

Advances and Challenges in Aerodigestive Epithelial Cancer: Meeting Summary and Research Opportunities

Jennifer R. Grandis,¹ David P. Carbone,² and Anil K. Rustgi³

¹Departments of Otolaryngology and Pharmacology, University of Pittsburgh, Pittsburgh, Pennsylvania; ²Department of Medicine, Vanderbilt University, Nashville, Tennessee; and ³Department of Medicine (GI Division) and Abramson Cancer Center, University of Pennsylvania, Philadelphia, Pennsylvania

Introduction

Malignant neoplasms arising in the upper aerodigestive tract (encompassing the oral cavity, pharynx, esophagus, larynx, and lung) are among the most common cancers in the United States and worldwide, accounting for nearly one third of all malignancies. These cancers are often fatal despite combined modality treatment consisting of surgery, radiation, and chemotherapy. Cancers arising in these sites often share common features such as environmental risk factors, genetic alterations, histopathology, and treatment approaches. Moreover, patients who survive their first aerodigestive tract cancer remain at increased risk for developing a second primary tumor at these anatomic sites throughout their lifetime. The addition of molecular targeting agents to treatment regimens may improve clinical outcome with reduced toxicities. The American Association of Cancer Research organized a special conference on the Advances and Challenges in Aerodigestive Epithelial Cancer: Genetics, Diagnosis, and Therapy (held in Charleston, South Carolina, February 6–9, 2007). Over a 2.5-day period, experts in head and neck squamous cell carcinoma (HNSCC), lung cancer, and esophageal carcinoma met to identify common areas for preclinical and clinical investigations. The overall consensus was that common biological features characterize cancers that arise in the aerodigestive tract and that research efforts should not be restricted by the anatomic site of the primary tumor. Collaborations between HNSCC, lung cancer, and esophageal cancer working groups will enhance the opportunities to impact these fatal malignancies.

Meeting Sessions

Two overview talks set the stage by discussing common biological features of upper aerodigestive tract cancers. D. Sidransky (Johns Hopkins University, Baltimore, MD) discussed the cancer *methylome*. Methylation seems to be a more common means to inactivate gene expression. The approach taken by his group has shown a high incidence of promoter methylation of a panel of genes in HNSCC patients. In lung cancer, the methylated genes detected are similar to those found in HNSCC. It is therefore possible that methylation markers may be used in conjunction with computed tomography screening for lung cancer. Of note, methylation is well annotated in esophageal squamous cell cancer as well as Barrett's esophagus, the signature precursor lesion to esophageal adenocarcinoma. He concluded that new discovery approaches are needed to define the cancer methylome.

J. Siegfried (University of Pittsburgh, Pittsburgh, PA) presented her work on targeting the gastrin-releasing peptide receptor in lung and head and neck cancers. Gastrin-releasing peptide receptor and its autocrine ligand gastrin-releasing peptide contribute to tumor formation and progression in HNSCC and non-small-cell lung cancer (NSCLC). Gastrin-releasing peptide receptor expression levels in buccal mucosa cells from NSCLC and HNSCC patients are elevated compared with control subjects without cancer. Therefore, not only can this pathway serve as a therapeutic target for these cancers but assessment of gastrin-releasing peptide receptor levels in surrogate tissues may also be an effective screening strategy.

In the session on preneoplasia, S. Lippman (University of Texas M. D. Anderson, Houston, TX) reviewed the experience with retinoids as proof-of-principle for the development of molecular targeting approaches to cancer prevention. He emphasized the need to embed correlative studies in chemoprevention trials to achieve eventually a goal of personalized cancer medicine. A. Gazdar (University of Texas Southwestern, Dallas, TX) discussed the molecular pathogenesis of lung cancer preneoplasia. He noted that the WHO seven-stage classification of bronchial preneoplasia cannot be used for risk assessment because conventional pathology is a poor predictor of progression for bronchial premalignant lesions. New technologies, such as morphometric analysis of aneuploidy, high-throughput cytology, genomic approaches, and three-dimensional cell culture systems, were discussed as means of linking to molecular imaging. V. Papadimitrakopoulou (University of Texas M. D. Anderson, Houston, TX) discussed interventions for premalignant lesions of the head and neck and biomarker development. She noted that studies to date have shown that no single drug can target all genetic changes, including retinoic acid, ketorolac, or celecoxib. The results of an ongoing phase III trial of erlotinib in high-risk premalignant oral cavity lesions will be informative.

