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The Biology of Tobacco and Nicotine: Bench to Bedside

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Abstract

Strong epidemiologic evidence links smoking and cancer. An increased understanding of the molecular biology of tobacco-related cancers could advance progress toward improving smoking cessation and patient management. Knowledge gaps between tobacco addiction, tumorigenesis, and cancer brought an interdisciplinary group of investigators together to discuss “The Biology of Nicotine and Tobacco: Bench to Bedside.” Presentations on the signaling pathways and pathogenesis in tobacco-related cancers, mouse models of addiction, imaging and regulation of nicotinic receptors, the genetic basis for tobacco carcinogenesis and development of lung cancer, and molecular mechanisms of carcinogenesis were heard. Importantly, new opportunities to use molecular biology to identify and abrogate tobacco-mediated carcinogenesis and to identify high-risk individuals were recognized.

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Introduction

Tobacco accounts for 30% of all cancer deaths and lung cancer is the most lethal cancer killing more Americans in 2004 than breast, prostate, and colorectal cancers combined (1). Tobacco addiction is mediated by nicotine. Despite strong epidemiologic evidence linking smoking and cancer and increased understanding of the molecular biology of tobacco-related cancers, progress in improving smoking cessation and patient management is modest. To bridge knowledge gaps between tobacco addiction, tumorigenesis, and cancer, an interdisciplinary group of investigators met on April 23, 2004, in Gaithersburg, Maryland, to discuss “The Biology of Nicotine and Tobacco: Bench to Bedside.” The format included oral presentations from speakers from five NIH institutes and the extramural community; a poster session is also included. Topics covered signaling pathways and the pathogenesis of head and neck cancers, mouse models of addiction, imaging and regulation of nicotinic receptors, the genetic basis for tobacco carcinogenesis and development of lung cancer, and molecular mechanisms of carcinogenesis. This meeting provided information exchange, facilitating interactions between investigators from different disciplines. Importantly, new opportunities using molecular biology to identify and abrogate tobacco-mediated carcinogenesis and to identify high-risk individuals were recognized.

Nuclear Factor-κB and the Proteasome in Pathogenesis and Therapy of Head and Neck Cancer

Dr. Carter Van Waes began by discussing nuclear factor-κB (NF-κB) as a transcription factor induced in response to injury by chemical toxins, such as carcinogens, infections, or growth factors and cytokines. NF-κB is constitutively activated in head and neck squamous cell carcinomas (HNSCC) and its activation is dependent on signal-activated degradation of inhibitor-κB by the proteasome. Proteasome inhibitors inhibit NF-κB activation, proliferation, resistance to apoptosis, and angiogenesis in preclinical studies. The proteasome inhibitor bortezomib has been shown to inhibit radiation-induced activation of NF-κB and have radiosensitizing activity against HNSCC. A phase I trial combining bortezomib with radiation therapy provided evidence for NF-κB inhibitory, proapoptotic, and antiangiogenesis effects in tumor tissue. His studies provided a rationale for targeting upstream or coactivating pathways of NF-κB in the prevention and therapy of HNSCC and other tobacco-related aerodigestive malignancies.

Head and Neck Cancer and Signaling Networks: Emerging Paradigms

Dr. J. Silvio Gutkind continued discussing important pathways using HNSCC as a model. He pointed out that although tobacco use and alcohol consumption are well-known risk factors, there is a lack of understanding in the nature of the deregulated molecular functions characterizing these lesions. In HNSCC, some underlying genetic alterations have been identified, but the molecular mechanisms responsible for this malignancy are not fully understood. His laboratory developed technologies for high-throughput analysis of gene and protein expression and function in HNSCC. These efforts have helped identify aberrant patterns of gene expression, including genes encoding differentiation markers, signal transducing and cell cycle regulatory molecules, growth and angiogenic factors, and matrix degrading proteases. These data suggested that alterations in the *wnt*/β-catenin and *notch* signaling pathways may contribute to carcinogenesis. Efforts in cell- and animal-based models are now under way to establish their causal or correlative relationship. Parallel studies addressing the aberrant function of signaling pathways have also revealed a number of highly interrelated alterations in key signaling routes, including Akt, Stat3, and NF-κB. The emerging information may identify markers for early detection of preneoplastic lesions

and targets for pharmacologic intervention in both chemoprevention and treatment of tobacco-related neoplastic diseases.

