Cutaneous Melanoma Subtypes Show Different BRAF and NRAS Mutation Frequencies

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Abstract

Purpose: BRAF mutations are present in two thirds of cutaneous melanomas and many of the rest have NRAS mutations. However, cutaneous melanoma is a heterogeneous disease with many clinicopathologic subtypes. Of these, the majority fits into four categories: superficial spreading, nodular, lentigo maligna, and acral lentiginous melanoma (ALM). Thus far, there is very limited data combining BRAF and NRAS mutation analysis to explore differences between cutaneous melanoma subtypes. The aim of this study was to address this issue.

Experimental Design: The frequency of BRAF and NRAS hotspot mutations, in exons 15 and 2, respectively, was assessed in 59 cutaneous melanomas comprising superficial spreading, nodular, lentigo maligna, and ALM using single-strand conformational polymorphism and RFLP-PCR analysis.

Results: Only 2 of 21 (9.5%) ALM showed BRAF exon 15 mutation compared with 9 of 14 (64.3%) superficial spreading malignant melanomas, 4 of 11 (36.4%) nodular melanomas, and 7 of 13 (53.4%) lentigo maligna melanomas ($P < 0.01$). However, our key finding is that the combined analysis of BRAF exon 15 and NRAS exon 2 showed that there were no significant differences in the overall mutation frequency between subtypes. In particular, 9 of 19 (47.4%) ALM without BRAF exon 15 mutation had an NRAS exon 2 mutation.

Conclusions: We show that the overall BRAF/NRAS frequency in mutation hotspots is not significantly different among cutaneous melanoma subtypes. These data show that mitogen-activated protein kinase pathway activation may be important in all major subtypes of cutaneous melanoma, although the mechanism by which this is achieved varies.

Cutaneous melanoma is the most serious form of skin cancer because it metastasizes so readily. Initially, it was regarded as a homogeneous entity with a uniformly poor prognosis, but the advent of specialized pigmented lesion clinics allowed detailed multidisciplinary study of cutaneous melanoma and, as a result, a number of different subtypes with distinct clinicopathologic features were described. The distinctions were based on the preferred site of origin, relative amount of UV light exposure, and duration of preinvasive growth, with four subtypes accounting for the vast majority of cutaneous melanomas. These are superficial spreading, nodular, lentigo maligna, and acral lentiginous melanoma (ALM), comprising ~70% to 75%, 20% to 25%, 5% to 10%, and 5% of cutaneous melanoma cases, respectively, in White Caucasian populations, with acral melanomas being the most frequent in dark-skinned races (1). More recently, molecular differences between these subtypes have come to light, corroborating the clinicopathologic observations. Compelling evidence for differences comes from patterns of DNA alterations observed in cytogenic and comparative genomic hybridization studies, where distinct alterations have been identified. For example, acral melanomas tend to have frequent genetic loci showing amplifications, some of which are early events that can even be detected in adjacent histologically normal melanocytes (2). An initial study indicated that 66% of melanomas had BRAF mutations, whereas NRAS mutations occurred in many of the remainder (3). BRAF and NRAS genes both encode mitogen-activated protein kinase (MAPK) pathway constituents. This pathway is important in several crucial cellular processes, such as proliferation and differentiation. Subsequent studies have shown that BRAF and NRAS mutation frequencies differ between cutaneous melanoma subtypes (4–6) and in addition BRAF mutation is uncommon in noncutaneous melanomas such as ocular and mucosal types (7–11). A recent study indicated that the degree of sun damage is more predictive of molecular pathology than cutaneous melanoma subtype, which poses a threat to the relevance of histologic subtype in clinical practice (12).

Nevertheless, there is relatively little data focusing on the frequency of BRAF mutations in cutaneous melanoma subtypes and there are only two studies that combine this with analysis of NRAS mutations (5, 13). These were both in Japanese populations where the overall frequency cutaneous melanoma is lower than in Western countries and where acral melanoma is the most common type. This lack of knowledge undermines

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an emerging goal in cutaneous melanoma research: to determine whether subtypes of cutaneous melanoma have different molecular pathways of tumor progression. Achieving this goal is critical for development of tailored treatment for all of the various forms of cutaneous melanoma. In this context, the aim of this study was to better characterize the frequency of BRAF and NRAS mutations in the four commonest cutaneous melanoma subtypes, the hypothesis being that clinicopathologic differences will be underpinned by molecular differences.