In the second session on preneoplasia, A. Dannenberg (Cornell University, Ithaca, NY) discussed how tobacco smoke regulates cyclooxygenase-2 (COX2) expression by both genomic and non-genomic mechanisms. Ligands of the aryl hydrocarbon receptor found in tobacco smoke can induce cleavage of the epidermal growth factor receptor (EGFR) proligand amphiregulin by tumor necrosis factor α (TNF- α)-converting enzyme. Aryl hydrocarbon receptor contributes to tobacco smoke-mediated induction of COX2 by an EGFR-dependent mechanism. Therefore, EGFR inhibitors (and/or COX2 inhibitors) may prevent the harmful effects of tobacco smoke. J. Viner (National Cancer Institute, Bethesda, MD) reviewed the progress in esophageal cancer prevention. In adenocarcinomas, *p53* mutation, tetraploidy, and aneuploidy in premalignant lesions predict progression to invasive cancer. She emphasized the need for consortia, more firm diagnostics, and national/international mechanisms to carry out these trials [e.g., stomach/esophageal neoplasia translation research network (SENTRNet)]. F. Hirsch (University of Colorado,

Requests for reprints: Jennifer Rubin Grandis, The Eye and Ear Institute, Room 105, 200 Lothrop Street, Pittsburgh, PA 15213. Phone: 412-647-5280; Fax: 412-647-0108; E-mail: jgrandis@pitt.edu.

©2007 American Association for Cancer Research.
doi:10.1158/0008-5472.CAN-07-1733

Boulder, CO) discussed predictive factors in preneoplasia in the bronchus, reiterating the need to define intermediate end points for prevention studies. He reviewed the University of Colorado high-risk cohort that has been studied since 1992. In this cohort, bronchial histology is strongly associated with smoking status and gender, and sputum cytology is associated with smoking status (but interestingly, not with smoking duration or pack-years) but not with bronchial histology.

The next session focused on mouse models of aerodigestive tract cancers. X.J. Wang (Oregon Health & Science University, Portland, OR) presented her models of HNSCC, noting that there were no native tissue-specific promoters. They examined HNSCC and found a high rate of *ras* activation, which was accompanied by loss of expression of transforming growth factor- β receptor (*TGF β RII*). Using an inducible *cre-lox* system in the mouse, the targeted inactivation of *TGF β RII* coupled with *ras* activation through *LSL-K-ras^{G12D/+}* led to HNSCC, which was further accelerated by the administration of 7,12-dimethylbenz(a)anthracene. These studies suggest that *ras* activation serves as an initiation event and *TGF β RII* loss promotes malignant progression. G. Lozano (University of Texas M. D. Anderson, Houston, TX) discussed the p53/murine double minute (Mdm) pathway in mouse models of tumorigenesis. Mdm2, a ubiquitin ligase that negatively regulates p53, is overexpressed in nearly 90% of HNSCC. Mdm2 and Mdm4 haploinsufficiency increases sensitivity to ionizing radiation, which is a p53-dependent phenotype. A. Rustgi (University of Pennsylvania, Philadelphia, PA) discussed models of esophageal cancer. Squamous cell carcinomas are characterized by EGFR activation, which induces transformation in cooperation with other genetic changes such as p53 mutation. Using an organotypic culture model, they can study these cells in the tumor microenvironment. Using the EBV ED-L2 promoter to target cyclin D1 to the oral-esophageal squamous epithelial cells, transgenic mice were generated. When these animals were crossed with p53-deficient mice, their offspring develop oral and esophageal squamous cell carcinomas by 6 months.