Pharmacology and Regulation of Neuronal Nicotinic Receptors

Dr. Kenneth Kellar presented his work toward understanding nicotine receptors. Nicotinic acetylcholine receptors (nAChR) are found throughout the central nervous system and peripheral nervous system and, additionally, in non-neuronal tissues, including bronchial epithelial cells, vascular endothelial cells, vascular smooth muscle, skin keratinocytes, and the immune system. nAChRs are composed of combinations of α and β subunits forming pentameric ligand-gated cation channels. Nine α subunits and three β subunits have been identified in vertebrates and different subunit combinations define specific receptor subtypes. However, the actual number of possible nAChR subtypes is not yet established and the subtypes exhibit diverse biophysical and pharmacologic properties. Radioligand binding studies show that the affinity of agonists is higher at heteromeric receptors containing α subunits in association with β_2 than with β_4 subunits. The pharmacologic characteristics of functional responses mediated by a nAChR can help identify that receptor. Pharmacologic characteristics combined with anatomic methods, such as receptor autoradiography, can help identify nAChR subtypes even in complex, heterogeneous tissues. Moreover, detailed pharmacologic knowledge has allowed development of *in vivo* imaging ligands for brain nAChRs. However, the precise identity of nAChRs subtypes ultimately requires determination of its subunit composition. Thus far, the results indicate the prevalent subtypes in forebrain are $\alpha_4\beta_2$ and α_7; nevertheless, other subtypes exist in the central nervous system and seem to predominate in some brain regions. Chronic exposure to nicotine leads to increased density of nAChR binding sites in rodent brains, and a similar increase is found in smokers. These receptors are desensitized but are functional after removal of the nicotine. Consistent with the apparent resistance to change of the other subtypes in brain, nAChRs in autonomic ganglia, adrenal gland, and pineal gland are not increased by chronic exposure to nicotine. The nicotine-induced increase in brain nAChRs may be directly related to the changes underlying nicotine addiction; therefore, the cellular mechanisms underlying these increases are vitally important targets for further study.

In vivo Imaging of Nicotinic Receptors: A New Tool for the Investigation of Nicotine Dependence

Dr. Alexey Mukhin suggested that noninvasive *in vivo* positron emission tomography imaging of nAChRs containing α_4 and β_2 subunits in human brain could be valuable for elucidating the role of the receptors in nicotine dependence and the pathogenesis of Alzheimer's and Parkinson's diseases. Previous research focusing on radioligands for *in vivo* imaging identified 5-[^{123}I]iodo-A-85380 and 2-[^{18}F]fluoro-A-85380 (2-[^{18}F]FA) as radioligands, which are analogues of 3-(2(*S*) azetidinylmethoxy) pyridine (A-85380) that allow visualization of brain regions with high densities of nAChRs in humans. Recent human studies confirmed that 5-[^{123}I]iodo-A-85380 and 2-[^{18}F]FA are suitable for quantifying thalamic nAChRs *in vivo*. However, the use of 5-[^{123}I]iodo-A-85380 and 2-[^{18}F]FA for quantitative studies of nAChRs in extrathalamic regions is difficult. To identify radioligands for quantitative imaging of extrathalamic nAChRs, his laboratory developed a series of high-affinity derivatives. Positron emission tomography studies in Rhesus monkeys with two radioligands from this series ([^{18}F]NIDA52189 and [^{18}F]NIDA522131) revealed binding potential values exceeding 2-[^{18}F]FA by 2- to 3-fold. These data and results from preliminary toxicity studies in mice show that they were comparable with 2-[^{18}F]FA, and suggest that [^{18}F]NIDA52189 and [^{18}F]NIDA522131 are promising ligands for studies of extrathalamic nAChRs in humans. Recent positron emission tomography studies with 2-[^{18}F]FA in the Rhesus monkey showed that continuous i.v. infusion of nicotine decreased *in*

vivo binding of radioligand to nAChRs in a dose-dependent manner. Demonstration of similar receptor occupancies by nicotine in human brain and the study of dynamic changes of nicotine-induced increase of the density of nAChRs in smokers' brain could be important in determining the role of nAChRs in nicotine dependence.

