Modern Pathology (2011) 24, 1031–1035

KIT mutations in ocular melanoma: frequency and anatomic distribution

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KIT **mutations are known to occur in ~15% of chronic sun damaged cutaneous, mucosal, and acral melanomas. Melanomas with demonstrated activating mutations in *KIT* or platelet-derived growth factor receptor A (*PDGFRA*) may benefit from treatment with tyrosine kinase inhibitors. Currently, the limited data regarding *KIT* mutational status in ocular melanoma suggest that activating mutations are extremely rare. *PDGFRA* mutational status in ocular melanoma has not been determined. Seventy-five ocular melanomas (53 choroidal, 6 iris, 11 ciliary body, and 5 conjuctival) were selected from the files of the Department of Ophthalmology. High-resolution melting curve analysis and sequencing were performed to detect mutations in *KIT* exons 9, 11, 13, and 17 and *PDGFRA* exons 12 and 18. Results of mutational analysis were correlated with anatomical site and *KIT* (CD117) immunohistochemistry. Eight of 75 (11%) ocular melanomas contained mutations in either the *KIT* or *PDGFRA* gene. Five of 53 (9%) choroidal melanomas were associated with mutations (*KIT* exon 11 = 3; *KIT* exon 17 = 1; *PDGFRA* intron 18 = 1). Two of six (33%) iris melanomas and a single (9%) ciliary body melanoma harbored *KIT* exon 11 mutations. No mutations were identified in conjunctival melanomas. The distribution of *KIT* and *PDGFRA* mutations by ocular melanoma anatomical site did not reach statistical significance ($P = 0.393$) CD117 positivity was not predictive of *KIT* mutational status as only 6 of 58 (10%) CD177-positive tumors harbored *KIT* mutations. In addition, a *KIT* exon 17 mutation was identified in one CD117-negative tumor. *KIT* and *PDGFRA* mutations do occur in ocular melanomas at a frequency (11%) that is similar to acral and mucosal melanomas. Limited correlation of CD117 positivity with mutational status suggests that all ocular melanomas should undergo mutational analysis to determine if imatinib therapy is appropriate.**

Modern Pathology (2011) **24,** 1031–1035; doi:10.1038/modpathol.2011.57; published online 8 April 2011

Keywords: CD117; *KIT*; ocular melanoma; *PDGFRA*

KIT signaling has been known to be important in melanocyte biology for approximately two decades.[1–5] However, the role of *KIT* during melanoma pathogenesis has been contentious and is still not fully elucidated. Early studies of murine models and observations that *KIT* expression was lost in the dermal component of invasive melanomas led to the hypothesis that melanocytes leaving the epidermal boundaries would lose *KIT* expression.[1,4,5] Thus, the invasive component of malignant melanomas would not demonstrate *KIT* expression but the *in situ*/junctional component would express *KIT*. Our initial investigation into the role of *KIT* signaling in malignant melanomas confirmed that the majority of *KIT* expression was limited to *in situ* melanomas and the junctional component of invasive lesions.[6] However, several metastatic melanomas in our study not only expressed *KIT* in their invasive components, but harbored *KIT*-activating mutations as well. Subsequently, others confirmed the presence of *KIT* mutations in the invasive portion of primary malignant melanomas and in their metastatic deposits.[7–9] These findings suggested that targeted molecular therapy with tyrosine kinase inhibitors could be successful for melanoma, which is a notoriously difficult malignancy to treat. Indeed, promising results have emerged from clinical studies investigating the use of imatinib for

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Received 30 December 2010; revised 18 February 2011; accepted 18 February 2011; published online 8 April 2011

melanoma patients with confirmed *KIT*-activating mutations.[10–13]

The prevalence of *KIT* mutations varies between melanoma subtypes, which are defined by the site of anatomical origin. Acral, mucosal, and chronic sun damaged skin melanomas have been shown to harbor *KIT* mutations while non-chronic sun damaged skin melanomas generally do not since they are often characterized by *BRAF* or *NRAS* mutations.[7–9,14,15] The frequency of *KIT*-activating mutations in specific melanoma subtypes is relatively low with reports of 15% for acral, 19% for mucosal, and 17% for chronic sun damaged skin melanomas.[15,16] Ocular melanoma, although a rare melanoma subtype, does account for the majority of all intraocular malignancies. Arising from melanocytes of the choroid, iris, ciliary body, and conjunctiva, ocular melanomas have been reported to express *KIT* (CD117) at frequencies between 63 and 91%.[17–22] *KIT* mutations, however, have only been reported in one conjunctival tumor.[8] Given the low frequency of *KIT* mutations (<1%) in ocular melanoma, it is not surprisingly that recent studies have failed to demonstrate the clinical efficacy for imatinib mesylate therapy in unselected patients.[6,18]

As there are only limited data on the prevalence of *KIT* mutations in ocular melanoma, we reviewed a rather large series of 75 ocular melanomas including cases arising in the choroid, iris, ciliary body, and conjunctiva. The frequency of *KIT*-activating mutations and *KIT* protein expression was determined for each anatomical site. In addition, we also investigated the frequency of platelet-derived growth factor receptor A (*PDGFRA*) mutations in ocular melanoma, which has not been reported to date. Herein, we report the findings of that study.