Materials and Methods

Melanoma tissues. Samples were selected from the archives of the University Hospitals of Leicester NHS Trust with local research ethics committee approval. Cases were identified from a search for melanoma samples over a 5-year period, comprising over 500 cutaneous melanomas, each diagnosis being verified by a dermatopathologist (G.S.). From these samples, melanomas were grouped into one of the four major subtypes. The criteria for these subtypes are well recognized by dermatopathologists, but are liable to some degree of subjectivity. We used the criteria summarized in a published table by Crowson et al. (14). Cases that could not be classified into these subtypes or that were other rarer subtypes were discarded. From 440 remaining samples comprising these major subtypes, 14 superficial spreading malignant melanomas (SSMM), 11 nodular melanomas (NM), 13 lentigo maligna melanomas (LMM), and 21 ALM were selected. The relatively large number of acral melanomas was chosen for statistical purposes to ensure that our finding of a low BRAF mutation frequency was genuine. Cases within each subtype were selected according to consecutive laboratory accession numbers. None of the cases have been reported previously. The mean (and median) Breslow depths were 2.4 mm (1.8 mm), 3.8 mm (3.0 mm), 2.4 mm (1.5 mm), and 3.0 mm (1.8 mm); mean ages were 56.5, 65.5, 70, and 67 years; and male to female ratios were 4:10, 5:6, 5:8, and 10:11 for SSMM, NM, LMM, and ALM respectively.

Sun damage was assessed in contiguous skin of cutaneous melanoma samples. Cases demonstrating coalescent areas of solar elastosis in the dermis were defined as showing chronic UV damage, cases with noncoalescent areas were defined as nonchronic UV damage and those with absent solar elastosis as minimal UV damage.

Mutation analysis. Mutations in *NRAS* and *BRAF* genes were detected using RFLP-PCR or single-strand conformational polymorphism (SSCP) analysis of PCR-amplified DNA from 1 to 5 × 10 μm tissue sections per sample. Enrichment of DNA from melanoma cells was done by removing the tumor tissue from 10 μm sections with a pipette tip. The tumor tissue was identified by comparison with a H&E-stained serial section. Microdissection, DNA extraction, and SSCP and RFLP-PCR analyses were done as described previously (15), except that the PCR primers for NRAS exon 2 SSCP analysis were as follows: forward 5′-CACCCCCAGGATTCTTACAG-3′ and reverse 5′-TCGCCTTTCCTCATGTATTG-3′. The RFLP-PCR primers for BRAF exon 15 analysis were as follows: forward 5′-GGTGATTTTGGTCTAGCTGCA-3′ and reverse 5′-GCTTGCTCTGATAGGAAAATGAG-3′. This primer detects T/A transversions at codon 600 (previously called codon 599) by restriction digestion with *Bts*I (New England Biolabs, Ipswich, MA). This restriction enzyme digests the wild-type sequence, but not the mutant, thus identifying mutations in codon 600.

Statistical analysis. A χ^2 test was done for analysis of categorical variables, except for 2 × 2 contingency tables, where Fisher's exact test was used. A Mann-Whitney *U* test was done for continuous variables. All analyses were two-tailed and a *P* value of <0.05 was regarded as statistically significant for the *a priori* (i.e., planned) comparison between cutaneous melanoma subtypes and mutation frequencies, whereas a conservative *P* value of <0.01 was regarded as significant for all other comparisons. All tests were done using SPSS for Windows, version 12.0.1.

Results

We analyzed the four main clinicopathologic subtypes of cutaneous melanoma for BRAF mutation. Full demographic data are shown in Table 1. Codon 600 in exon 15 of the gene was first assessed using a RFLP-PCR method to detect the T/A transversion that represents the vast majority of alterations seen in cutaneous melanoma. Detected mutations were confirmed by DNA sequencing. SSCP was subsequently used to screen exon 15 of the *BRAF* gene for alternative sequence alterations in those cases where T/A transversions were absent, but no further mutations were detected. *BRAF* exon 11 mutations, which are relatively rare in cutaneous melanoma, were not assessed. Examples of RFLP-PCR analysis are shown in Fig. 1A and the *BRAF* mutation results are summarized in Table 2. The overall *BRAF* exon 15 mutation frequency was 22 of 37 cases (37.3%). There was a significant difference in *BRAF* exon 15 mutation frequencies between subtypes, with only 2 of 21 (9.5%) ALM showing this alteration compared with 9 of 14 (64.3%) SSMM, 4 of 11 (36.4%) NM, and 7 of 13 (53.4%) LMM ($P < 0.01$).

Exposure to UV radiation is the best characterized environmental risk factor for cutaneous melanoma and is one of the factors that distinguish some of the melanoma subtypes. There was a significant difference in the frequency of BRAF mutation between cutaneous melanomas with chronic, nonchronic, and minimal UV exposure, being 10 of 18 (55.6%), 10 of 20 (50%), and 2 of 21 (9.5%), respectively ($P = 0.004$; see Table 2). However, this reflected the fact that acral melanomas lacked BRAF mutations and are, by definition, from sites that are relatively protected from UV exposure. Repeating this analysis, excluding ALM, showed no significant relationship between UV exposure and BRAF mutation status ($P = 0.732$). There was no correlation between BRAF mutation status and other clinical variables, namely age, sex, Breslow's depth, or Clark's level, as shown in Table 2.

