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Immunohistochemical determination of HER-2/neu overexpression in malignant melanoma reveals no prognostic value, while c-Kit (CD117) overexpression exhibits potential therapeutic implications

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Abstract

Background: HER-2/neu and c-kit (CD117) onco-protein are increasingly being recognized as targets for therapy in solid tumors, but data on their role in malignant melanoma is currently limited. We studied the prevalence of overexpression of HER-2/neu and c-Kit in 202 patients with malignant melanoma to evaluate a possible prognostic value of these molecular targets in malignant melanoma.

Methods: Overexpression of HER-2/neu and c-Kit was evaluated using immunohistochemical assays in 202 archival tissue specimens.

Results: Between 1991 and 2001, 202 subjects (109 males; 54% and 93 females; 46%) with malignant melanoma were studied with a mean age of 57 years (age range: 15–101 years). The most common histologic type was amelanotic melanoma (n = 62; 30.7%) followed by superficial spreading melanoma (n = 54; 26.7%). The depth of penetration of melanoma (Breslow thickness, pT Stage) ranged from 0.4 mm (stage pT1) to 8.0 mm (stage pT4A). Mean thickness was 2.6 mm (stage pT3A). The ECOG performance scores ranged from 0 to 3. Only 2 patients (0.9%) revealed HER-2/neu overexpression, whereas 46 (22.8%) revealed c-Kit overexpression. Multivariate analysis performed did not show a significant difference in survival between c-Kit positive and negative groups (p = 0.36). Interestingly, not only was c-Kit more likely to be overexpressed in the superficial spreading type, a preliminary association between the presence or absence of c-Kit overexpression and the existence of another second primary tumor was also observed.

Conclusions: The results of our large study indicate that the HER-2/neu onco-protein neither has a role in melanogenesis nor is a potential target for clinical trials with monoclonal antibody therapy. This indicates there is no role for its testing in patients with malignant melanoma. Although c-Kit, expressed preferentially in the superficial spreading type, may not have prognostic value, it does have significant therapeutic implications as a molecular target warranting further investigation.

Background

The molecular basis of human malignant melanoma progression has remained largely unknown despite the fact that the incidence of melanoma is rapidly increasing [1].

Current staging methods have limitations in their ability to accurately identify all of the patients who will develop melanoma metastases making the targeting of therapeutic interventions very important. These limitations, along with the poor prognosis once metastases are discovered, have led to the recent emphasis on the study of melanoma genetics. Melanoma development from genomic alterations has been classified into either tumor suppressors, that are down-regulated with malignant transformation, or oncogenes, whose functions are up-regulated. It is believed that oncogenes often represent activated growth factor or growth factor receptor genes. Mutations at the proto-oncogenic loci can lead to enhanced gene product function and provide the needed substrate for an increased susceptibility to malignant conversion [2]. Several attempts have been made to identify various prognostic and diagnostic molecular factors like HER-2/neu and c-Kit (CD117) in patients with malignant melanoma. However, to date, there is limited and contradictory information available in the literature regarding the role of these possible molecular targets for cancer therapy.

HER-2/neu proto-oncogene was originally isolated from a human mammary carcinoma and a salivary gland adenocarcinoma as an amplified gene related to but distinct from epidermal growth factor (EGF) receptor. HER-2/neu oncogene encodes a 185-kDa cell surface glycoprotein, which has intrinsic tyrosine kinase activity with multiple transduction pathways and involvement in the regulation of cell growth and differentiation [3]. The overexpression of HER-2/neu has been demonstrated in a variety of malignancies including breast, ovarian, lung, gastric, endometrial, and glioblastomas [4–8]. However, the extent to which HER-2/neu is overexpressed in melanomas is unclear. There have been a few smaller studies done to demonstrate the status of HER-2/neu overexpression in malignant melanoma [9–12]. A recent study demonstrated that emodin, a tyrosine kinase inhbitor, can inhibit HER-2/neu's tyrosine kinase activity and block the increase of HER-2/neu-overexpressing cells in nude mice and culture. This study also targeted the HER-2/neu promoter in tumor-bearing mice resulting in extended survival with suppression of tumor [13]. These earlier findings propose the possibility of using therapeutics to target HER-2/neu-overexpressing cancer cells.

