von Hippel–Lindau Gene Mutations in Human and Rodent Renal Tumors—Association With Clear Cell Phenotype

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Renal cell carcinoma (RCC) is the most common cancer in the adult kidney, accounting for about 85% of all renal cancers. It is estimated that in 1998, there will be approximately 29,900 new cases of RCC diagnosed in the United States (17,600 in men and 12,300 in women), and 11,000 people will die of RCC (1). Risk factors for developing RCC include cigarette smoking and hypertension (2) as well as obesity, diet, end-stage renal disease, and occupational exposure to asbestos or cadmium (3,4). With an incidence rate that has increased more than 45% between 1973 and 1995 (5), gaining an understanding of the etiology of this disease is clearly important.

There are several genetic diseases associated with RCC. Affected patients in high-risk families often develop multiple, bilateral renal tumors. These include patients with von Hippel–Lindau (VHL) disease (an inherited syndrome in which 40% of the affected patients will develop RCC) (6), patients with a form of RCC termed hereditary papillary renal carcinoma (HPRC) (7), and patients with tuberous sclerosis (8,9). The genes underlying each of these diseases have been cloned and germline mutations in affected patients have been identified (Table 1).

The classification of RCC is complicated and renal tumors are often diagnosed according to a mixture of histologic and cytologic criteria. A cytomorphologic classification method for RCC was developed by Thoenes et al. (10). This scheme classifies tumor cells into five cell types and variants. Based on antigenic markers, the basic cell types also appear to correspond to specific sites of origin in the kidney. The most common forms of RCC are the clear cell and chromophilic types, which likely arise from the proximal renal tubule. The clear cell phenotype results from cells that are rich in glycogen and lipid. Therefore, after hematoxylin–eosin staining, the cells appear to have an empty cytoplasm. Chromophilic cells, on the other hand, have little glycogen and show strong basophilic hematoxylin–eosin staining. Clear cell RCCs tend to have a compact growth pattern and account for about 75%–80% of all RCCs. Chromophilic RCCs tend to have a papillary growth pattern and comprise about 10%–15% of all RCCs. RCC genotypes appear to associate to an extent with phenotypes. Patients with VHL disease develop clear cell RCC. In addition, VHL is inactivated in the majority of sporadic clear cell RCC tumors [reviewed in (11)]. RCC tumors from patients with HPRC are chromophilic with a papillary growth pattern, but sporadic tumors with this phenotype may not commonly show c-met proto-oncogene activation (12). RCCs associated with tuberous sclerosis also appear to have a clear cell phenotype (8), but these tumors appear not to have VHL gene involvement. Therefore, further studies will be needed to understand better the complex interactions and activities of the VHL and tuberous sclerosis tumor suppressors and the c-met proto-oncogene and the role that each of these gene products plays in RCC.