Previous data indicates that many cutaneous melanoma that lack BRAF mutation harbor NRAS mutation. Thus, the low frequency of BRAF mutations in ALM could be due to an increase in NRAS alterations. We therefore looked for NRAS mutations in cases where *BRAF* exon 15 mutation was absent. Only exon 2 of the *NRAS* gene was analyzed (using SSCP) because this is the commonest site for alterations in cutaneous melanoma, with codon 61 representing a mutation hotspot. The results are shown in Fig. 1B and C and Table 2. Nine of 19 (47.4%) ALM that lacked *BRAF* exon 15 mutation had an *NRAS* exon 2 mutation, although the frequency may be higher, because in three cases PCR with the NRAS primers was unsuccessful, despite several attempts. We also looked for *NRAS* exon 2 mutations in the SSMM, NM, and LMM cases that lacked *BRAF* exon 15 mutations. They showed 2 of 5 (40%), 6 of 7 (87.1%), and 1 of 6 (16.7%) mutations, respectively. All *NRAS* exon 2 mutations were at codon 61, being either Q61R (CAA→CGA) or Q61K (CAA→AAA) alterations. The frequency of *NRAS* exon 2 mutations in these cases showed no statistically significant differences ($P = 0.091$). The frequency of *BRAF* exon 15 and *NRAS* exon 2 mutation was combined to determine the overall frequency in SSMM, NM, LMM, and ALM. This was 11 of 14 (78.6%), 10 of 11 (90.9%), 8 of 13 (61.5%), and 11 of 21 (52.4%), respectively ($P = 0.114$). This indicates that when NRAS mutations are accounted for, cutaneous melanoma subtypes show no statistically significant difference in the