Inhibiting the AP-1 Transcriptional Factor in the Mouse Striatum Potentiates the Addictive Properties of Cocaine: Is This a Good Model for Nicotine Addiction?

A mouse model for nicotine addiction based on cocaine was presented by Dr. Charles Vinson. Cocaine administration produces changes in transcriptional activity in the striatum and is proposed to be critical for its addictive properties; it is also shown to increase the activity of many transcription factors in the striatum, including AP-1. To investigate the effect of AP-1 activity on cocaine addiction, they selectively expressed an inhibitor of AP-1 DNA binding, A-FOS, in striatal neurons of adult mice. In these mice, no obvious locomotion changes were observed and following a single injection of cocaine, there was no difference in the increased locomotion in the A-FOS-expressing mice compared with wild-type controls. However, following repeated injections of cocaine, the A-FOS-expressing mice show increased locomotion relative to controls. Additionally, a condition place preference assay indicates that A-FOS-expressing mice have a greater preference for the cocaine-associated environment. These data indicate that AP-1 suppresses the addictive properties of cocaine. To identify genes misregulated by A-FOS expression and mediating increased sensitization to the addictive properties of cocaine, a microarray analysis of the striatum before and after acute cocaine treatment was done. These experiments did not identify numerous genes mis-regulated by A-FOS in the basal state. Many previously identified genes induced by cocaine in both the wild-type and A-FOS mice were identified. However, several genes are identified that are misregulated between wild-type and A-FOS mice and, thus, may mediate the increased sensitization to the addictive properties of cocaine.

The Aryl Hydrocarbon Receptor as a Molecular Target of Chemopreventive Phytochemicals

Dr. Grace Yeh presented epidemiologic evidence that diets rich in fruits and vegetables are associated with decreased lung cancer risk. Tobacco smoke contains several known carcinogens requiring the aryl hydrocarbon receptor (AhR)-mediated pathway for activation. AhR is a ubiquitous protein regulating expression of genes involved in activation and detoxification of carcinogens. Carcinogens activate AhR and increase the expression of genes encoding cytochrome P450 1A1 (CYP1A1) and other members of the AhR "battery" of genes. These proteins catalyze oxidative catabolism of the parent carcinogen generating genotoxic metabolites or they may be conjugated by detoxification enzymes and eliminated. The AhR pathway is central to carcinogenesis induced by environmental carcinogens, such as benzo(*a*)pyrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. During the development of this project, the only known natural ligand of AhR was a metabolite of a phytochemical found in cruciferous vegetables, indole-3-carbinol. Furthermore, several phytochemicals were shown to be effective inhibitors of the initiation of aryl hydrocarbon-induced tumorigenesis in rodent models. This led Dr. Yeh's laboratory to hypothesize that dietary flavonoids and curcuminoids may be candidate ligands of the AhR. Her laboratory has identified several flavonoids and curcuminoids as natural ligands of the receptor. Resveratrol is a phytoalexin present in plants and may be responsible for the protective effect of wine against heart disease. Resveratrol was shown to be a potent inhibitor of 7,12-dimethylbenz(*a*)anthracene–induced carcinogenesis and has received a great deal of interest in delineating its mechanism

of action. Carcinogen-induced CYP1A enzyme activity was inhibited by two mechanisms: by blocking the increase in *CYP1A1* and *CYP1A2* expression caused by carcinogens and by a direct, competitive inhibition of the enzyme itself. As a result, there was less activation of carcinogen in resveratrol-treated cells. These dual actions of resveratrol may be an important chemopreventive activity toward AhR-induced cancer. Dr. Yeh also found no evidence that resveratrol itself binds the AhR or induces CYP1A1 expression. It is, therefore, a pure antagonist of the receptor, and is the first and only such compound yet discovered.