In this issue of the Journal, Shiao et al. (13) present a study in which archival tissues from N-nitrosodimethylamine (NDMA)-induced rat renal tumors were analyzed for mutations in the VHL gene. The treatment protocol, in which rats were maintained on a protein-free diet prior to NDMA challenge, resulted in renal tumor formation in 90% of the animals treated. This method, protein deprivation for induction of renal tumors in rats, is interesting in light of the association between a high-protein diet and RCC in humans (4). Differences in dietary and drug metabolism between humans and rats apparently influence induction of renal tumors. Of the renal tumors induced and analyzed, approximately 90% had a granular phenotype, while the remaining 10% had a clear cell or mixed clear/granular cell phenotype. Previous work by these and other authors (14,15) showed that chemically induced nonclear cell rat renal tumors did not contain VHL mutations. In the current study, Shiao et al. report that VHL mutations were identified in three of eight clear cell renal tumors. This is the first study to link VHL gene mutations to chemical exposure, and the results are particularly interesting, since they link NDMA, a chemical found in cigarette smoke, to VHL mutations.
The demonstration of VHL mutations in clear cell or mixed clear cell rat tumors suggests an association between VHL and this cell type. This is supported by the absence of VHL mutations in nonclear cell renal tumors (14,15), the majority of tumors arising in the rat. This is also in agreement with previous analyses of human RCC tumors (11). However, the critical question, which is beyond the scope of the current study, is whether the VHL mutations detected are linked to the etiology of the disease. Unfortunately, the data are not straightforward. In human clear cell tumors, VHL inactivation is apparent. From our own study, 53% of the detected VHL mutations introduced protein coding frameshifts, while the remaining resulted in nonconservative amino acid substitutions (11). Of the rat clear cell tumors reported, the point mutations do not result in striking amino acid substitutions: I20V (isoleucine to valine at position 20), R142K (arginine to lysine at position 142), and Q165Q (glutamine to glutamine at position 165, a silent change). A fourth nucleotide substitution was reported, a G→A transition near the 5’ splice donor site in intron 2. The intronic sequences surrounding this substitution are not conserved among rat, mouse, or human VHL sequences, and based on 5’ splice donor sequence recognition requirements (16), it is unlikely that such a change would adversely affect splicing. Therefore, to determine whether the mutations identified by Shiao et al. (13) affect VHL function, appropriate VHL mutant complementary DNA constructs expressing the appropriate amino acid substitutions would need to be generated and tested for the ability or inability to suppress tumorigenicity in appropriate RCC cell lines.

The study by Shiao et al. (13) also raises the issue of the availability of animal models for RCC. The necessity for animal models is evident. We need to evaluate susceptibility to various carcinogens. In addition, the progression of events from tumor initiation to metastasis must be determined. RCC is a disease in which a great number of patients present with advanced disease, making analysis of early lesions difficult if not impossible. However, only 10% of the NDMA-induced tumors had the clear cell phenotype. This is the inverse of what is seen in human RCC, with 75%–80% of tumors having the clear cell phenotype. Therefore, additional studies examining different chemical carcinogens and/or different strains or species is warranted to determine whether the “human” form of RCC can be recapitulated.

The Eker rat has been extensively studied as an animal model for RCC (17). This strain has a germline disruption of the TSC2 gene. While the −/− genotype is embryonic lethal, the +/− rats are viable and develop spontaneous or chemically induced chromophilic RCC at a high incidence. Tumorigenesis is associated with inactivation of the wild-type TSC2 allele. From the Eker model and the model presented by Shiao et al. and others, it is evident that chromophilic RCC arises at much higher frequencies in rats than they are seen in humans. This may be due to the types of chemical carcinogens administered [we simply have not identified the drug(s) that induce the clear cell phenotype] or differences in drug metabolism between rats and humans.

Tumor suppressor gene inactivation requires two allelic mutations. In VHL-associated RCC and in sporadic clear cell RCC, this involves mutation of one allele and deletion of the other (11). The frequency of second allele-inactivating events may be the central difference between human and rodent RCC. In humans, the VHL gene is located telomERICALLY on chromosome 3p25.5. With two common fragile sites at 3p24.2 and 3p14.2 (FRA3A and FRA3B, respectively), the VHL gene finds itself in an unstable position. The occurrence of chromosome 3p breakage has been associated with the effects of cigarette smoke (18). Peripheral lymphocytes of young smokers (five or more pack-year history) showed a high frequency of chromosome breakage, particularly at several fragile sites, including 3p14.2 (FRA3B). Therefore, chemical carcinogens in cigarette smoke may induce both VHL mutations and VHL loss of heterozygosity (LOH) in humans. Rats, in the absence of a syntenic fragile site, may not exhibit a high frequency of VHL LOH, therefore decreasing the susceptibility for developing clear cell tumors.

