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.


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 observa-
tions 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 be-
came invasive and metastatic (3,4). It was
observed that more than a third of the me-
lanomas without detectable KIT mutation,
or increased copy number showed overex-
pression of KIT by IHC. This observation
led the authors to consider other mech-
anisms 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 melan-
omas 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 pre-
vious studies the KIT mutations prevalence
in acral lentiginous/ mucosal melanomas is
relatively low (not more than 15–20%) but
they can have profound therapeutic impli-
cations for localized high risk or metastatic
disease (6). In this study, KIT protein ex-
pression by IHC in a large series of mel-
nomas 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 affil-
iated hospitals of Shiraz University of Med-
multistage sampling method, sampling size
was selected to be 50. We targeted a con-
fidence interval of 95% (Z=1.96), range of
variation of 0 to 100 (S= 16.67), and criti-
cal difference of 4.5% (d=4.5). The diagno-
ses were confirmed by morphological fea-
tures. 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 prima-
ry mucosal, 14 primary nodular, 14 meta-
static, and two primary uveal melanomas.

H&E (Hematoxylin and Eosin stain)
slides and paraffin tissue blocks were re-
trieved from the archives. Immunohisto-
chemical analysis for KIT was performed
using an anti-CD117 polyclonal rabbit an-
tihuman antibody (Dilution 1:1000, Dako-
Cytomation, Carpinteria, CA, USA) on tis-
ue sections. Negative controls were pre-
bred 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 (neg-
ative), <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 (moder-
ate), 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-square test was used for statistical eva-
luations.

Results
A total of 50 cases of cutaneous and mu-
cosal (primary and metastatic) melanoma
including 31 (62%) male and 19 (38%) fe-
male 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 fre-
frequent site, followed by anorectal area and
colon mucosa. Lymph nodes were the sites
most frequently involved by metastatic tu-
mors in our series (7 cases) followed by
skin subcutaneous (6 cases) and bone mar-
row metastases (1 case). Table 1 summa-
izes patients’ demographics and clinical
features of the analyzed tumors.

The cases were also evaluated for neurot-
ropism 4 out of 50 (8%), tumor lymphocyt-
ic infiltration 4 out of 50 (8%), vascular
invasion 5 out of 50 (10%), microsatellites
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

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

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

**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).
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 seem 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.

<table>
<thead>
<tr>
<th>Table 2. Immunohistochemical expression of KIT (percentage and intensity of positive cells) in primary and metastatic melanoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immunohistochemical expression of KIT (%)</strong></td>
</tr>
<tr>
<td>Intensity</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1+</td>
</tr>
<tr>
<td>2+</td>
</tr>
<tr>
<td>3+</td>
</tr>
<tr>
<td>Diagnosis</td>
</tr>
<tr>
<td>Acral lentiginous, Primary</td>
</tr>
<tr>
<td>Mucosal, primary</td>
</tr>
<tr>
<td>Nodular, primary</td>
</tr>
<tr>
<td>Uveal, primary</td>
</tr>
<tr>
<td>Metastatic</td>
</tr>
</tbody>
</table>

*P-value from Chi-Square test
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 have 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 metastatic 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 lentigious, 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.

Acknowledgments
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Expression in primary and metastatic melanoma


