SHORT REPORT

Absence of BRAF mutations in UV-protected mucosal melanomas


Background: Mutations in BRAF have recently been identified in a significant percentage of primary and metastatic cutaneous malignant melanomas. As ultraviolet (UV) exposure may play a role in the development of cutaneous melanomas lesions with BRAF mutations, BRAF mutation frequency in melanomas arising in sites protected from sun exposure may be lower than those from sun-exposed areas. Thus, we determined the BRAF mutation frequency in a panel of 13 mucosal melanomas and compared those data with data from all currently published series of cutaneous melanomas.

Methods: BRAF exon 15 DNA from 13 archival primary mucosal melanomas (eight vulvar, four anorectal, and one laryngeal) was sequenced using intron-based primers. As archival DNA occasionally produces poor-quality template, results were confirmed with a TspRI restriction fragment length polymorphism (RFLP) that distinguishes wild-type BRAF from the common mutant form V599E. A binomial test was used to compare the mutation frequency in the mucosal melanomas with the published mutation frequency in cutaneous melanomas.

Results: None of the 13 mucosal melanomas in this series had an exon 15 BRAF mutation, as compared to 54/165 (33%) primary cutaneous melanomas with BRAF mutations in a compilation of all current published studies (p = 0.006).

Discussion: These data suggest that UV exposure, plays a role in the genesis of BRAF mutations in cutaneous melanoma, despite the absence of the characteristic C>T or CC>TT mutation signature associated with UV exposure, and suggests mechanisms other than pyrimidine dimer formation are important in UV-induced mutagenesis.

Cutaneous malignant melanoma is a lethal form of skin cancer that is epidemiologically linked to solar UV exposure (reviewed in Gilchrest et al1). Recently, oncogenic mutations in the DNA sequence encoding the kinase domain of BRAF have been identified in the majority of primary cell lines derived from cutaneous melanomas.2,3 The observation that about 90% of these mutations in melanomas are due to a recurrent single nucleotide substitution (T1796A; V599E)2,3 raises the possibility that a specific environmental exposure contributes to the genesis of this mutation. However, the common T1796A BRAF mutation is not a characteristic UV signature mutation. UV exposure causes DNA damage via two distinct mutational mechanisms. Cyclobutane pyrimidine dimers (CPD) and 6–4 photoproducts are precursors to the C/T and CC/TT transitions that are the classic ‘‘UV signature’’ mutations.4 UV exposure also causes oxidative damage via generation of oxygen free radicals, which in turn can lead to replicative errors and, ultimately, to base substitutions.4,5 In order to evaluate the role of UV exposure in the genesis of the T1796A BRAF mutation in cutaneous melanoma, we examined a series of melanomas arising in sites protected from sun exposure and compared our results with the published frequencies of BRAF mutations in cutaneous melanoma.

METHODS

Tumour specimens

Tissue blocks for 13 formalin-fixed paraffin-embedded mucosal melanomas (12 primary, one local recurrence), comprised of eight vulvar, four anorectal, and one laryngeal lesion, were retrieved from the Surgical Pathology archives of the Fox Chase Cancer Center and Pennsylvania Hospital under an IRB-approved protocol. The original histopathology data for each case were obtained and haematoxylin and eosin stained sections from all tumours were reviewed by a surgical pathologist (RHE).

DNA extraction

Tumour tissue was manually microdissected from surrounding normal tissue using 5×5 μm sections that were first stained using a modified H&E protocol. In serial order, the steps for this protocol were incubation in: xylene×5 min×2, 100% ethanol×30 s×2, 95% ethanol×30 s, 80% ethanol×30 s, ddH2O×30 s, Harris’ modified haematoxylin×30 s, ddH2O×30 s, ammonium hydroxide×30 s, 80% ethanol×30 s, 95% ethanol×30 s, eosin Y×30 s, 95% ethanol×30 s×2, 100% ethanol×30 s, and xylene×5 min. Slides were air-dried then placed in a vacuum dessicator for a minimum of 2 h to ensure complete dehydration of tissue. DNA extractions were performed using the QiAquick DNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol with one modification: during microdissection of H&E stained sections, tissue was placed directly into Qiagen ATL buffer for proteinase K digestion.

Mutation detection

As archival material may yield poor quality DNA in some cases, mutation detection was performed by two independent methods. First, BRAF exon 15 was amplified by polymerase chain reaction (PCR) using previously published primers: BRAF exon 15F (forward): TCATAATGCTTGCTCTGATAGGA, BRAF exon 15R (reverse): GGCACAAAAATTTAATCAGTGGA. The components of the PCR reaction mix were: 1× GeneAmp PCR Buffer II (Applied Biosystems, Foster City, CA), 2 mM MgCl2, 250 μM dNTPs, 1 μM primers, 2.5 U AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA), 5%
RESULTS
Using a combination of direct sequencing (12/13 samples) and TspRI RFLP analysis (13/13 samples), we did not identify any exon 15 Braf mutations in 13 mucosal melanomas (0/13). Representative results of the RFLP analysis are shown in figure 1. This result represents a highly significantly different Braf exon 15 mutation prevalence in mucosal melanomas as compared to the cumulative exon 15 Braf mutation frequency published for primary cutaneous melanoma (54/165; 33%; p = 0.006) (see table).

