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Expression and characterization of nicotinic receptor polymorphisms in lung cancer cell lines

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Worldwide approximately 5 million deaths annually are attributed to tobacco-associated diseases. Identifying genetic factors that modify individual risk associated with nicotine dependence, metabolism and disease susceptibility is therefore a high priority. Multiple independent genome-wide association studies (GWAS) have recently identified that the 15q24/15q25 gene locus encoding α3, α5 and β4 nicotinic acetylcholine receptor (nAChR) subunits is linked to nicotine dependence and lung cancer risk. One particular variant of the α5 nAChR subunit, rs16969968, a non-synonymous single nucleotide polymorphism (SNP) has been identified as being highly associated with both nicotine dependence and lung cancer. In the major allele of rs16969968, residue 398 of α5 is aspartate, in the minor, cancer-associated allele, residue 398 is asparagine. Consistent with the role of nAChR signaling in lung cancer, our laboratory has previously demonstrated that lung cancers express an upregulated autocrine cholinergic signaling pathway in which exogenous nicotine and endogenous acetylcholine (ACh) stimulate the growth of lung cancer cells by interacting with nAChR and muscarinic ACh receptors (mAChR) expressed by the lung cancers. The aim of this study was to characterize expression of nAChR polymorphisms in 15q24/15q25 in small cell lung cancer (SCLC) cell lines and to examine if expression levels and function varied with genotype at rs16969968. A panel of 28 SCLC cell lines was analyzed using real-time PCR and all lines were found to express the α5 nAChR subunit and most expressed the α3 and β4 subunits as well. Cell lines were genotyped using real-time PCR for rs16969968 and also for rs1051730, a synonymous SNP in α3, which are tightly linked together, $R^2 \geq 0.8$. Consistent with described allele frequencies in Caucasians (in which the minor A, cancer associated-allele frequency is 33–42%) the AA, GA and GG genotypes were respectively found in 8, 5 and 15 in the SCLC cell lines. In addition, consistent with other published studies, there was perfect linkage between the genotype of rs16969968 with rs1051730 in the cell lines. Statistical analysis of nAChR mRNA subunit levels showed no difference in nAChR mRNA levels based on genotype. This suggests that the increased cancer risk associated with the A allele at rs16969968 is not due to alteration of the mRNA levels, but rather may be associated with nAChR receptor function. Studies are currently in progress with SCLC cell lines expressing either one or the other of the two homozygous forms of rs16969968 to determine if functional differences in cell proliferation or nicotine-induced currents in the cancer cells can be attributed to the altered structure of α5.

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"Neuron-like" communication between nicotinic signaling and GABA signaling in bronchial epithelial cells: Implications for the link between smoking, asthma and COPD

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Our laboratory has previously shown that prenatal nicotine exposure alters normal lung development by upregulating a cholinergic paracrine loop in which airway epithelium synthesizes and secretes acetylcholine. Chronic nicotine exposure both activates and increases nicotinic receptor expression in developing lung. Xiang et al. [1] have recently reported that GABA signaling by BEC is essential for mucus overproduction in asthma. We now report that activation of nicotinic receptors (nAChR) in BEC by chronic nicotine upregulates GABA signaling in BEC to increase mucus overproduction in monkey bronchial epithelia cells (BEC). As shown by both immunofluorescence and RT-PCR, monkey BEC and lung express the GABA synthesizing enzyme GAD, GABA transporters (GAT1-3) and $GABA_A$ receptor subunits α5, β1, β2, γ2 and ρ. Real-time PCR showed that chronic nicotine exposure increased mRNA expression of GAD, $GAT_{1\text{-}3}$, and $GABA_A$ receptor subunits α5, β1, β2, γ2 in both cultured BEC and in lungs from newborn monkeys exposed to nicotine *in utero*. In cultured monkey BEC, 0.5 mM GABA induced a transient inward current. Incubation of BEC with 1 μM nicotine for 48 h, increased the amplitude of nicotinic receptor currents as we have previously reported [2], but also increased the GABA-induced current by 48%, and GABA current density (pA/pF) was enhanced by 33%. The nicotine-induced increase in GAD and GABA receptors was blocked by the nicotinic antagonists mecamylamine (Mec) and methyllycaconitine (MLA). As shown by both PAS staining and immunofluorescence, prenatal nicotine increased mucin and mucus expression in developing lung. In cultured BEC, incubation with 1 μM nicotine for 48 h significantly increased MUC5AC mRNA expression. This increase in mucin mRNA by nicotine was suppressed by the nicotinic antagonists, Mec and MLA, but also by the $GABA_A$ receptor antagonists, picrotoxin and bicuculline. Nicotine also increased mRNA expression in BEC of two proteins shown to regulate $GABA_A$ receptor expression in brain, Plic-1 and $GABA_A$ receptor associated protein (GABARAP). Suggesting a role for GABARAP in mediating the effects of nicotine on BEC GABA signaling, siRNA knockdown of GABARAP in BEC prevented the nicotine-induced increase in $GABA_A$ receptor subunit γ2. These results show that activation of nAChR in BEC leads to activated GABA signaling in BEC leading in turn to increased mucin expression in BEC. This data thus suggests a new mechanism underlying the connection between smoking, asthma and COPD. This data also suggests a new paradigm of communication between non-neuronal transmitter systems in BEC that will likely provide a new target for development of drugs for common lung diseases.

References

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