Materials and methods

Sources of Tissue

Seventy-five ocular melanoma cases were retrieved from the files of the Department of Ophthalmology at the University of Utah. All cases were in-house cases and consecutively accessioned over a 15-year period. Cases were reviewed to confirm the original diagnosis and to ensure that sufficient tumor was present for analysis. The use of human tissue for this analysis was approved by the University of Utah Institutional Review Board.

DNA Extraction

Unstained slides from formalin-fixed paraffin-embedded tissue blocks were deparaffinized and rehydrated prior to manual microdissection of the tumor. Genomic DNA was isolated by an overnight proteinase K digestion. Following heat inactivation of the proteinase K, the crude extract was used directly in PCR.

High-Resolution Melting Analysis and Sequencing

These procedures have been described previously.[23] Briefly, *KIT* exons 9, 11, 13, and 17 and *PDGFRA* exons 12 and 18 were screened for activating mutations by PCR and high-resolution melting analysis on a LightScanner32 (Idaho Technology). Each 10 μl PCR reaction contained 5X master mix (0.2 U/μl KlenTaq1 DNA polymerase (AB Peptides), 44 ng/μl TaqStart Antibody (Clontech), 2.5 mg/ml bovine serum albumin (Sigma), 1 mM each nucleotide mix (dATP, dCTP, dGTP, dTTP) (Roche), 10 mM $MgCl_2$ (Sigma), 5X LCGreen PLUS (Idaho Technology), and 250 mM Tris pH 8.3), 0.5 μM forward primer, 0.5 μM reverse primer, and 1 μl crude DNA extract. Additional BSA (1.2 μg/μl) was added to heavily pigmented samples. PCR included an initial activation of 95°C for 120 s, followed by 45 cycles of 95°C for 5 s, 62°C for 5 s, and 74°C for 15 s. Melting curves were generated by heating samples from 65 to 95°C. Samples with abnormal melting curves, as determined by comparison to an internal negative control, were evaluated by Sanger sequencing at the University of Utah DNA sequencing core facility. The following Genbank reference sequences were utilized: *KIT* (HSU63834) and *PDGFRA* (M21574).

CD117 Staining

Immunohistochemical staining for *KIT* protein was performed with the rabbit polyclonal CD117 antibody from Dako Cytomation (Carpinteria, CA, USA). Automated staining was performed on 4-μm thick sections of formalin-fixed, paraffin-embedded tissues. Sections were air-dried and then melted in a 60°C oven for 30 min. Slides were loaded onto the Ventana (Tucson, AZ, USA) XT automated staining instrument where deparaffinization with EZ Prep solution and application of primary antibody (1:200; 32 min at 37°C) were performed. The slides were detected using the IView Enhanced Alkaline Phosphatase RED detection kit (Ventana) and counterstained for 4 min with hematoxylin.

Statistics

The Fisher's exact test was used to correlate ocular melanoma anatomical location with CD117 positivity and *KIT*/*PDGFRA* mutational status.

Results

Fifty-eight of the 75 (77%) ocular melanomas displayed at least weak focal staining with CD117. The distribution of CD117 positivity by anatomical site indicated that the prevalence of *KIT* expression was approximately equal in melanomas of the choroid, iris, and ciliary body (81, 83, and 82%, respectively) (Table 1). *KIT* expression was the least

prevalent in conjunctival melanomas (20%), but this failed to reach statistical significance ($P=0.641$).

KIT and *PDGFRA* mutational analysis of the 75 ocular melanomas in our series identified 7 (9%) with *KIT* mutations and 1 (1%) with an intronic *PDGFRA* mutation. In total, 8 of 75 (11%) ocular melanomas in our series had mutations in either *KIT* or *PDGFRA*. Five of 53 (9%) choroidal, two of six (33%) iris, and 1 of 11 (9%) ciliary body melanomas had mutations (Table 1). No mutations were identified in our five conjunctival melanomas. The distribution of *KIT* and *PDGFRA* mutations by ocular melanoma anatomical site did not reach statistical significance ($P=0.393$). Six of the eight mutations occurred in *KIT* exon 11 of which 2 were identical (P573L) while the remaining 4 were unique (Table 2). One of these cases harbored a nonsense mutation while two cases harbored two missense mutations, alleles unknown. One mutation each was present in *KIT* exon 17 (R804W) and *PDGFRA* intron 18.