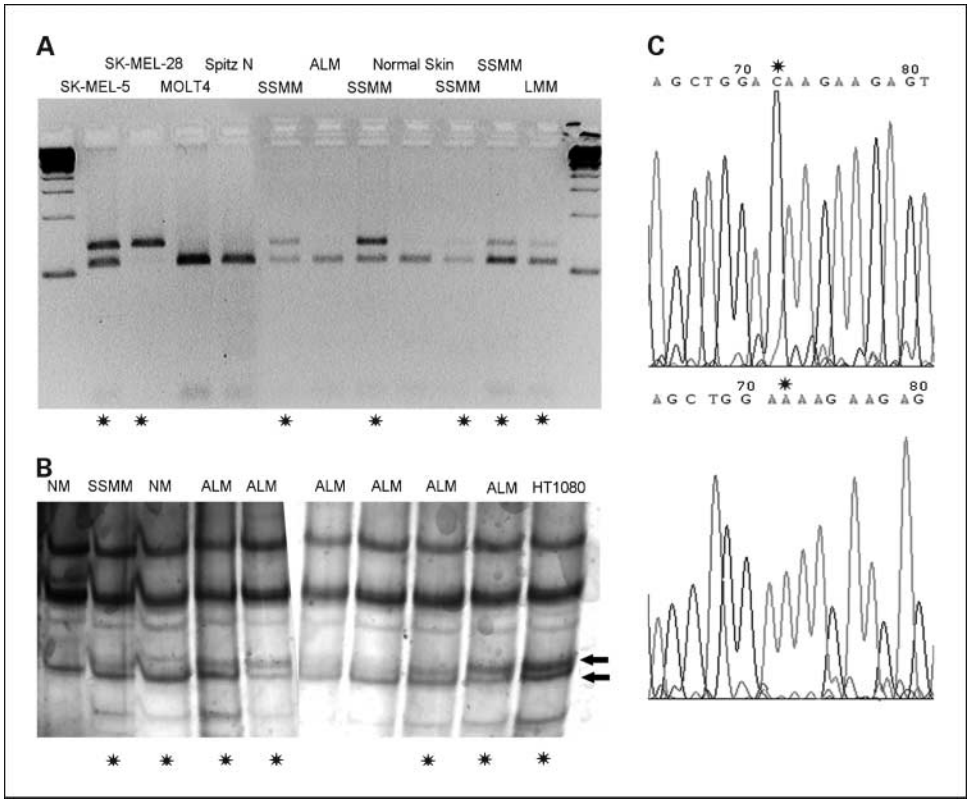

Fig. 1. *BRAF* exon 15 and *NRAS* exon 2 mutation analysis. *A,* RFLP-PCR analysis, where a *Bts*I restriction site was introduced into the PCR product, is shown. Mutation in codon 600 removes the restriction site, thus wild-type sequences show the digested lower molecular weight band, whereas mutant sequences show the undigested higher molecular weight band. SK-MEL-5, SK-MEL-28 (both melanoma cell lines), and MOLT 4 (acute lymphoblastic leukemia cell line) are controls having heterozygous codon 600 mutation, homozygous codon 600 mutation, and wild-type sequences, respectively. Spitz N, a Spitz nevus that we have previously shown to have a wild-type BRAF sequence (see ref. 13). *, lanes where mutations were present. *B, NRAS* exon 2 SSCP analysis, showing melanoma cases alongside the sarcoma cell line, HT1080, which is known to have a codon 61 mutation. Top arrow, aberrant mutant bands; bottom arrow, wild-type bands. *, lanes with mutant bands, all these being codon 61 CAA→AAA, except lane 3, which was CAA→CGA. *C,* sequencing results from the ALM in lane 4 of the SSCP gel picture. The bands corresponding to the two arrowed positions in this lane were picked from the gel with a needle and sequenced. Top, sequencing result for the lower wild-type band; bottom, result for the upper CAA→AAA mutant band. *, the first nucleotide of codon 61.

overall frequency of *BRAF* exon 15 and *NRAS* exon 2 mutations. Forty of 59 cases (67.8%) harbored one or other mutation. Because these mutations are predicted to activate the MAPK pathway, the latter seems to be important in all of the four common subtypes of melanoma. The overall frequency of *BRAF* exon 15 and *NRAS* exon 2 mutations showed no significant correlation with patient sex, Breslow depth, Clark's level, or sun damage (including an analysis that excluded ALM, $P = 0.184$), but there was a correlation with patient age, namely mutant samples were from younger patients, at the 5% significance level, but not at the more conservative 1% level used for unplanned comparisons, as described in Materials and Methods (see Table 2).

Discussion

This study extends current knowledge of the prevalence of *BRAF* and *NRAS* mutations in cutaneous melanoma. Few studies have looked at both mutations in a range of cutaneous melanoma subtypes. This study shows that the BRAF mutation frequency differs between cutaneous melanoma subtypes. However, our key finding is that the overall *BRAF*/*NRAS* frequency is not significantly different among cutaneous melanoma subtypes, most notably because ALM, which have low frequency of BRAF mutation, have a high level of *NRAS* mutations. Although this may support the notion that cutaneous melanoma subtypes follow different roads to malignancy, both *BRAF* and *NRAS* mutations have a common effect: activation of the MAPK pathway. This study therefore shows that activation of this pathway has an important role in all major subtypes of cutaneous melanoma, but the precise way of achieving this varies. However, it should be noted that our study did not incorporate a stratified selection strategy to account for sun damage, and so this confounding effect is not addressed. This is an important consideration when our data are interpreted.

The results of this study show the critical importance of MAPK pathway signaling in cutaneous melanoma, which is not surprising given the role of that pathway in cell growth (16). However, our data reveals that the way of activating the pathway differs between subtypes, possibly reflective of the differences in site and/or sun exposure. It is unclear why NRAS should be the favored mechanism for MAPK pathway activation in ALM compared with other cutaneous melanoma types. UV exposure is the best known environmental risk factor for cutaneous melanoma, but ALM occurs in a relatively UV-protected sites, such as the hairless skin of palms, soles, and nail bed. Thus, differences in UV exposures could account for the differences in *BRAF* and *NRAS* mutation frequency. However, the relationship between cutaneous melanoma occurrence and UV exposure is complex because there is no clear relationship between dose and response (4). Thus, *BRAF* mutations are generally more common in intermittently exposed sites, such as the trunk, than sites of chronic damage, such as the face (4). Furthermore, *BRAF* mutations are seen in some internal malignancies, such as thyroid papillary carcinoma, colorectal carcinoma, pancreatic carcinoma, and borderline ovarian tumors, demonstrating that there are other factors apart from UV exposure that lead to *BRAF* mutation. One possibility is that inflammation and resulting oxidative damage could be the theme that links the occurrence of *BRAF* mutation at diverse sites, with UV damage being one of several ways that an inflammatory response can be generated. Also, the commonest alteration in *BRAF*, a T/A transversion, is not a signature change of UV damage, although there is evidence that psoralen plus