Like clear cell RCC, human lung cancers show a high frequency of chromosome 3p deletions, and a small number of lung cancers contain VHL mutations. Sekido et al. (19) analyzed 72 lung cancers and found five with VHL mutations. These included two nonconservative amino acid substitutions, G106D (glycine to aspartic acid at position 106, a G:C to A:T transition) in a small-cell lung cancer and L89H (leucine to histidine at position 89, a T:A to A:T transversion) in a mesothelioma (19). Two small-cell lung cancers contained silent mutations (nonamino acid change), while another contained a mutation in intron 2 (19). Whether VHL plays any role in human lung cancer development is unclear. However, it appears that VHL mutations do occur in tumors such as small-cell lung cancer, with which smoking has been associated.

Whether there is a direct relationship between VHL mutations and smoking remains to be determined. VHL mutation analyses have now been performed on several hundred RCC samples from the United States, Europe, and Japan (11). Acquisition of smoking histories from these patients and association with genotype should provide the answer. Since smoking increases the risk for developing RCC, it is important to determine whether VHL mutations in RCC are also associated with smoking. In addition, the relationship between smoking and chromosome 3p breakage also calls for a detailed study in patients with VHL disease. Do smokers with germline VHL mutations show either a high frequency of RCC or a distinct pattern of disease manifestation compared with VHL nonsmokers?

REFERENCES

Dietary Mutagens and the Risk of Breast Cancer

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In this issue of the Journal, Zheng et al. (1) report a positive association between the preference for well-done red meat and risk of postmenopausal breast cancer. Using color photographs of hamburger, bacon, and beefsteak showing increasing levels of doneness (from extremely rare to very well done), women who selected a cooking preference for “very well done” for each of these foods had a risk nearly five times higher than that for women who consistently selected a preference for rare/medium cooking. Overall consumption of red meat itself had only a weak association with risk, and white meats, including chicken, turkey, and fish, were not statistically significant risk factors regardless of cooking method. The specificity of the findings to meat preparation tends to rule out saturated fat as the causative factor and is consistent with the possibility that mutagens, including heterocyclic amines, formed during high-temperature cooking of meats may be mammary carcinogens in humans.

The browned and charred surface of meats contains a hash of laboratory carcinogens. Among these are the heterocyclic amines, formed through chemical reactions involving amino acids. The formation of heterocyclic amines requires high temperatures, such as are found in radiative or conductive cooking (e.g., grilling or frying) but not as a rule in indirect convection (e.g., poaching or steaming) methods (2). These substances are capable of inducing tumors in a number of species (2) and are reportedly among some of the most powerful mutagenic agents, based on the Ames test (3). Heterocyclic amines involving creatinine as a precursor are numerically most important. Among these, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) predominates, accounting for approximately one half of the total heterocyclic amine intake in the U.S. diet. Five compounds, i.e., PhIP, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), and 2-amino-9H-pyrido[2,3-b]indole (AαC), make up approximately two thirds of the total intake (4). Based on animal bioassay data, the relative mutagenic potency of these compounds varies by a factor of more than 20, with IQ and DiMeIQx at the high end, PhIP and AαC at the low end, and MeIQx at an intermediate level of potency (4). Thus, the absolute concentration of specific heterocyclic amines in foods must be weighed against their individual potential for genetic damage when they are evaluated as potential human carcinogens. Major dietary sources of these compounds vary from population to population depending on a number of factors, including cultural mores and meat availability. In a study of female registered nurses (5), the major predictor of PhIP in the diet was grilled or broiled chicken, whereas the chief source of DiMeIQx was pan-fried steak.

In the study by Zheng et al. (1), the absence of an association for chicken and fish is unexpected assuming that heterocyclic amines are causal in breast cancer, since these meats in some data contain among the highest levels of heterocyclic amines in compounds. Exposure from chicken would depend heavily on whether the meat was cooked with the skin and if the cooked skin was actually eaten. Not taking these factors into account may have obscured associations for chicken in the authors’ data.

Meat and meat-cooking preferences have been investigated in previous studies of breast cancer, and a few studies have found suggestive associations. Those that have examined intensity of cooking have tended to show positive associations (6–8), although this is not a universal finding (9). In a prospective study from Finland (6), there was a statistically significant el-

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