Correlation of CD117 positivity with *KIT* mutational analysis revealed that 6 of 58 (10%) CD117-positive cases displayed a mutation in the *KIT* gene. All six of the mutations were located in *KIT* exon 11. One additional CD117-positive case harbored a mutation in *PDGFRA* intron 18. Of the 17 non-reactive CD117 cases, only one (6%) harbored a *KIT* mutation, which was in exon 17. None of the CD117-negative cases harbored *PDGFRA* mutations. Fifty-two of 75 (69%) ocular melanomas demonstrated CD117 positivity but lacked mutations in *KIT*. These results indicate that CD117 positivity is a poor predictor of *KIT* mutational status.

Discussion

KIT mutations have been identified in ~15% of acral, mucosal, and chronic sun damaged melanomas.[6–9,14,15] Most of these reported mutations are predicted to be sensitive to tyrosine kinase inhibitors. Indeed, clinical studies using imatinib in melanoma patients with confirmed *KIT*-activating mutations have yielded promising results.[10–13] Although it is arguably premature, the reported high frequency of *KIT* overexpression in ocular melanoma (63–91%)[17–22] has led to an interest in the use of imatinib therapy for this melanoma subtype. In initial studies, however, overexpression of *KIT* protein in ocular melanoma does not appear to imply response to imatinib.[18,24] Currently, the cause of this insensitivity of ocular melanomas to imatinib is not entirely clear. The most plausible explanation is the near absence of *KIT*-activating mutations in ocular melanoma, as reported to date.[8,17,18,21] Only one conjunctival melanoma has demonstrated a *KIT* mutation.[8] It is important to note that most studies have performed *KIT* mutational analysis on a small subset of CD117 expressing ocular tumors.[17,18,21] The most extensive mutational analysis performed to date was by Beadling *et al*[8] who examined 60 choroidal and 13 conjunctival melanomas. Our study set was of similar magnitude and included 53 choroidal, 5 conjunctival, 11 ciliary body, and 6 iris melanomas. We observed a *KIT* mutation frequency of 9% in our series of ocular melanomas, which is greater than the frequency of 1.4% demonstrated by Beadling *et al*.[8] Our study is the first to report *KIT* mutations

Table 1 *KIT/PDGFRA* mutations and CD117 expression in ocular melanoma by anatomical site

Tumor site	*Percentage with mutations*	*Exons involved (number of cases)*	*% CD117 positive*	*% Mutation+of total CD117+*	*% Mutation+of total CD117−*
Choroidal	9% (5/53)	*KIT* exon 11 (3) *KIT* exon 17 (1) *PDGFRA* intron 18 (1)	81% (43/53)	9% (4/43)	10% (1/10)
Iris	33% (2/6)	*KIT* exon 11 (2)	83% (5/6)	40% (2/5)	0% (0/1)
Ciliary body	9% (1/11)	*KIT* exon 11 (1)	82% (9/11)	11% (1/9)	0% (0/2)
Conjunctiva	0% (0/5)	N/A	20% (1/5)	0% (0/1)	0% (0/4)

Table 2 *KIT* and *PDGFRA* mutations indentified in this study

Mutation location	*Ocular melanoma subtype*	*Mutations deduced at protein level*	*Mutations identified at DNA level*	*Mutation type at DNA level*
KIT exon 11	Choroidal	p.Val555Ile+p.Ser590Asn	c.1684G>A+c.1790G>A	Missense/missense
	Choroidal	p.Val569Ile+p.Ser590Asn	c.1726G>A+c.1790G>A	Missense/missense
	Iris	p.Asp572Asn	c.1735G>A	Missense
	Iris	p.Pro573Leu	c.1739C>T	Missense
	Choroidal	p.Pro573Leu	c.1739C>T	Missense
	Ciliary body	p.Trp582X	c.1767G>A	Nonsense
KIT exon 17	Choroidal	p.Arg804Trp	c.2431C>T	Missense
PDGFR intron 18	Choroidal		c.2701+6G>A	Splice site

in melanomas of the choroid, iris, and ciliary body. In combination with the data from Beadling *et al*, these data demonstrate that *KIT* mutations do occur in melanomas derived from all ocular anatomical locations.