The proto-oncogene c-Kit encodes a transmembrane tyrosine kinase receptor related to the platelet-derived growth factor PDGF/CSF-1 (c-fms) receptor subfamily [14]. C-Kit has been found to play a pivotal role in the normal growth and differentiation of embryonic melanoblasts. Mutations in c-Kit and its ligand result in white spotting in mice and humans [15,16]. Malignant transformation of melanocytes is associated with changes in the expression of the c-Kit receptor. Several studies have demonstrated that the progression of human melanoma is associated with the loss of expression of the c-Kit proto-oncogene [17–19]. These studies revealed that expression of the tyrosine kinase receptor encoded by the c-KIT proto-oncogene gradually declines during the tumor growth and invasion of human melanoma. The explanation for the loss of c-KIT gene expression in melanoma advancement has still not been fully explained. Recent research looked at the correlation between c-KIT expression and a transcription factor, AP-2, that binds to the c-KIT promotor. Results signified c-KIT expression was directed by AP-2 and AP-2 expression suppressed tumorigenicity and metastatic potential of human melanoma cells [20]. These findings emphasize the importance of this proto-oncogene's role as a molecular target in melanogenesis.

Since HER-2/neu and CD117 (c-Kit) are now potential targets for site-specific therapy in certain solid tumors, the identification of overexpression of the above mentioned markers by standard immunohistochemical techniques would be a significant scientific advance in our understanding of melanogenesis. This could possibly lead to the development of effective treatment protocols for patients with malignant melanoma. Hence, we analyzed overexpression of HER-2/neu and c-Kit in 202 patients with biopsy-proven malignant melanoma to determine their prevalence and to evaluate their possible role in malignant melanoma.

Methods

After appropriate approval from our institutional review board, a retrospective study was initiated to review the medical records of all patients with a diagnosis of malignant melanoma between 1991 and 2001. An extensive chart review was performed to collect relevant patient demographic and clinical data to include: age, gender, smoking history, presenting symptom(s), histological type, Breslow stage at diagnosis, performance status (ECOG score) at presentation, stage of the melanoma, modality/type of therapy, associated primary malignancies and survival. HER-2/neu and c-Kit overexpression were assayed using immunohistochemical techniques (IHC) on archival tissue specimens.

HER-2/neu testing

HER-2/neu testing was performed using the most widely used technique for assessment of HER-2/neu status i.e. immunohistochemistry (IHC), which measures overexpression of the HER-2/neu onco-protein. IHC was carried out on formalin fixed, paraffin embedded material, using the Hercep test developed by DAKO. Immunostaining was classified as follows: 0 = no staining, 1+ = faint, incomplete membranous pattern, 2+ = moderate, complete membranous pattern, 3+ = strong membranous pattern [21]. A trained pathologist who was blinded from the

clinical history of the patient interpreted these results. An IHC score of 2+ or greater was considered as HER-2/neu overexpression [22].

CD117 (c-Kit) testing

C-Kit testing was also performed using IHC and recorded on a semi-quantitative scale: the number of positive cells (0%, <10%, 10–50%, and >50%) and the intensity of the reaction. C-Kit results were interpreted by a single pathologist (S.K.) who was blinded from the clinical data, and reported as positive/negative. Immunohistochemical staining for KIT (CD117) was performed using a 1:250 dilution of the rabbit polyclonal antibody A4502 (IMPATH, CA) with the EnVision detection system. Antigen retrieval method was not utilized. Appropriate positive and negative controls were used throughout the testing process. For the purposes of our present study, we used the rabbit polyclonal antibody A4502, which has shown consistent performance even against a low background. We selected the A4502 polyclonal antibody because it is the most widely used KIT antibody and because it is the antibody specified for CD117 (c-Kit) testing in the large co-operative clinical trials involving STI571 (Gleevec®).