Table 1. Clinical data for melanoma cases

Type	Depth	Clark	Age	Sex	Sun damage	Mutation*
SSMM	3.0	4	56	Male	Nonchronic	WT
SSMM	1.3	3	43	Female	Nonchronic	BRAF
SSMM	1.1	4	21	Female	Nonchronic	NRAS
SSMM	1.2	3	74	Female	Chronic	BRAF
SSMM	0.4	2	49	Female	Nonchronic	BRAF
SSMM	0.9	4	61	Male	Nonchronic	NRAS
SSMM	0.4	2	56	Male	Nonchronic	BRAF
SSMM	4.2	5	81	Female	Nonchronic	WT†
SSMM	2.5	4	52	Female	Nonchronic	BRAF
SSMM	6.4	5	67	Female	Nonchronic	BRAF
SSMM	1.5	3	45	Female	Nonchronic	BRAF
SSMM	3.2	4	65	Female	Chronic	WT
SSMM	6.0	4	66	Female	Nonchronic	BRAF
SSMM	2.0	3	55	Male	Nonchronic	BRAF
NM	4.3	4	77	Female	Nonchronic	NRAS
NM	3.0	4	58	Male	Nonchronic	WT
NM	3.0	4	81	Male	Nonchronic	BRAF
NM	2.5	4	80	Female	Chronic	BRAF
NM	6.0	5	58	Male	Nonchronic	BRAF
NM	1.1	3	45	Female	Nonchronic	NRAS
NM	2.0	3	62	Female	Chronic	NRAS
NM	3.2	4	39	Male	Nonchronic	NRAS
NM	7.2	5	81	Male	Chronic	BRAF
NM	8.0	5	63	Female	Nonchronic	NRAS
NM	1.2	3	76	Female	Nonchronic	NRAS
LMM	4.0	5	45	Female	Chronic	BRAF
LMM	0.7	2	68	Male	Chronic	BRAF
LMM	0.6	3	54	Male	Chronic	BRAF
LMM	0.0	1	49	Female	Chronic	WT
LMM	10.0	4	91	Female	Chronic	NRAS

(*Continued on the following page*)

UVA (i.e., PUVA) can induce this alteration (17). Likewise, the NRAS mutations that we found do not show a UV damage signature. An alternative possibility underlying the variable pattern of mutations is that there are intrinsic differences between melanocytes from different regions of the body. Perhaps subtle variations in gene expression and local environment mean that acral melanocytes get greater advantage from *NRAS* rather than *BRAF* mutation. Alternatively, because acral melanocytes have extremely little exposure to the carcinogenic effects of UV, it may be critical that they progress by the least number of molecular "hits." In this respect, mutations in *NRAS* would be more advantageous than *BRAF*. This is because NRAS acts proximally to BRAF in the MAPK pathway and has additional effects on other cellular pathways, especially activation of the AKT pathway, which seems to be important in cutaneous melanoma (18, 19). Thus, *NRAS* mutation simultaneously activates two key pathways at a stroke. This multipathway effect is supported by a recent study that reported a melanoma model in human skin, where the presence of *NRAS* mutations rendered mutations in *BRAF* or the AKT pathway unnecessary, whereas *BRAF* mutations required additional events to activate AKT (20). However, according to this argument, one might also expect *NRAS* mutations to be relatively more common in SSMM than LMM because they also have relatively low levels of UV exposure. On the contrary, these cases have frequent *BRAF* mutations. However, this type of cutaneous melanoma is particularly associated with benign common nevi (21). The majority of common nevi have a BRAF mutation (22) and thus the high prevalence of mutations in both lesions may reflect the fact that common nevi and SSMM share a related tumorigenic pathway, as suggested by a proportion of SSMM arising *ex nevus*, a phenomenon that is relatively uncommon in some other types of cutaneous melanoma (21). This idea of distinct tumorigenic pathways is supported by recent studies on a group of melanocytic tumors with a characteristic morphology, Spitz nevi and Spitzoid melanoma, which seem to progress via a BRAF/NRAS-independent mechanism (23).

The results of the present study differ from those of Takata et al. (5) and Sasaki et al. (13). Sasaki et al. identified *BRAF* mutation in 4 of 8 SSMM, 5 of 15 ALM, none of 6 NM, 5 LMM, and 1 mucosal melanoma. Takata et al. looked at only 13 primary acral lesions, finding one BRAF mutation. Neither study identified an *NRAS* mutation in primary ALM. By contrast, the

Table 1. Clinical data for melanoma cases (Cont'd)

