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ROLE OF GENETIC POLYMORPHISM IN HUMAN SUSCEPTIBILITY TO ENVIRONMENTAL CARCINOGENESIS

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Humans are exposed daily to numerous environmental chemicals, including many carcinogens. However, it is a well-known fact that there is a large inter-individual difference in human susceptibility to environmental carcinogens and other toxic compounds. Elucidation of the mechanisms of susceptibility and identification of the individuals or the subpopulations that are particularly susceptible to environmental carcinogens are critical in cancer prevention.

Recently, the role of genetic polymorphism in human susceptibility to environmental carcinogenesis has received increasing attention. Genetic polymorphism refers to the frequently occurring DNA sequence variations, mostly at the single nucleotide level (single nucleotide polymorphisms, SNPs), in the genome. In contrast to the somatic mutations often identified in oncogenes or in tumor suppressor genes in many human tumors, the polymorphic sequence variations in human genome are conserved, inheritable changes. A DNA sequence variation is viewed as a genetic polymorphism when its frequency distribution in a given population is at least 1%.

Although the existence of the functional polymorphic variant alleles, including the "null" alleles (e.g. human glutathione *S*-transferase M1 and T1), has been demonstrated, it is important to bear in mind that most of the genetic polymorphisms in human genome have no functional significance. Even though a particular gene is known to be critical in the process of environmental carcinogenesis, the polymorphism(s) of that particular gene may or may not play an important role in carcinogenesis, depending on whether the polymorphism has a significant functional consequence. This concept should be

emphasized while designing all population-based studies to assess the role of genetic polymorphism in human risk to cancer and other environmental toxicity.

The general approaches used in studying the role of genetic polymorphism in human susceptibility to environmental carcinogenesis include: selection of the target gene(s) known or believed to play an important role in carcinogenesis, identification of novel genetic polymorphisms in the target genes, functional characterization of the genetic polymorphisms including the correlation of genotype to phenotype *in vivo*, and population-based studies.

In recent years, our group has taken these approaches and has focussed on the genetic polymorphisms of two environmentally relevant human enzymes, O^6-alkylguanine-DNA alkyltransferase (AGT) and cytochrome P450 2A6 (CYP2A6). AGT is a DNA repair protein that specifically repairs O^6-alkylated guanine in DNA, a major premutagenic lesion induced by alkylating carcinogens and chemotherapeutic drugs. It plays a critical role in protecting cells against the carcinogenicity and cytotoxicity of alkylating agents. Human CYP2A6 has been reported as a major enzyme responsible for the metabolism of nicotine and for the metabolic activation of two tobacco-specific carcinogens, N(-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Remarkable inter-individual variations in the activity and expression levels of both AGT and CYP2A6 have been observed in humans, and functional genetic polymorphisms of AGT and CYP2A6 could be a major factor responsible for these variations. Consequently, the functional genetic polymorphisms of these two enzymes may serve as an important determinant in an individual's susceptibility to relevant environmental cancers. We have identified a missense polymorphism (converting isoleucine to valine) at codon 143 ofthe human AGT gene, a position within the active site of the protein and only one amino acid apart from the alkyl acceptor Cys145. This codon 143 polymorphism appears to coexist with another polymorphic alteration in codon 178 (converting arginine to lysine). The functional characterization work on the codon 143/178 genetic polymorphism is in progress. We have also identified a BanI genetic polymorphism in the promoter region of the human AGT gene and this polymorphism has been demonstrated to have little effects on AGT promoter activity, as determined by a reporter gene assay, and on AGT mRNA expression level in human esophageal mucosa, as determined by a real-time quantitative RT-PCR (Taqman) method. In the protein coding region of the human CYP2A6 gene, we have identified three novel missense polymorphic variations (Ser29Asn, Arg64Cys, and Lys476Arg) and confirmed the existence of a reported CYP2A6v1 variant (Leu160His). To determine the functional significance of these polymorphic alterations, wild-type and variant CYP2A6 proteins were produced by site-directed mutagenesis/heterologous expression, and were

utilized for the metabolism of nicotine, NNK, and other CYP2A6 substrates. In comparison to the wild-type CYP2A6, the Ser29Asn, Arg64Cys, and Lys476Arg variant proteins did not show a significant difference in their activities in metabolizing the CYP2A6 substrates, suggesting that these polymorphic variations are of little functional importance. In contrast, the 2A6v1 (Leu160His) variant protein showed a total loss of the activities in the metabolism of the CYP2A6 substrates tested, suggesting that this polymorphic variation results in a "null" allele. These results provide important information for designing future molecular epidemiological studies on the relationship between CYP2A6 genetic polymorphism and smoking-related cancer risk in humans.

Although the current research on genetic polymorphism holds great promise in providing molecular mechanisms of inter-individual variations in responses to environmental carcinogens and in identifying the most susceptible individuals or subpopulations for protection, more extensive work is needed to establish the role of genetic polymorphism in environmental carcinogenesis. It will be difficult, if not impossible, to accomplish this task without the intensive collaborations amongst the scientists in different fields such as molecular biology, biochemistry, and epidemiology. In addition, many challenging issues need to be addressed. These include 1) To develop high-throughput methods for functional characterization of the polymorphic variants; 2) To elucidate the structure/activity relationship of the target molecules, with the aid of computer modeling and bioinformatics, for predicting the functional significance of a particular polymorphic alteration; 3) To determine gene-gene interaction by incorporating into the epidemiological studies the functional genetic polymorphisms of the multiple genes, each representing a different important pathway in carcinogenesis; and 4) To assess gene-environmental interaction by correlating the biomarkers of carcinogen exposure. (Research supported by the NIH grants RO1ES 10048, RO1ES 09885, and NIEHS Center Grant 05022).

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