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Comparison of c-Kit expression between primary and metastatic melanoma of skin and mucosa

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Abstract

Background: Melanoma causes the greatest morbidity and mortality of all skin cancers. Mucosal melanoma is a rare but highly aggressive neoplasm. According to previous studies the prevalence of KIT mutations in acral lentiginous and mucosal melanomas is relatively low (less than 15–20%), but it can have profound therapeutic implications for localized high risk or metastatic diseases. Our goal was to evaluate c-Kit expression in different types of primary and metastatic melanoma to discriminate potential candidates for targeted therapy.

Methods: We designed a cross-sectional study and selected 50 cases of malignant melanoma (primary, metastatic cutaneous, and mucosal) from the affiliated hospitals of Shiraz University of Medical Sciences in the period of 2008 to 2012. Immunohistochemistry for KIT expression was performed. Multistage sampling method was selected for sampling and chi-square test was used for statistical analysis.

Results: In our study, male to female ratio was 1.77. The male sex was correlated with higher tumor stage ($p< 0.05$). 62% (n= 31) of cases showed at least 5% of KIT-positive cells, consist of 18% (n= 9) with 5–50%, 16% (n= 8) with 51–95%, and 28% (n= 14) of cases showed more than 95% of cells expressing KIT. But in 38% (n= 19) of cases KIT expression was less than 5% of positive cells. Tumor stage was positively correlated with tumor cell immunoreactivity and intensity ($p< 0.05$). Metastatic melanoma showed lower percentage (43%) of positivity. Intensity of staining and percentage of positive cells were positively correlated ($p< 0.001$).

Conclusion: In primary melanomas, significant KIT expression was found by immunohistochemistry, which may be useful to screen the patients for advising to KIT mutation analysis and targeted therapy.

Keywords: Melanoma, Proto-Oncogene Proteins c-kit.

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Introduction

Melanoma causes the greatest morbidity and mortality between all skin cancers. Mucosal melanoma of the oral cavity is a rare but a highly aggressive neoplasm. The extensively studied role of KIT signaling in melanocyte biology has been reported. The interaction of stem cell factor with KIT, and its receptor, is critical for the survival, proliferation, differentiation and migration of melanocytes (1). However, the regulation of KIT pathway is complex and depends on other multiple cellular factors. KIT is a trans-membrane receptor tyrosine kinase encoded by the proto-oncogene KIT at 4q11-12 (1). KIT activation mutations are associated with a variety of malignant human tumors as well as malignant melanoma (1,2). It was speculated that melanoma cells should lose KIT expression to acquire proliferative activity and escape from the epidermal boundaries, (2). This hypoth-

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esis was supported by previous observations in which KIT expression in melanoma was strong in the in situ and junctional components of invasive lesions, while KIT expression was lost once the melanoma became invasive and metastatic (3,4). It was observed that more than a third of the melanomas without detectable KIT mutation, or increased copy number showed overexpression of KIT by IHC. This observation led the authors to consider other mechanisms than gene mutation or amplification to explain the KIT overexpression (5). In literature the role of IHC in the assessment of KIT in cutaneous and mucosal melanomas and their metastases, and its relation to the mutational status of the KIT gene is not well established, and a few articles are found on this subject. According to the previous studies the KIT mutations prevalence in acral lentiginous/ mucosal melanomas is relatively low (not more than 15–20%) but they can have profound therapeutic implications for localized high risk or metastatic disease (6). In this study, KIT protein expression by IHC in a large series of melanomas with emphasis on cutaneous and mucosal melanomas and their metastasis was evaluated.

Methods

We designed a cross-sectional study and selected 50 cases of malignant melanoma including primary cutaneous, mucosal and their metastasis that were referred to affiliated hospitals of Shiraz University of Medical Sciences (2008–2012). According to multistage sampling method, sampling size was selected to be 50. We targeted a confidence interval of 95% (Z=1.96), range of variation of 0 to 100 (S= 16.67), and critical difference of 4.5% (d=4.5). The diagnoses were confirmed by morphological features. Pathological staging of melanoma was done based on AJCC 2009 revised melanoma staging system. The analyzed melanomas, totally 50 cases, consisted of the 10 primary acral lentiginous, 10 primary mucosal, 14 primary nodular, 14 metastatic, and two primary uveal melanomas.