Type	Depth	Clark	Age	Sex	Sun damage	Mutation*
LMM	0.3	2	79	Male	Chronic	BRAF
LMM	0.4	2	83	Female	Chronic	BRAF
LMM	2.4	4	48	Male	Chronic	BRAF
LMM	4.9	4	93	Female	Chronic	WT
LMM	1.0	4	82	Female	Chronic	WT
LMM	2.0	4	69	Male	Chronic	BRAF
LMM	1.5	3	83	Female	Chronic	WT
LMM	3.1	4	73	Female	Chronic	WT
ALM	1.6	4	83	Female	Minimal	NRAS
ALM	2.2	3	77	Male	Minimal	WT†
ALM	0.0	1	70	Male	Minimal	NRAS
ALM	4.0	4	70	Female	Minimal	WT
ALM	1.3	2	77	Female	Minimal	WT
ALM	1.7	3	41	Female	Minimal	WT
ALM	12.6	5	81	Female	Minimal	WT†
ALM	0.0	1	62	Female	Minimal	NRAS
ALM	0.4	2	39	Female	Minimal	NRAS
ALM	0.0	1	19	Female	Minimal	NRAS
ALM	5.6	5	54	Male	Minimal	NRAS
ALM	3.0	4	82	Male	Minimal	NRAS
ALM	9.0	4	76	Male	Minimal	WT
ALM	3.6	4	83	Male	Minimal	WT†
ALM	1.6	4	63	Male	Minimal	WT
ALM	3.0	4	86	Male	Minimal	WT
ALM	1.5	3	79	Male	Minimal	NRAS
ALM	4.0	4	77	Male	Minimal	BRAF
ALM	0.6	2	33	Female	Minimal	BRAF
ALM	1.8	4	79	Female	Minimal	WT
ALM	5.0	5	90	Female	Minimal	NRAS

Abbreviation: WT, Wild Type.
*Mutation in BRAF exon 15 or NRAS exon 2.
†PCR failure during NRAS analysis.

present study found 11 of 21 mutations in ALM, of which two were in BRAF. This discrepancy could have arisen because of inherent differences in melanoma pathogenesis in Japanese and United Kingdom populations. Also, the frequency at which mutations are detected is dependent on the detection method (24). Both previous studies used direct sequencing, the least sensitive method. Lang et al. (25) analyzed *BRAF* mutation (but not NRAS mutation) in cutaneous melanoma subtypes, and found 2 of 13 in ALM, in agreement with our data. They found the prevalence of mutation was highest in SSMM, followed by NM, LMM, and ALM. In our data, there is a similar trend except for LMM, where we found a much higher frequency of mutations. Also, their overall frequency of *BRAF* mutations in all cutaneous melanomas was relatively low (13 of 52; 25%). The discrepancy is due to our higher frequency of mutations in LMM and SSMM. This is possibly due to differences in the mutation detection techniques, although their methods are sensitive (24). Another potential bias is that the cutaneous melanomas used by Lang et al. were relatively thick (mean 6.3 mm versus 2.9 mm). Lang et al. also found that *BRAF* mutation frequency was independent of sun damage, whereas we found a significant relationship. This is likely to be because our sample was biased toward ALMs. Indeed, this significant effect disappeared when ALMs were excluded from our analysis ($P = 0.732$). Lang et al. also used a different criterion for assessing sun damage, namely site of origin rather than a direct assessment of solar elastosis in surrounding tissue. This strategy leads to unverified assumptions about the degree of solar damage and is likely to be less reliable than direct assessment of sun damage that we used.

Comparative genomic hybridization data suggest that there are important differences between ALM and SSMM. Our finding of low BRAF mutation frequency in ALM compared with other types of cutaneous melanoma supports this difference. Comparative genomic hybridization shows that ALMs have frequent and early gene amplifications that are often at loci containing known oncogenes such as *cyclin D1*, *CDK4*, and *HRAS*, although some loci contain unknown targets (2). Bastian et al. (26) reported, in the proceedings abstract from the XIIth annual Pan-American Society for Pigment Cell Research conference, a low overall frequency of *NRAS* mutations, but made no specific mention of *NRAS* mutation in acral lesions. It is difficult to make systematic comparisons of our findings to the limited amount of information presented in an abstract. However, in a full study presenting similar data, Curtin et al.

