NRAS Hypermutability in Familial Melanoma With CDKN2A Mutations—Cause and Effect?

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The frequency of cutaneous melanoma has been increasing worldwide for several decades. Approximately 8%–12% of melanomas occur in individuals with a history of cutaneous melanoma in a blood relative. Germline mutations in the CDKN2A tumor suppressor gene have been identified in approximately 20% of familial melanoma families, including many with dysplastic nevi (DN) (1). The CDKN2A region of chromosome 9p21 codes for p16 (INK4A), which functions in the retinoblastoma (Rb) protein cell-cycle control pathway, and also p14ARF, the product of an alternatively spliced transcript, which participates in the p53-mediated cell-cycle control pathway. Melanoma cell lines that do not express p16, or that express a mutant form of p16, do not show the normal G2 cell-cycle delay after UV exposure (2). In this issue of the Journal, Eskandarpour et al. (3) describe a study in which NRAS mutations in melanomas from 25 patients in six Swedish melanoma-prone families carrying germline CDKN2A mutations were compared with NRAS mutations in melanomas from patients with sporadic melanomas. Using sophisticated laser capture microscopy techniques, they isolated melanoma cells from microscope slides and sequenced the tumor DNA. They found activating NRAS mutations in 95% (20/21) of familial melanomas but in only 10% (1/10) of sporadic melanomas (P < .001). These data demonstrate a remarkable hypermutability in the familial cancers.

Are these NRAS mutations related to sunlight exposure? A molecular analysis of the types of mutations found in the NRAS gene may provide some clues. All of the NRAS mutations that the authors observed in the melanomas involved alteration of CAA (codon 61), which codes for glutamine. They found approximately equal frequencies of substitution of arginine (CGA) or lysine (AAA) for glutamine. UV radiation in sunlight damages DNA, forming predominantly cyclobutane dimers or 6-4 pyrimidine pyrimidone lesions at adjacent pyrimidines (TT, TC, CC, or CT) (4). Because the strand opposite the CAA contains a TT sequence, this site is a prime candidate for UV damage. Cellular attempts to repair UV-induced DNA damage or replicate past DNA photoproducts may occasionally result in mutations (i.e., altered DNA sequences). In general, however, most UV-induced mutations do not occur at TT sites, which are the locations of the greatest damage, but at the cytosines in TC or CC sites (4). In the absence of selection, the most common UV-induced mutations are C to T or CC to TT transitions (4). T to C transitions of the type found in NRAS codon 61 that would change glutamine to arginine are found at a much lower frequency than C to T transitions. C to A transversion mutations at isolated pyrimidines, as in the glutamine to lysine mutations in NRAS codon 61, are not typical of UV exposure (4).

In studies of non-melanoma skin cancer, the finding of “signature” CC to TT mutations that inactivate the p53 tumor suppressor gene is often cited as strong evidence for the role of sunlight in induction of squamous cell carcinomas (5,6). By contrast, p53 is rarely mutated in melanoma. Oncogenes such as NRAS, however, are activated in many cancers, including melanomas. Although many mutations can inactivate the p53 tumor suppressor gene, only a limited number of mutations can activate the NRAS oncogene to transform cells. An earlier study demonstrated that in vitro UV treatment of NRAS DNA resulted in transformation of cultured rat cells (7). The authors found codon 61 glutamine mutated to arginine and lysine in the transformed cells. This result indicates that, in the human melanomas studied by Eskandarpour et al. (3), these mutations could be the result of UV damage. However, other agents may also produce the same mutations. Thus, the link between the observed NRAS hypermutability and UV exposure must be viewed as tentative. The discovery of inactivation of a tumor suppressor gene in human melanomas might resolve this issue.

Studies of another genetic disorder may provide insight into the hypermutability in familial melanoma. Xeroderma pigmentosum (XP), like familial melanoma with DN, is a cancer-prone genetic disease with multiple pigmented lesions and an increased frequency of melanoma. XP patients have marked sun sensitivity and a 1000-fold increase in melanomas (and non-melanoma skin cancers) in association with defective DNA repair (8–10). Cells from patients with the variant form of XP (XP-V), like cells from patients with familial melanoma with DN, have normal or near normal post-UV cell survival and normal, unscheduled DNA synthesis (a measure of nucleotide excision repair). Noncancerous, normal-appearing cells from XP-V patients are hypermutable to UV and introduce more mutations into UV-treated plasmids compared with cells from normal individuals (11). Plasmid mutability is measured by the use of a host-cell reactivation assay in which a UV-damaged replicating shuttle vector plasmid is transfected into the human host cell. The plasmid is repaired and replicated by the cell’s enzymes, and mutations that are introduced into the plasmid are assessed by transformation of indicator bacteria, followed by DNA sequencing (11). Thus, XP-V may be a model for possible somatic cellular abnormalities in familial melanoma with DN. Noncancerous lymphoblastoid cell lines from familial melanoma with DN individuals were found to be hypermutable to UV, thus providing evidence of a systemic abnormality (12). Like XP-V cells, familial melanoma lymphoblastoid cell lines show post-UV plasmid hypermutability (13,14). In the absence of selection, analysis of 69 UV-mutated plasmids revealed that the types of single and tandem mutations that these cells introduced into plasmids were 56% C to T transitions, and 26% mutations at T with no detectible T to C transitions. These studies were expanded to examine post-UV plasmid mutability in 31 lymphoblastoid cell lines from six familial melanoma with DN kindreds (15). Lymphoblastoid cell lines from all 13 individuals...
who had melanoma and DN were plasmid UV-hypermutable compared with none of 16 lymphoblastoid cell lines from normal controls. Furthermore, lymphoblastoid cell lines from five of eight individuals in these kindreds with DN without melanoma were plasmid UV hypermutable. Thus, there appears to be a systemic hypermutability in addition to a tumor hypermutability in familial melanoma with DN.

What is the relationship of the UV hypermutability at NRAS to the inherited mutation in the CDKN2A gene? The study by Eskandarpour et al. (3) examined melanomas in individuals who had germline mutations in the CDKN2A gene. They did not study familial melanoma kindreds that did not have this germ line mutation. In our study of lymphoblastoid cell lines from six familial melanoma kindreds, five of the kindreds had germline CDKN2A mutations and one did not (1,15). The relationship of these mutations to plasmid UV-hypermutability is complex. Whereas lymphoblastoid cell lines from 11 individuals with melanoma plus DN had germline mutations and plasmid UV-hypermutability, two had plasmid UV-hypermutability without germline mutations. One melanoma patient without DN had a germline mutation without plasmid UV-hypermutability, and another had neither a germline mutation nor plasmid UV-hypermutability. In addition, four individuals with DN without melanoma showed plasmid UV-hypermutability without germline mutations. Recently, the defect in the XP-V patients was determined to be a recessive germline mutation in an error-prone DNA polymerase gene (pol-eta) that has a role in the bypassing of UV photoproducts (16–19). Hence, other yet-to-be-identified abnormalities in DNA repair may be present in cells from patients with familial melanoma that contribute to the observed in vitro and in vivo hypermutability.

REFERENCES

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