H&E (Hematoxylin and Eosin stain) slides and paraffin tissue blocks were retrieved from the archives. Immunohistochemical analysis for KIT was performed using an anti-CD117 polyclonal rabbit antihuman antibody (Dilution 1:1000, Dako-Cytomation, Carpinteria, CA, USA) on tissue sections. Negative controls were prepared by substituting the primary antibody with non-immune rabbit serum. Sections of strongly positive c-Kit GIST were used as positive control. The slides were evaluated for both tumor cell percentage and intensity of immunoreactivity. Percentage of positive cells was recorded as following: 0 (negative), <5% of cells staining, 5–50% of cells staining, 51–95% of cells staining, and >95% of cells staining. Intensity was scored as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong) (7). The IHC data were collected along clinical and morphological findings including age, sex, site, Breslow thickness, stage and other specifications. Chi-squre test was used for statistical evaluations.

Results

A total of 50 cases of cutaneous and mucosal (primary and metastatic) melanoma including 31 (62%) male and 19 (38%) female with ratio of 1.77 were enrolled in the study. The age range was wide (1–87) with mean±SD age of 53±17.5 years. Foot was the most common anatomical primary site for cutaneous melanoma. Six (60%) out of 10 mucosal melanoma patients were male. For the mucosal malignant melanoma the head and neck primaries were the most frequent site, followed by anorectal area and colon mucosa. Lymph nodes were the sites most frequently involved by metastatic tumors in our series (7 cases) followed by skin subcutaneous (6 cases) and bone marrow metastases (1 case). Table 1 summarizes patients' demographics and clinical features of the analyzed tumors.

The cases were also evaluated for neurotropism 4 out of 50 (8%), tumor lymphocytic infiltration 4 out of 50 (8%), vascular invasion 5 out of 50 (10%), microsatellites

Table 1. Patient demographics and clinical characteristics of primary and metastatic melanoma cases

Diagnosis	Total N (%)	Male N (%)	Female N (%)	Anatomical Site
Acral lentiginous, Primary	10(20)	5(10)	5(10)	Foot and heel
Mucosal, primary	10(20)	6(12)	4(8)	Nasal cavity, nasopharynx, parotid gland and buccal mucosa, anorectal & colon
Nodular, primary	14(28)	11(22)	3(6)	Ankle, foot, arm, hand and scalp
Uveal, primary	2(4)	0	2(4)	Eye
Metastatic	14(28)	9(18)	5(10)	Lymph node, subcutaneous and bone marrow

0 out of 50, regressive changes 1 out of 50 (2%).

We also investigated the chi-square test between sex and site of the lesions and stage of the tumors. Results revealed that male sex correlated with higher stage ($p<0.05$) but there was no correlation between site and stage of the tumors.

Immunohistochemical analysis for KIT: Overall, 19 (38%) cases showed 0 to less than 5% of positive cells, 9 (18%) cases 5–50%, 8 (16%) cases 51 -95%, and 14 (28%) cases showed greater than 95% of cells expressing KIT (Fig. 1).

A high percentage of cutaneous and mucosal melanomas, both primary and metastatic, showed at least 5% of KIT-positive cells, 30 of 48 cases (62.5%). One of the