The most commonly reported *KIT* mutation in acral, mucosal, and chronic sun damaged melanomas is L576P in exon 11.[6,7,9,15,25] While we did not observe the L576P mutation in any of our ocular melanomas, all but one of our *KIT* mutations were located in exon 11, which encodes the juxtamembrane domain of the receptor. It is widely believed that this domain normally functions as a negative regulator of kinase activity. Mutations that alter the conformation of this domain are therefore oncogenic due to the disruption of negative regulation. With the exception of W582X, we predict that the remaining exon 11 mutations (Table 2) are likely to be sensitive to imatinib. Conversely, the exon 17 R804W mutation is likely to be imatinib resistant, given its location within the activation loop of *KIT*. However, additional *in vitro* studies would be needed to ultimately determine the efficacy of imatinib or other *KIT* inhibitors against these mutations.

In addition to *KIT* mutational analysis, we performed mutational analysis for the highly homologous receptor tyrosine kinase *PDGFRA* on our complete series of ocular melanomas. The *PDGFRA* gene, like *KIT*, is located at chromosome 4q12, which is a region that displays copy number variation in melanoma.[9] Although no *PDGFRA* mutations have been reported to date in melanoma,[7,9,26,27] no data are available for the ocular subtype. While we did not identify any *PDGFRA* mutations in exons 12 or 18, we did identify an intron 18-point mutation in one CD117-positive choroidal melanoma. Given the location of the mutation near the exon/intron boundary, it is predicted to effect splicing. Additional studies would be needed to confirm that this mutation alters splicing and to evaluate whether it confers imatinib sensitivity. Our results suggest that *PDGFRA* mutations are extremely rare in ocular melanoma, which is in general agreement with the data from other melanoma subtypes.[7,9,26,27]

The utility of CD117 staining as a screen for *KIT* mutational status in ocular melanoma appears to be limited. Although 6 of 7 *KIT* mutated melanomas were CD117 positive in our study, the correlation failed to reach statistical significance. The majority of cases were CD117 positive and mutation negative (68%), yielding a positive predictive value of only 12%. One exon 17 mutation was discovered in a CD117-negative tumor, which would have been missed if mutational analysis was limited to only CD117-positive cases. Our results regarding the limited utility of CD117 staining in ocular melanoma are in accordance with the majority of data from other melanoma subtypes. Studies of acral, mucosal, and cutaneous melanomas by Beadling *et al*,[8] Curtin *et al*,[9] and Rivera *et al*[28] have all shown limited correlation between CD117 staining intensity and *KIT* mutational status. Although Torres-Cabala *et al*[15] recently demonstrated a significant correlation between *KIT* mutational status and the percentage of CD117-positive tumor cells, irrespective of staining intensity, in their large series of acral, lentiginous, and mucosal melanomas, their positive predictive value was still low (14%). Overall, this poor correlation between CD117 positivity and the presence of *KIT*-activating mutations may in part explain the non-responsiveness of these melanomas to imatinib therapy when patient eligibility is determined solely by CD117 status.

While we have demonstrated that *KIT*-activating mutations may be one method of driving *KIT* overexpression in ocular melanoma, additional mechanisms must also exist. Gene amplification, constitutive phosphorylation of the receptor, and dysfunction or deficiency of phosphatases that normally turn off the activated signal could all play a role.[7,29] Alternatively, a decrease in protein turnover could result in the accumulation of *KIT* protein within neoplastic cells. *In vitro* studies have also implicated the stem cell factor/*KIT* autocrine loop in the malignant transformation of ocular melanocytes.[17,19] Clearly, the role of *KIT* in the oncogenesis of ocular melanoma is not fully elucidated. However, the discovery of *KIT*-activating mutations in even a small proportion of ocular melanomas has important therapeutic implications for this malignancy, which is notoriously difficult to treat.

The present study is the first to show that *KIT* and *PDGFRA* mutations do occur in ocular melanomas at a frequency similar to that seen in acral, mucosal, and chronic sun damaged melanomas. Although no single mutation site appears to dominate, the majority of mutations are found in *KIT* exon 11. Melanomas of the iris may harbor *KIT* mutations more frequently than other anatomical sites but this correlation is hindered by a small sample size. CD117 immunoreactivity is not a good predictor for the presence of *KIT* mutations in ocular melanoma as only 10% of CD117-positive cases demonstrated a mutation. Screening of all ocular melanomas for *KIT* and *PDGFRA* mutations, irrespective of CD117 status, may be warranted to identify patients that could benefit from imatinib therapy.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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