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Mechanisms of Differential Expression of the CYP2A13 7520C/G Alleles in Human Lung: Allelic Expression Analysis of CYP2A13 Heterogeneous Nuclear RNA and Involvement of Multiple Cis-Regulatory Single Nucleotide Polymorphisms

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CYP2A13 has been implicated in lung cancer susceptibility among smokers. A common 7520C>G single nucleotide polymorphism (SNP) of CYP2A13 is associated with decreased allelic expression of CYP2A13 mRNA in human lungs. The aim of this study was to explore the mechanisms of the decreased allelic expression of the 7520G allele. Initial studies measuring mRNA stability in CYP2A13-transfected cells indicated that the 7520C>G SNP does not play a direct role in the low allelic expression. Allele-specific expression analysis for heterogeneous nuclear RNA (hnRNA) demonstrated a consistently low expression of the 7520G allele in lung samples from nine heterozygotes, findings that implicated decreased transcriptional activation of the 7520G allele. We speculate that regulatory region SNPs commonly occurring among the nine subjects, including -1479T>C, -3100T>G, and -7755G>A, are likely responsible for the altered transcriptional regulation. These SNPs impacted the ability of the associated cis-elements to interact with DNA binding proteins in gel-shift assays. Reporter gene assays indicated that each of the three SNPs led to decreased promoter activities, and the -3100T>G and -7755G>A SNPs had an additive effect in their suppression of CYP2A13 promoter activities. The -1479T>C variation generated a new CpG site, which was at least partially methylated in human lung DNA. In vitro methylation of CYP2A13 promoter constructs containing either -1479T or -1479C led to greater decreases in promoter activity for the -1479C than for the -1479T construct. Thus, the decreased expression of the 7520G allele probably resulted from cumulative effects of multiple sequence variations, with each having a small effect.