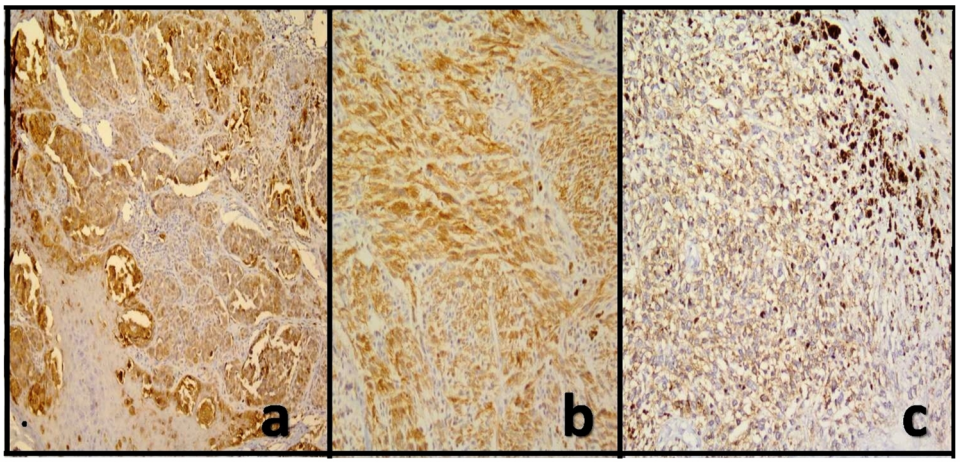

Fig. 1. C-Kit positive immunohistochemical staining in melanoma. a: 3+ positivity b: 2+ positivity, c: 1+ positivity, some melanin pigment for comparison is seen at upper outer quadrant of the lesion (X 200).

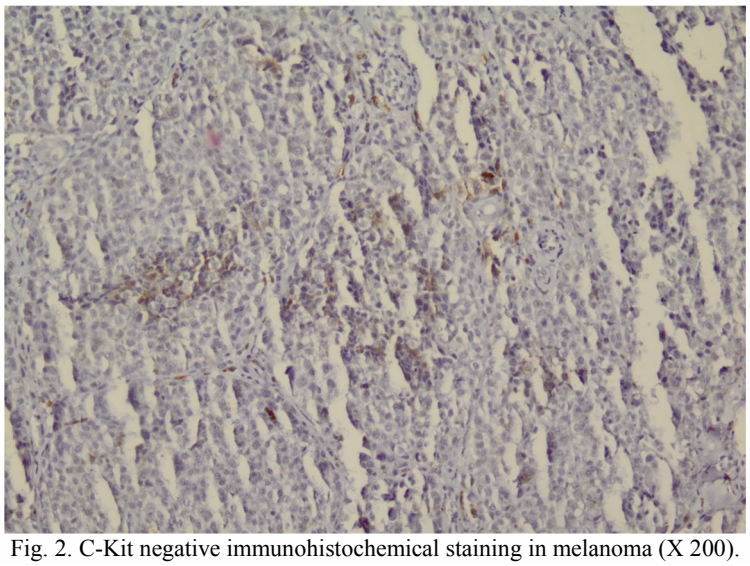

Fig. 2. C-Kit negative immunohistochemical staining in melanoma (X 200).

Table 2. Immunohistochemical expression of KIT (percentage and intensity of positive cells) in primary and metastatic melanoma

Immunohistochemical expression of KIT (%)					
	0	5-50	51-95	>95	p
Intensity			N (%)		
0	19(38)	0	0	0	<0.001*
1+	0	5(10)	1(2)	3(6)	
2+	0	4(8)	4(8)	1(2)	
3+	0	0	3(6)	10(20)	
Diagnosis					
Acral lentiginous, Primary	2(4)	1(2)	3(6)	4(8)	>0.05*
Mucosal, primary	2(4)	4(8)	2(4)	2(4)	
Nodular, primary	6(12)	1(2)	3(6)	4(8)	
Uveal, primary	1(2)	0(0)	0(0)	1(2)	
Metastatic	8(16)	3(6)	1(2)	2(4)	

* P-value from Chi- Square test

two uveal melanoma cases showed more than 95% of tumor cells positive for KIT, whereas another case was negative for KIT staining (Fig. 2).

Tumor cell positivity for KIT IHC staining as well as its intensity did not correlate with type of melanoma. ($p > 0.05$) Intensity of staining and percentage of positive cells were positively correlated ($p < 0.001$, Table 2). Cytoplasmic staining with membranous accentuation was noted in most of the cases (Fig. 1). Acral lentiginous and nodular types of cutaneous melanoma and mucosal primary melanomas showed a comparable level of KIT IHC expression. While cases showing at least 5% of positive cells were outstanding (8 out of 10 primary acral lentiginous, 8 out of 10 primary nodular melanomas, and 8 out of 10 primary mucosal melanomas). Though cases are not quite sufficient for statistical analysis, metastatic melanomas seems to be less likely to be KIT-positive than non-metastatic melanomas (6 out of 14 cases, 43% v/s 24 out of 36 cases, 66%, respectively). Tumor stage was also positively correlated with tumor cell immunoreactivity and intensity ($p<0.05$).

