresponding targets, development of a molecular signature for bevacizumab using molecular and clinical data from patient-based clinical trials, examining the effects of different anti-angiogenic drugs (including tyrosine kinase inhibitors and antibody-based therapies) on early- versus late-stage lung cancer, and identification of tumor-derived factors that mediate resistance to anti-vascular endothelial growth factor-based therapies in NSCLC.

Drug Design and New Targets

The MD Anderson BATTLE program was discussed as a possible route for personalized therapy in lung cancer using an adaptive design to allocate treatment decisions with molecular targeting agents using tumor biomarkers. Other approaches towards personalizing chemotherapy for advanced lung cancer were discussed, including ERCC1- and RRM1-based biomarker strategies, as well as strategies attacking new targets for the chemoprevention of lung cancer.

Summary

The meeting provided a unique lung cancer focused translational science meeting that brought together various experts and stakeholders in lung cancer research. The meeting had breadth by examining a number of unique technologies for tumor profiling, including both genomic and proteomic approaches, as well as examined tumor biology from early pre-neoplastic changes to targeting tumor pathways in metastatic and advanced stages of disease. Tentative plans are to have this meeting every other year. One theme of this meeting is the emerging picture of uniqueness amongst lung cancer defined by molecular subtyping offered by emerging technologies and the ability to optimize patient care through an improved understanding of each individual's tumor biology. As this approach is showing promise, the deployment of novel therapeutic and preventative strategies will benefit from continued efforts in molecular profiling of lung cancer.

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