

## **Workshop on Salivary Gland Tumor Research: Current Status and Future Directions**

November 17–18, 2008  
Bethesda, Maryland

### **Executive Summary**

#### **Background**

Jointly sponsored by the NIH Office of Rare Diseases and the Adenoid Cystic Carcinoma Research Foundation (ACCRF), a nonprofit organization whose main mission is to find a cure for adenoid cystic carcinoma (ACC), this workshop was the second sponsored by NIH on salivary gland tumors (SGTs). Following the first workshop 2 years ago, it became clear that critical gaps existed in the research infrastructure to support a meaningful research program on salivary gland tumors, although some activities were sponsored by ACCRF. For example, no tissue resources and very few cell lines were available to facilitate basic and translational research in this field. To address these urgent needs, the NIDCR has taken the lead in creating the Salivary Gland Tumor Biorepository (SGTB), hosted at the M.D. Anderson Cancer Center, and in forming the Salivary Gland Tumor Consortium. Through such resources, NIDCR has started to fill an important gap in SGT research with the ultimate goal of improving public health.

#### **Leadership**

This workshop was co-chaired by Adel K. El-Naggar, M.D., Ph.D., professor of pathology and head and neck surgery, director, Head and Neck Pathology, Division of Pathology, and Kenneth D. Müller Professor of Cancer Research at the University of Texas M.D. Anderson Cancer Center and by Frederic J. Kaye, M.D., head, Mechanisms of Oncogene Action Section, Center for Cancer Research, National Cancer Institute, NIH. The workshop scientific planning committee consisted of Yasaman Shirazi, Ph.D., director, Epithelial Cell Regulation and Transformation Program, Integrative Biology and Infectious Diseases Branch, National Institute of Dental and Craniofacial Research (NIDCR), NIH; Dr. El-Naggar; Dr. Kaye; Arlene A. Forastiere, M.D., professor of oncology at Johns Hopkins University; and J. Silvio Gutkind, Ph.D., chief, Oral and Pharyngeal Cancer Branch, NIDCR, NIH.

#### **Goals**

The present workshop was organized to facilitate collaborations among investigators, identify and set priorities for scientific endeavors, and prepare workshop recommendations. Its overall goal was to engage national and international researchers with expertise and interest in the biology, pathology, and treatment of SGTs in an effort to identify research gaps and opportunities and to develop research agendas in basic and clinical research over the next 3 to 5 years. Longer-term objectives included publishing and disseminating information for the broad scientific community as well as crafting future research initiatives in SGT research.

## **State of the Science**

### *Basic Science Research*

A session entitled, “Genomic and Epigenomic Platforms for Target Discovery,” offered an opportunity for the group to discuss the status of various platforms to determine if they are at a stage at which they could be used for analyzing SGTs. Dr. Paul Meltzer of the NCI presented several cutting-edge techniques for tumor profiling that may be useful for target discovery and clinical implementation. Transporting specimens to the laboratory remains a significant challenge, however. New methods for using formalin-fixed paraffin blocks are being explored.

The subject of a presentation given by Dr. Nicole Solomini, Brigham and Women’s Hospital, was multiplex RNA interference screens for cancer therapeutic target discovery. Her group has developed genome-wide small hairpin RNA (shRNA) libraries—both in retroviral and lentiviral delivery systems—to look for genetic dependencies. The technology can be used to perform “cancer lethal screens” to identify specific dependencies in cancer cells. The greatest interest is in genes that, if knocked out, would kill the cancer cells because the cells require those genes for support.

The cancer epigenome was the subject addressed by Dr. Jean-Pierre Issa of the University of Texas M.D. Anderson Cancer Center. The epigenetic code consists of signals that are necessary and sufficient to establish and/or perpetuate an epigenetic state, usually through DNA methylation or histone modifications. A few SGT samples have been studied for aberrant DNA methylation patterns, revealing that up to 9% of genes were differentially methylated.

Studies using carbohydrate microarrays, according to Dr. Jeffrey Gildersleeve of NCI, have shown that cancer cells undergo important changes during the onset of cancer, including the loss of some natural antigens and gain of unusual, atypical antigens termed tumor-associated carbohydrate antigens. Some cancer carbohydrate antigens may serve as diagnostic or predictive biomarkers for detecting cancer or predicting response to treatment. Because carbohydrates are difficult to detect, isolate, and characterize, new tools and technologies are greatly needed.

Mouse models can provide a basis for understanding SGTs and for testing therapies. Therefore, another session, “Conditional and Xenograft Mouse Models,” was devoted to answering two main questions: (1) Can we develop new and better animal models for studying SGTs? (2) How can we improve upon existing models? Most interesting are mouse models that have head and neck cancers analogous to those in humans. Dr. James Jackson, University of Texas M.D. Anderson Cancer Center, described progress in developing a mouse model of muco-epidermoid carcinoma (MEC) that recapitulates what happens in humans from an expression standpoint. Dr. Carlos Caulin, also of the M.D. Anderson Cancer Center, spoke about how his group is developing an inducible system that permits the focal accumulation of somatic mutations such as those that occur in sporadic human cases.

Dr. Christopher A. Moskaluk remarked on xenograft model systems of ACC and described the ACC xenografting effort at the University of Virginia. Most material comes from resected primary and metastatic tumors. In 27 attempts at establishing unique xenografts, 17 have been successful (71% take rate); 3 are pending (< 1 year), and 7 were unsuccessful. The tumor-suppressive phenotype of myoepithelial cells derived from SGTs was the topic of the presentation by Dr. Sanford H. Barsky, Ohio State University College of Medicine. He has

established four transplantable xenografts and several cell lines derived from myoepithelial tumors.