Discussion

Controversy still exists about the expression of KIT protein in melanomas. The role of IHC in the assessment of KIT status in melanomas is not well established yet. Although the reported prevalence of KIT mutations in acral lentiginous/mucosal melanomas is relatively low, the detection of that mutation can have profound therapeutic implications (7). A study reported KIT expression in 96% of primary melanomas, while its expression was 55% in metastatic melanomas (8). Another study showed a possible role of KIT in some types of melanoma, such as mucosal melanomas (21% KIT mutations, and 61% KIT overexpression), acral cutaneous melanomas (11% KIT mutations, and 75% c-Kit overexpression) and cutaneous melanomas on skin with chronic sun damage (17% KIT mutations, and 100% c-Kit overexpression). In another study KIT mutation was detected in 14 out of 39 (35%) (10). In contrast, KIT mutations are rarely found in the major subtype of cutaneous melanoma originating from skin without chronic sun damage (5); it is not common in unselected cutaneous melanomas (2 out of 100) (9). This suggests that c-Kit may have pathogenetic relevance and used as a therapeutic target in these subtypes of melanoma. Cytoplasmic c-Kit staining was significantly correlated with poor survival in patients with acral melanoma. There is significant difference between c-Kit immunoreactivities and the mortality risks of melanomas on acral and non-acral sites. It may change site-specific targeted therapeutic concepts in melanoma in future (11). Among patients with advanced melanoma harboring KIT alterations, treatment with imatinib mesylate resulted in significant clinical response in a subgroup of patients (12). Foot was the most common anatomical location of cutaneous melanoma in our study.

The aim of this study was to further clarify c-Kit alterations in patients with cutaneous and mucosal melanomas and their metastasis. Our results are in keeping with those recent studies showing that some types of melanoma has significant expression of KIT which can be detected by IHC. We used a highly sensitive detection method employing a rabbit polyclonal antibody, widely tested in previous trials (7, 9). We set a cutoff point of 5% for KIT expression positivity. In our study, c-Kit expression was detected in 66.6% of cutaneous melanomas, while other authors have reported the expression of c-Kit from 22.8% (12) up to 84% (13). In cases of mucosal melanomas we achieved a positive rate of 80% which is similar to previous studies, including primary mucosal melanomas of the anal/rectal mucosa 12 out of 16 (75%) (13), oral cavity 16 out of 18 (88%) (14), and primary mucosal melanoma 35 out of 39 (90%) (15). However, one study reported lower number, 6 out of 26 (23%) of c-Kit positivity in mucosal melanomas of the anal/rectal tract (15). The high range of differences between these studies could be explained with the different qualities of IHC and different cutoff point for tumor cell positivity (e.g. 20%). c-Kit expression appears to be in a similar range in mucosal melanomas and cutaneous melanomas in our study. In contrast to other categories, metastatic melanoma revealed the lowest percentage of positivity in c-Kit expression which is similar to the results of metastaic cutaneous melanomas in other studies (4,7). Our results in cases of metastatic melanomas were in contrast to another study in which the expression was found in 9 of 9 metastases of primary mucosal melanoma. That study included only mucosal melanomas (39 patients) of different locations (15). Other study also detected c-Kit reactivity in 6 out of 6 metastases from 20 cases of anal melanomas (16). One of the sources of these discrepancies may be due to evaluating the metastatic cutaneous and mucosal melanomas in a single group. The other reason was studying only mucosal melanomas in large groups. Immunohistochemistry may be not sufficient to detect tumors with mutations susceptible for KIT blockade, as overexpression can also occur in tumors without mutation (14). Moreover, therapeutic studies with the KIT blocker imatinib in unselected melanoma patients without known mutation status were disappointing (17-19).

Conclusion

In summary, according to our findings, primary melanomas including acral lentiginous, nodular, mucosal, and uveal melanomas shows high KIT expression. Therefore, IHC evaluation may be a useful tool for screening patients that are subjected to KIT mutation and can be used as a patient selection method for targeted therapy. Mucosal and metastatic melanomas showed highest and lowest KIT expression, respectively. Tumor stage was also positively correlated with tumor cell immunoreactivity and intensity. We have also encountered some limitations including melanin interference with KIT expression within IHC study.

We recommend further study to evaluate mutational status of the KIT gene to assess the efficacy of immunohistochemistry in order to predict mutations in KIT.

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