DISCUSSION
In the present study, we did not identify any exon 15 Braf mutations in 13 mucosal melanomas. This finding is in sharp contrast to the cumulative published rate of 33% in primary cutaneous malignant melanoma and thus represents a distinct genetic difference in melanomas arising from cutaneous v mucosal sites. Exon 11 was not evaluated in this study as mutations in this region account for a very small percentage of total Braf mutations in both cutaneous melanoma and cancers arising from non-sun-exposed tissues. The lack of Braf mutations identified in mucosal sites suggests that the prevalence of Braf mutations in melanoma varies depending upon the anatomical origin of the tumour, possibly in direct relation to the extent of sun exposure for the tissue of origin. However, we cannot exclude the possibility that an inherent feature of the tissue type of origin is the determinant of Braf mutagenesis in melanoma. Further studies of mutation prevalence in different subtypes of cutaneous melanoma (superficial spreading, nodular, acral lentiginous, and lentigo maligna melanoma) may be informative in this regard, as the extent and type of sun exposure varies between these subtypes.

While the mechanism of Braf mutagenesis in melanoma is unknown, a role for UV exposure must be considered. The major UV-generated DNA lesions, cyclobutane pyrimidine dimers and 6–4 photoproducts, lead to C/T or CC/TT transitions, not the commonly described T/A transversion in Braf. However, several additional minor photoproducts also are generated. The role of these compounds in UV mutagenesis is not well-studied, but they include thymine glycol, pyrimidine hydrate, 8,8-adenine dehydrate, and...
Biochemical and molecular approaches to UV-induced DNA damage and cancer. 1 In summary, these data demonstrate that exon 15 mutation is generated, UV exposure might generate single-strand breaks, and repressive interactions with nearby cells with wild-type BRAF. Thus while a direct role for UV-induced oxidation in BRAF mutagenesis has not been demonstrated, it is possible that an as yet undetermined oxidative lesion serves as a precursor for the T/A transversion. Of note, oxidative damage also occurs in the context of inflammation, which is a component of UV-induced sunburn. The detection of BRAF mutations in several additional tumour types potentially etiologically linked to inflammatory processes—papillary thyroid cancer with Hashimoto’s thyroiditis,12–14 pancreatic cancer with chronic pancreatitis,15–17 and ovarian cancer18 with cyclic, ovulation-induced inflammation—raises the possibility that inflammation-associated oxidative changes play a role in the genesis of BRAF mutations.

Once a BRAF mutation is generated, UV exposure might also promote melanocytic tumour progression. BRAF is a component of the MAPK signalling pathway, with activation of BRAF leading to increased levels of phosphorylated ERK (reviewed in Smalley19). Depending upon the cellular context and level of activity, ERK activation may lead to cell proliferation, differentiation, or apoptosis.20 In normal melanocytes, UV-induced activation of MAPK induces differentiation through α-MSH/MC1R-mediated increases in cAMP that directly or indirectly induce tyrosinase transcription.19 In melanoma, however, constitutive ERK activation provides proliferative signals and inhibits differentiation. In support of this, dominant negative RAS mutations, ERK mutations, and MEK inhibitors lead to differentiation.21 Thus it is possible that in addition to inducing mutations, UV irradiation provokes a stronger proliferative response in melanocytes with an activating BRAF mutation than in neighbouring cells with wild-type BRAF, further promoting melanoma progression in UV-exposed sites.

In summary, these data demonstrate that exon 15 BRAF mutations are much less frequent in mucosal than cutaneous melanomas. Additional studies are needed to further assess the relationship between prevalence of this mutation in specific melanoma subtypes and ultraviolet exposure. Finally, the absence of BRAF mutations in mucosal melanoma suggests that mucosal lesions may not respond as well as cutaneous lesions in clinical trials of BRAF/MEK inhibitors.

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Research support was received from the National Cancer Institute, grant numbers 1K08CA93748 (to RHE) and P50CA093372 (to MH), the National Institute of Arthritis and Musculoskeletal and Skin Diseases, grant number K24 AR02102 (to SRL), and from the Abramson Family Cancer Research Institute (to BLW).
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\textit{J Med Genet} 2004 41: 270-272
doi: 10.1136/jmg.2003.016667