Table 2. A comparison of mutation frequencies and clinical features

	BRAF mutant, *n* = 22 (37.3%)	BRAF wild type, *n* = 37 (62.7%)	*P*	BRAF or NRAS* mutant, *n* = 40 (67.8%)	BRAF and NRAS wild type, *n* = 19 (32.2%)	*P*
Subtype						
SSMM	9 (64.3%)	5 (35.7%)		11 (78.6%)	3 (21.4%)	
NM	4 (36.4%)	7 (63.6%)		10 (90.9%)	1 (9.1%)	
ALM	2 (9.5%)	19 (90.5%)		11 (52.4%) †	10 (47.6%)	
LMM	7 (53.4%)	6 (46.6%)	0.005	8 (61.5%)	5 (38.5%)	0.114
Sun damage ‡						
Minimal	2 (9.5%)	19 (90.5%)		11 (52.4%)	10 (47.6%)	
Nonchronic	10 (50%)	10 (50%)		17 (85.0%)	3 (15.0%)	
Chronic	10 (55.6%)	8 (44.4%)	0.004	12 (66.7%)	6 (33.3%)	0.082
Age, y (±SD)	62.0 (±14.9)	67.2 (±18.7)	0.140	62.0 (±18.2)	72.3 (±13.6)	0.031
Sex						
Male	11 (45.8%)	13 (54.2%)		17 (70.8%)	7 (29.2%)	
Female	11 (31.4%)	24 (68.6%)	0.286	23 (65.7%)	12 (34.3%)	0.780
Breslow depth, mm (±SD)	2.5 (±2.2)	3.1 (±2.9)	0.505	2.6 (±2.5)	3.4 (±2.9)	0.148
Clark level						
I	0 (0%)	4 (100%)		3 (75%)	1 (25%)	
II	6 (75%)	2 (25%)		7 (87.5%)	1 (12.5%)	
III	5 (41.7%)	7 (58.3%)		9 (75.0%)	3 (25.0%)	
IV	7 (26.9%)	19 (73.1%)		14 (53.8%)	12 (46.2%)	
V	4 (44.4%)	5 (55.6%)	0.286§	7 (77.8%)	2 (22.2%)	0.161§

*Only BRAF exon 2 wild-type cases were analyzed.
† *NRAS* exon 2 analysis was unsuccessful in three cases because of inability, despite several attempts, to amplify DNA with NRAS exon 2 primers.
‡ These data includes 21 ALM that inevitably show minimal sun damage and have low BRAF mutation frequency. Revised *P* values that exclude ALM are 0.732 for BRAF mutants and 0.184 for BRAF/NRAS mutants (see main text).
§Small counts invalidated χ test, therefore thin (Clark I-III) versus thick (Clark IV-V) lesions were analyzed using Fisher's exact test.

(12) show that the underlying molecular pathology of melanoma is strongly correlated with UV damage, which was a stronger predictor of genetic changes than subtype. They reported that the frequency of *BRAF* mutations was significantly different on chronic versus nonchronic sun-damaged skin when acral lesions were discounted. By contrast, a comparable analysis using our data, where ALMs were excluded, showed that sun damage and mutation status were independent. This is because the LMMs in our study, which all had chronic sun damage, had a relatively high *BRAF* mutation frequency. This same discrepancy also distinguishes our findings from those of Maldonado et al. (4), who also assessed mutations in relation to solar damage. There are several possibilities for this specific discrepancy. It is possible that the mixture of cutaneous melanoma subtypes that were assigned to the chronic sun damage category was different. There are also inherent problems in the precise way that that chronic sun damage is defined because this assessment is somewhat subjective. Additionally, LMM mutation frequencies in any of these studies could be skewed by purely stochastic events. Despite these differences, there is a broadly similar theme in our, Maldonado et al.'s, and Curtin et al.'s studies: All found the highest proportion of cutaneous melanomas with *BRAF*/*NRAS* wild-type status in those from acral and chronically sun damaged skin, whereas those from nonchronic sun-damaged skin had the lowest frequency. Curtin et al. additionally identified common, focused *CDK4* and *CCND1* amplifications in cutaneous melanomas from acral and chronically sun damage sites, respectively, at the expense of *BRAF* and *NRAS* mutations. Taken together, these findings would be consistent with cutaneous melanomas from all three studies having relatively frequent amplifications in cutaneous melanomas from acral and chronically sun damaged skin compared with nonchronically damaged skin. However, this latter point is somewhat speculative because it assumes that the findings of Curtin et al. can be generalized, given that neither we nor Maldonado et al. did *CCND1*/*CDK4* gene dosage analyses. Notably, both our and Curtin et al.'s data emphasize the critical need for cell cycle deregulation in all cutaneous melanoma subtypes. Our study emphasizes MAPK pathway activation, whereas Curtin et al. emphasize both MAPK pathway activation and amplification of downstream targets, such as CCND1 and CDK4.

In summary, the findings of the present study refine our understanding of the role of *BRAF* and *NRAS* mutation in cutaneous melanoma. Our principal finding was to show the importance of MAPK pathway activation in each of the major cutaneous melanoma subtypes, although other studies provide evidence that amplification of downstream MAPK pathway targets may also be important. An emerging and critical aspect of current research is to use molecular pathology to generate new treatments, notably drugs targeted at relevant kinases (27). This study directly informs future use of these new treatments, but more work will be needed to further clarify the relative importance gene amplifications versus *BRAF*/*NRAS* mutation and also to assess whether cutaneous melanoma subtype or degree of sun damage are better predictors of molecular pathology.