Therapeutic strategies were presented in the next session. A. Forastiere (Johns Hopkins University, Baltimore, MD) reviewed multimodality therapeutic approaches for HNSCC and esophageal cancer. Cisplatin remains the dominant chemotherapy reagent and EGFR-directed therapies represent the primary molecular targeting approach in HNSCC. The EGFR monoclonal antibody cetuximab was Food and Drug Administration (FDA) approved for use in HNSCC in 2006 based on its efficacy when combined with radiation. In esophageal cancer, molecular targets are less well understood but EGFR targeting may be an effective strategy. H. Choy (University of Texas Southwestern, Dallas, TX) discussed recent advances in management of NSCLC with a focus on how to incorporate targeted therapies. EGFR expression in NSCLC correlates with radiation sensitivity, and the EGFR tyrosine kinase inhibitor erlotinib is FDA approved for use in NSCLC but the biological basis for response to EGFR targeting is likely multifactorial and incompletely understood. W. El-Diery (University of Pennsylvania, Philadelphia, PA) discussed modeling tumor progression and developing novel therapies for esophageal cancer, noting that there were few therapeutic options for patients with advanced disease. Molecular changes in esophageal cancer include inactivation of p53 and p16 and overexpression of cyclin D1 and EGFR. In preclinical models, primary esophageal cells are immortalized with hTERT and large T antigen, and then followed by introduction of different oncogenes, thereby resulting in tumors. The tumors can be monitored on a serial temporal basis through

bioluminescence. R. Herbst (University of Texas M. D. Anderson, Dallas, TX) discussed targeted therapy for aerodigestive cancers with an emphasis on EGFR and antiangiogenic approaches. Vascular endothelial growth factor (VEGF) is the primary mediator of angiogenesis and is induced by multiple tumor-relevant stimuli. The anti-VEGF monoclonal antibody bevacizumab has shown a significant clinical benefit in patients with nonsquamous cell NSCLC in a randomized phase III trial wherein the addition of bevacizumab to chemotherapy with paclitaxel plus carboplatin provided a significant survival benefit over chemotherapy alone. Ongoing studies are evaluating bevacizumab in other NSCLC settings and are attempting to identify predictive factors for responses to this antiangiogenic agent. E. Vokes (University of Chicago, Chicago, IL) presented the concept of virus-mediated delivery of TNF- α to HNSCC. TNFerade is an adenoviral vector engineered to deliver the transgene for human TNF- α to solid tumors. Further investigation has shown that ionizing radiation can be used as a genetic switch for this cancer therapy.

In the session on carcinogenesis, M. Spitz (University of Texas M. D. Anderson, Houston, TX) discussed predictors of lung cancer risk and outcome with an emphasis on a molecular epidemiologic approach. She emphasized the importance of performing a pathway-based genotyping approach to assess the combined effects of a panel of polymorphisms that interact in the same pathway analysis to identify common genetic variations that effect risk. With the assessment of all nine genes in the nucleotide excision repair pathway in combination with a functional assay to measure DNA repair capacity, they found that current smokers have the best DNA repair capacity and individuals with poor DNA repair have a better response to chemotherapy. E. Taioli (University of Pittsburgh, Pittsburgh, PA) discussed gene-environment interactions in tobacco-related cancers, noting that an assessment of genes that metabolize carcinogens (and other products) may identify susceptible individuals. In a meta-analysis of published data and a pooled analysis of individual case control studies on *CYP1B1* and lung cancer, they found a significant association between the homozygous variant of the *CYP1B1* Leu⁴³²Val polymorphism and lung cancer.