Pro-oxidants and Injury Effects: Contributors to Tobacco Carcinogenesis in the Lung?

Drs. Lucy Anderson, Anna Maciag, and Gunamani Sithanandam presented their work on injury effects and reactive oxygen species (ROS) in the lung. Interestingly, recent epidemiologic analyses reveal that lung cancer risk increases more as a function of duration of smoking than of smoking intensity. An analogous phenomenon where tumorigenesis is dependent on exposure may be represented in animal models for tumor promotion. In these models, chronic exposure to a weakly genotoxic or nongenotoxic agent is required to develop tumors initiated by low doses of strong genotoxic carcinogens. Common features of these models include inflammation and increased ROS, notable changes in smokers' lungs. Dr. Anderson's laboratory hypothesized that tumor promotion-like processes play a rate-determining role in smoking-related lung cancer and should receive attention as specific targets for prevention and intervention. Two aspects of lung adenocarcinoma and its causation in this context were presented. The first is that transforming growth factor α (TGFα) is characteristically up-regulated during lung injury and inflammation. They showed that in lung cancer cells, TGFα stimulates a pathway recently implicated in cancer cell growth and survival: the ErbB1/ErbB2/ErbB3 complex, phosphatidylinositol 3 kinase activation through its p85 subunit, activation of Akt, deactivating phosphorylation of glycogen synthase kinase 3β, and up-regulation of cyclin D1 levels. Experiments down-regulating this pathway confirmed that it is essential for maintenance of cell division and invasive morphology. Thus, TGFα may contribute to promotion/ progression of lung cancer by synergizing with up-regulation of ErbB3 during the disease process.

The second aspect is that ROS are present in and generated by tobacco smoke, and inflammatory cells show reduced antioxidant capacity in lungs and blood of smokers. Her laboratory investigated ROS production as part of the mechanism of oncogenicity for mutant K-ras, an oncoprotein frequently mutated in lung adenocarcinoma. High expression of a K-ras^{v12} expression vector in nontransformed peripheral lung epithelial cells in culture results in increased ROS of several types, as well as marked DNA damage; however, after several days, these effects return to baseline, concomitant with pronounced up-regulation of antioxidant defenses. These cells show morphologic changes but are not transformed and grow more slowly than normal. Thus, the potent antioxidant response of the nontransformed lung epithelial cells effectively protects them. However, increased ROS might become transforming if the antioxidant defenses are overwhelmed by stable redox cyclers, such as hydroquinone and catechol, present in high concentrations in tobacco smoke. Additionally, ROS may become transforming in collaboration with single nucleotide polymorphisms or mutations that reduce effectiveness of antioxidant components and/or DNA damage repair. If ROS are found to be transforming under these conditions, it could indicate that maintenance of antioxidant levels is especially important for prevention/intervention efforts.

Genetic Susceptibility to Tobacco Carcinogenesis

Dr. Margaret Spitz presented an integrative epidemiologic approach to studying the carcinogenic spectrum from propensity to cigarette smoking, through predisposition and

early diagnosis, to outcome, by applying the principles of epidemiology to the notable advances in molecular genetics. Interindividual variation in susceptibility to tobacco carcinogenesis may explain why only a fraction of smokers will develop lung cancer. Tobacco dependence exhibits characteristics of a complex genetic trait with gene-to-gene and gene-to-environment interactions and phenotypic/genotypic heterogeneity. Two examples of genetic variants modulating smoking behavior were presented—the dopamine receptor and nicotine metabolic pathways.

One cellular process that may explain variation in tumor susceptibility is DNA repair capacity, which is critical in maintaining genome integrity. Her laboratory applies a functional assay to measure DNA repair capacity and there is a statistically significant trend for increasing lung cancer risk with decreasing DNA repair capacity. Functional assays are labor-intensive and not amenable to large-scale, high-throughput application in population studies, and her goal is to identify sequence variations in DNA repair genes in the DNA repair capacity pathway to predict the repair phenotype, and also to predict outcome in patients treated with platinum-based chemotherapy. How well markers in surrogate tissues (peripheral lymphocytes) reflect genetic events in the target tissue is still unknown. There is a need to develop novel repair assays, build robust multivariate models, and perform genotype/phenotype and surrogate/target tissue correlations.