### *Basic/Translational Science Research*

The focus of a session entitled, “Status of Biomarker Research on Salivary Gland Tumors, was on defining etiologic biomarkers, subgrouping tumors accordingly, and initiating clinical trials.

Dr. Frederic Kaye of the NCI spoke about the 11;19 translocation as the karyotypic abnormality implicated in MEC. How might the *Crtc1-Maml2* fusion oncogene serve as a biomarker for diagnosis and treatment of SGTs? How can it be used in clinical trials? Can the biology of *Crtc1-Maml2* be exploited to design targeted therapies? Dr. Isabel Fonseca, Instituto Português de Oncologia de Francisco, offered additional current perspectives on biomarkers in salivary neoplasia. One notable finding was that the *Crtc1-Maml2* fusion oncogene appears to have prognostic implications and might serve as a basis for identifying patient subgroups.

Several genetic events can lead to the malignant transformation of benign pleomorphic adenoma (PA). Using genome-wide high-resolution arrays for comparative genomic hybridization analysis of SGTs, Dr. Göran Stenman of the Sahlgrenska Academy at Goteborg University, has shown that in ACC, losses are much more common than gains; fewer than 10 copy number alterations are implicated in the majority of ACC tumors. Gene amplifications are very rare. Dr. Stenman also compared his group’s findings to other recently published CGH studies of ACC.

Dr. Patrick Ha, Johns Hopkins University, spoke about promoter hypermethylation, which is an effective means of silencing tumor suppressor genes by inhibiting transcription and gene discovery efforts. The hope is to use expression to try to discover genes that are relevant in SGT development.

The aim of studies by Dr. Charlotta Lindvall of the Van Andel Institute was to characterize the synergy between the Wnt/ $\beta$ -catenin and mTOR signaling pathways in tumorigenesis. She described efforts to develop a Wnt/mTOR mouse model by crossing Pten-flox mice with Apc-flox mice. Although it was expected that cross genotype would have a mammary phenotype, the mice developed SGTs (mostly in the parotid gland) and none had mammary tumors. The histologic subtype is similar to human acinic cell carcinoma.

In another session, “Current and Evolving Concepts in Clinical Trials for Salivary Gland Tumors,” several presenters commented on ongoing and recently completed trials and offered some ideas for future clinical trials of molecularly targeted agents for treating malignant SGTs. Dr. Lillian Siu, Princess Margaret Hospital, University of Toronto, reviewed several clinical trials of molecularly targeted agents in ACC and pointed out some challenges in carrying out randomized clinical trial designs with small patient populations. To carry out such studies, a global effort will be needed.

Dr. Merrill Kies, University of Texas M.D. Anderson Cancer Center, suggested that clinical trials of molecularly targeted agents in SGTs could be accomplished with adaptive designs that could correlate molecular markers with clinical outcomes while evaluating safety and efficacy of the agents. This new research paradigm calls for mandatory biopsies and offers an opportunity

for biomarker discovery and confirmation. The group decided that it would be worthwhile to have a power analysis done by a biostatistician to see how many patients would be needed for the proposed marker-based design.

Mr. Jeffrey Kaufman, the executive director of the Adenoid Cystic Carcinoma Research Foundation (ACCRF), described the organization's beginnings and its purpose: to accelerate the development of improved therapies and find a cure for ACC. He described several research projects supported by the ACCRF. A particular strength of the foundation is its outreach to patients, a strength that will help recruit patients for clinical trials.

### **Action Items**

Workgroup participants agreed on several initiatives for proceeding. In the realm of basic and translational science, participants considered establishing new models for the study of SGT. For example, it would be useful to develop normal and SGT continuous cell lines and xenograft models, gene-targeted mouse models, and MMTV-associated transgene mouse models.

Participants also agreed on the value of gaining access to veterinary specimens for study. For example, if ACC-type or MEC are found, studying them might lead to a greater understanding of the biology of the human disease.

Other important initiatives suggested include: 1) investigating amplification of *erbB2* (*Her2*) (and nearby genes) because it is a very common amplicon in a wide variety of SGTs, 2) tracking SGT epidemiologic data, including incidence and prevalence rates, as well as geographic distribution of salivary gland cancer, would be useful to document whether the number of cases is increasing.

In the area of clinical research, participants agreed that the most important goal is to establish infrastructure and a network for clinical trials. They discussed the need for designing and initiating collaborative trials and encouraging patients to participate in clinical trials. They agreed on:

1. Forming a clinical trials network or group of committed clinicians.
2. Defining the patient populations and types of trials.
3. Deciding what biomarkers and tissue acquisitions are needed for these trials, and what retrospective studies are required to support the concept.

This can be best achieved through the Rare Tumor Risk Force (RTTF), under the auspices of the NCI's Head and Neck Steering Committee. The "concepts" for future clinical trials on SGTs will be developed through the former group (co-chaired by Dr. Barbara Conley, chief, Division of Hematology/Oncology, Michigan State University), and then vetted through the Head and Neck Steering committee, which has representations not only from the cooperative groups, but also the investigators from the Head and Neck Specialized Program of Research Excellence (SPORE). One suggestion was a trial of vandetinib (ZD6474), a dual VEGF/EGFR inhibitor, on (1) patients with ACC (exclusive of ductal cases), and (2) patients with MEC and rarer SGTs. Investigators could collaborate within the network to decide upon a rational study design and the ACCRF would have a role in the planning process.

Finally, the participants agreed on the need for a Web site to serve as a centralized resource to delineate which cell lines, animal models, and xenografts are available. Such a site could also include general information about salivary glands, mouse histology, staining techniques, and so on, to aid investigators who do not normally work on salivary glands.