In the session on molecular biology and pathway analysis, J. Grandis (University of Pittsburgh, Pittsburgh, PA) discussed signaling aberrations that could serve as molecular targets in HNSCC. In addition to ubiquitous EGFR overexpression, HNSCC are characterized by increased activation of signal transducers and activators of transcription and Src family kinases. Further investigation has shown persistent transactivation of EGFR by G-protein-coupled receptor as well as the presence of the mutant receptor EGFRvIII. All of these aberrations can be targeted by molecular therapeutic approaches, alone or in combination with EGFR blockade. M. Meyerson (Dana-Farber Cancer Institute, Boston, MA) presented the results of genomic studies in human aerodigestive cancers. Using several methods including single-nucleotide polymorphism arrays, exon resequencing, and computational subtraction (for viruses), these efforts are focused on finding "drugable" genes. A comprehensive strategy showed EGFRvIII in ~10% of NSCLC. Therapies that target this mutant EGFR could be tumor specific. Sequencing a large series of lung cancer will allow the discovery of targets through cancer genomics.

In the final session on molecular signatures, D. Carbone (Vanderbilt University, Nashville, TN) discussed blood-based assays for early detection of NSCLC. Many resected lung nodules are benign and there are no tests to determine which preneoplastic

lesions will progress to cancer. Direct measurement of abnormally expressed or modified proteins in the tumor and/or blood may be an effective approach for discovering new biomarkers. Proteomics has the significant advantage of being able to discern not only changes in expression levels but also in posttranslational modifications. Thus, a proteomics approach to protein profiling and biomarker discovery may discriminate sensitive and resistant tumors, predict response to therapy, and provide a prognostic signature. He emphasized that targeted therapies required targeted patient selection. R. Ferris (University of Pittsburgh, Pittsburgh, PA) discussed early detection in HNSCC using multiplexed immunobead-based biomarker profiling. In a series of cases with active HNSCC, subjects that were free of disease, and noncancer controls, they showed the feasibility of this assay. The multimarker panel offering the highest diagnostic power was composed of 25 proteins including EGFR and interleukin-8. Simultaneous assessment of a series of serum biomarkers may aid in early detection. C. Chung (Vanderbilt University, Nashville, TN) next presented her work on an activated EGFR signature in HNSCC. An *EGFR* gene signature has been associated with a poor prognosis in HNSCC. More recent works suggest that the activated EGFR signature correlates with fluorescence *in situ* hybridization status/gene amplification. Using a renal capsule xenograft model, they are determining EGFR activation pathways in squamous epithelial cells. B. Reid (Fred Hutchinson Cancer Center, Seattle, WA) presented the biology of BE, emphasizing that Barrett's esophagus (BE) represents an ideal model for the study of neoplastic clonal evolution. In their prospective cohort study, 34 of 243 subjects have developed adenocarcinomas. Markers of cancer development include 17p loss

of heterozygosity (LOH), tetraploidy, aneuploidy, and 9p LOH. The use of nonsteroidal anti-inflammatory drugs seems to decrease the incidence of esophageal cancer in subjects with BE.

Summary

Translational research programs that focus on a specific tumor may not necessarily be informed by discoveries in related cancers. Epithelial malignancies (primarily squamous cell carcinomas and adenocarcinomas) that arise in the mucosa of the upper aerodigestive tract share many features. They are associated with common risk exposures (such as tobacco and alcohol), genetic and epigenetic alterations (e.g., EGFR overexpression/activation, cyclin D1 overexpression, and *p53* mutation), and response to therapy (including chemoradiation and EGFR targeting). By establishing working groups to develop and share preclinical models, design early detection strategies, and implement chemoprevention approaches, progress may be accelerated in these frequently fatal malignancies. An upper aerodigestive tract cancer consortium could oversee early-phase biomarker-driven clinical trials, surveillance, and intervention studies and the testing of chemopreventive agents in high-risk cohorts. Collectively, these malignancies are the most common in the United States and worldwide. There are clear opportunities to change the morbidity and mortality of aerodigestive cancers. The complex biology and the substantial barriers to clinical translation require that future efforts enlist the participation of investigators with diverse yet complementary perspectives and expertise. There is much optimism to change the landscape as a result of these burgeoning interactions.