Genetic Susceptibility in Lung Cancer Kindreds

Dr. Joan Bailey-Wilson reviewed the literature demonstrating that lung cancer may be a heritable trait. In fact, lung cancer is frequently cited as an example of a malignancy solely determined by the environment. Over 40 years ago, Tokuhata and Lilienfeld (2) provided epidemiologic evidence for familial aggregation of lung cancer after accounting for smoking, suggesting the possible interaction of genes, shared environment, and common lifestyle factors in the etiology of lung cancer. Fraumeni et al. (3) reported an increased risk of lung cancer mortality in siblings of lung cancer probands, and positive family history has consistently been a risk factor for lung cancer in case-control studies. Genetic modeling studies also suggest familial aggregation of lung cancer may be due to inheritance genetic factors. Segregation analyses provide evidence for inheritance of a rare major autosomal gene acting in conjunction with cigarette smoking to produce earlier age of onset of the cancer.

Based on this evidence, Dr. Bailey-Wilson and her collaborators did a genome-wide linkage scan in families selected for aggregation of lung cancer. The high case-fatality rate and low resection rate makes studying lung cancer families particularly challenging due to limited numbers of biospecimens available for analysis. Dr. Bailey-Wilson reported 54 families have undergone complete genome scanning through the efforts of the Genetic Epidemiology of Lung Cancer Consortium and that a region of chromosome 6 has reached a genome-wide significant likelihood ratio of evidence for linkage (LOD score). This work has recently been published (4). Dr. Bailey-Wilson concluded that only a multidisciplinary, collaborative effort is likely to be successful in identifying and accruing large numbers of families necessary to test the hypothesis that genetic variations greatly increase the risk of lung cancer.

Conclusions

Following the presentations, the attendees discussed key questions and explored options to extend established research efforts into new interdisciplinary areas. Are mechanisms of tobacco-related carcinogenesis similar in all tobacco-related cancers? Are molecular alterations in NF-κB or signaling pathways in head and neck cancer also observed in

bladder, pancreatic, kidney, and lung cancers? An integrated approach combining genomics, proteomics, and tissue microarrays would be best to address this question and could generate a uniform approach to prevent tobacco-related carcinogenesis.

The participants agreed that a better understanding of the molecular basis for addiction is essential. Specifically, nicotinic receptors are neglected components of tobacco-related research. Although the complexity of these receptors and poor experimental tools have limited study of these receptors in the past, researchers can now profile these receptors, modify their function, and detect the presence or absence of specific receptors using imaging techniques *in vivo*. These developments could allow the development of targeted addiction therapies and imaging of nicotinic receptors in target tissues during tobacco-related carcinogenesis.

Similar to the need to develop a better molecular understanding of addiction, defining mechanisms of actions for chemopreventive agents is required. Can unifying mechanisms be identified for chemically dissimilar chemopreventive agents? The ability of chemopreventive agents to prevent activation of carcinogens, rather than cause regression of preneoplastic lesions, is a laudable goal. This approach could provide intervention before the onset of carcinogenesis.

Finally, have reliable predictive factors for tobacco-related cancers been identified? Can these predictive factors be used to stratify patients for screening and/or early intervention? The recent localization of a major susceptibility locus influencing lung cancer risk to chromosome 6q23–25 offers the opportunity to coalesce a multidisciplinary team to study these rare families (4). The identification of tumor suppressor genes or oncogenes that are altered within this region of the genome may help us to better understand the molecular alterations in sporadic, tobacco-related lung cancers. The participants agreed that augmenting genetic analyses with newer technologies, such as proteomics and molecular tissue analyses, could potentially add power to predictive studies. Applying state-of-the-art science to study addiction, tobacco-related carcinogenesis, and patients most at risk could provide benefit to individuals and society burdened by the effects of tobacco.

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