References

1. Barnhill RL. Pathology of melanocytic nevi and malignant melanoma. Boston: Butterworth-Heinemann; 1995.
2. Bastian BC, Kashani-Sabet M, Hamm H, et al. Gene amplifications characterize acral melanoma and permit the detection of occult tumor cells in the surrounding skin. Cancer Res 2000;60:1968–73.
3. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417: 949–54.
4. Maldonado JL, Fridlyand J, Patel H, et al. Determinants of BRAF mutations in primary melanomas. J Natl Cancer Inst 2003;95:1878–90.
5. Sasaki Y, Niu C, Makino R, et al. BRAF point mutations in primary melanoma show different prevalences by subtype. J Invest Dermatol 2004; 123:177–83.
6. Davison JM, Rosenbaum E, Barrett TL, et al. Absence of V599E BRAF mutations in desmoplastic melanomas. Cancer 2005;103:788–92.
7. Edmunds SC, Cree IA, Di Nicolantonio F, Hungerford JL, Hurren JS, Kelsell DP. Absence of BRAF gene mutations in uveal melanomas in contrast to cutaneous melanomas. Br J Cancer 2003;88:1403–5.
8. Rimoldi D, Salvi S, Lienard D, et al. Lack of BRAF mutations in uveal melanoma. Cancer Res 2003;63: 5712–5.
9. Cohen Y, Rosenbaum E, Begum S, et al. Exon 15 BRAF mutations are uncommon in melanomas arising in nonsun-exposed sites. Clin Cancer Res 2004;10: 3444–7.
10. Edwards RH, Ward MR, Wu H, et al. Absence of BRAF mutations in UV-protected mucosal melanomas. J Med Genet 2004;41:270–2.
11. Wong CW, Fan YS, Chan TL, et al. BRAF and NRAS mutations are uncommon in melanomas arising in diverse internal organs. J Clin Pathol 2005; 58:640–4.
12. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med 2005;353:2135–47.
13. Takata M, Goto Y, Ichii N, et al. Constitutive activation of the mitogen-activated protein kinase signaling pathway in acral melanomas. J Invest Dermatol 2005; 125:318–22.
14. Crowson AN, Magro CM, Mihm MC. Malignant melanoma. In: The melanocytic proliferations. A comprehensive textbook of pigmented lesions. New York: Wiley-Liss; 2001. p. 283.
15. Saldanha G, Purnell D, Fletcher A, Potter L, Gillies A, Pringle JH. High BRAF mutation frequency does not characterize all melanocytic tumor types. Int J Cancer 2004;111:705–10.
16. Kolch W. Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. Biochem J 2000;351:289–305.
17. Besaratinia A, Pfeifer GP. Biological consequences of 8-methoxypsoralen-photoinduced lesions: sequence-specificity of mutations and preponderance of T to C and T to A mutations. J Invest Dermatol 2004;123:1140–6.
18. Dhawan P, Singh AB, Ellis DL, Richmond A. Constitutive activation of Akt/protein kinase B in melanoma leads to up-regulation of nuclear factor-κB and tumor progression. Cancer Res 2002;62: 7335–42.
19. Stahl JM, Sharma A, Cheung M, et al. Deregulated Akt3 activity promotes development of malignant melanoma. Cancer Res 2004;64:7002–10.
20. Chudnovsky Y, Adams AE, Robbins PB, Lin Q, Khavari PA. Use of human tissue to assess the oncogenic activity of melanoma-associated mutations. Nat Genet 2005;37:745–9.
21. Skender-Kalnenas TM, English DR, Heenan PJ. Benign melanocytic lesions: risk markers or precursors of cutaneous melanoma? J Am Acad Dermatol 1995; 33:1000–7.
22. Pollock PM, Harper UL, Hansen KS, et al. High frequency of BRAF mutations in nevi. Nat Genet 2003; 33:19–20.
23. Lee DA, Cohen JA, Twaddell WS, et al. Are all melanomas the same? Spitzoid melanoma is a distinct subtype of melanoma. Cancer 2006;106:907–13.
24. Miller CJ, Cheung M, Sharma A, et al. Method of mutation analysis may contribute to discrepancies in reports of (V599E) BRAF mutation frequencies in melanocytic neoplasms. J Invest Dermatol 2004;123: 990–2.
25. Lang J, Mackie RM. Prevalence of exon 15 BRAF mutations in primary melanoma of the superficial spreading, nodular, acral, and lentigo maligna subtypes. J Invest Dermatol 2005;125:575–9.
26. Bastian BC, Curtin J, Fridlyand J, et al. Braf and melanocytic diseases. Pigment Cell Res 2004;17:428.
27. Sawyers C. Targeted cancer therapy. Nature 2004; 432:294–7.

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