Results

Between 1991 and 2001, 202 subjects (109 males; 54% and 93 females; 46%) with malignant melanoma were studied. Mean age of the study group was 57 years (age range: 15–101 years). Sixty-seven (33.1%) were smokers, 108 (53.5%) were non-smokers and in 27 (13.4%) patients, smoking history was not documented. The most common histologic type was amelanotic melanoma (n = 62; 30.7%) followed by superficial spreading melanoma (n = 54; 26.7%). The ECOG performance scores ranged from 0 to 3. Other histologic types identified in the study group included lentigo maligna melanoma (n = 30; 14.9%), nodular melanoma (n = 27; 13.4%) and acral lentiginous melanoma (n = 23; 11.4%). The depth of penetration of melanoma (Breslow thickness, pT Stage) ranges from 0.4 mm (stage pT1) to 8.0 mm (stage pT4A). Mean thickness was 2.6 mm (stage pT3A). The most common presenting symptom was change in skin lesion (n = 166; 82.2%) and only 12 patients (5.9%) had evidence of distant metastasis at presentation. Treatment modalities

Table 1: Demographic and clinical features of c-Kit positive/negative subjects.

	c-Kit positive (n = 46)	c-Kit negative (n = 156)	P-value
Age:	54 years	58 years	0.17*
Sex :			
Males n = 109	26 (23.9%)	83 (76.1%)	0.69**
Females n = 93	20 (21.5%)	73 (78.5%)	
Performance Status:			
ECOG – 0 n = 87	23 (26.4%)	64 (73.6%)	
ECOG – 1 n = 65	24 (36.9%)	41 (63.1%)	
ECOG – 2 n = 42	20 (47.6%)	22 (52.4%)	
ECOG – 3 n = 8	4 (50%)	4 (50%)	
Smoking Status:			
Smoker n = 67	13 (19.4%)	54 (80.6%)	0.76**
Non-Smoker n = 108	23 (21.3%)	85 (78.7%)	
Unknown n = 27	10 (37.0%)	17 (63.0%)	
Second Primary Cancers:			
Absent n = 154	41 (26.6%)	113 (73.4%)	0.02**
Present n = 48	5 (10.4%)	43 (89.6%)	
Presenting Symptom:			
Change in skin lesion n = 166	44 (26.5%)	122 (73.5%)	0.01**
Others n = 19	0 (0.0%)	19 (100%)	
Unknown n = 17	2 (11.8%)	15 (88.2%)	
Histologic type:			
Amelanotic Melanoma n = 62	3 (4.8%)	59 (95.2%)	
Superficial Spreading n = 54	29 (53.7%)	25 (46.3%)	
Lentigo maligna n = 30	7 (23.3%)	23 (76.7%)	
Nodular Melanoma n = 27	4 (14.8%)	23 (85.2%)	
Acral lentiginous n = 23	3 (13.0%)	20 (87.0%)	
Others/unclassified n = 6	0 (0.0%)	6 (100%)	
Mean Breslow thickness	2.9 mm	3.1 mm	
Survival (Mean)	96 months	91 months	0.22†

* Test of significance by unpaired t-test † Test of significance by Log Rank Test ** Test of significance by chi-2 test

included surgical excision alone or adjuvant chemotherapy with either single agent DTIC, combination therapy with CVD (cisplatin, vincristine and dacarbazine), high dose interferon-alpha or interleukin therapy. HER-2/neu onco-protein was overexpressed in only 2/202 patients (0.9%) while c-Kit was overexpressed in 46 patients (22.8%). Demographic and clinical features of c-Kit positive subjects are summarized in table 1.

After adjusting for age, sex, smoking status and treatment history, survival analysis was performed using the Cox proportional regression model. Survival data was disease specific to melanoma. Multivariate analysis did not show a significant difference in survival between the c-Kit positive and negative groups (p = 0.36) (Figure 1). Sex was a significant predictor of survival (p = 0.02) in this model. The mean survival in males was 109 months as compared to 93 months in females.

Interestingly, c-Kit overexpression was seen in 10.4% (n = 5) of melanoma subjects who also had another co-existent second primary tumor. Also, absence of c-Kit overexpression was statistically related to the absence of other associated second primary malignancies in patients with melanoma as c-Kit was overexpressed in only 26.6% (n = 41) of melanoma patients (n = 154) without associated malignancies (p = 0.01). Among the various sub-types of melanoma, c-Kit was overexpressed in 29/54 (53.7%) of patients with superficial spreading melanoma. Also 29/46 (63%) of c-Kit positive patients had superficial spreading melanoma, indicating that c-Kit overexpression is preferentially seen in the superficial spreading type.

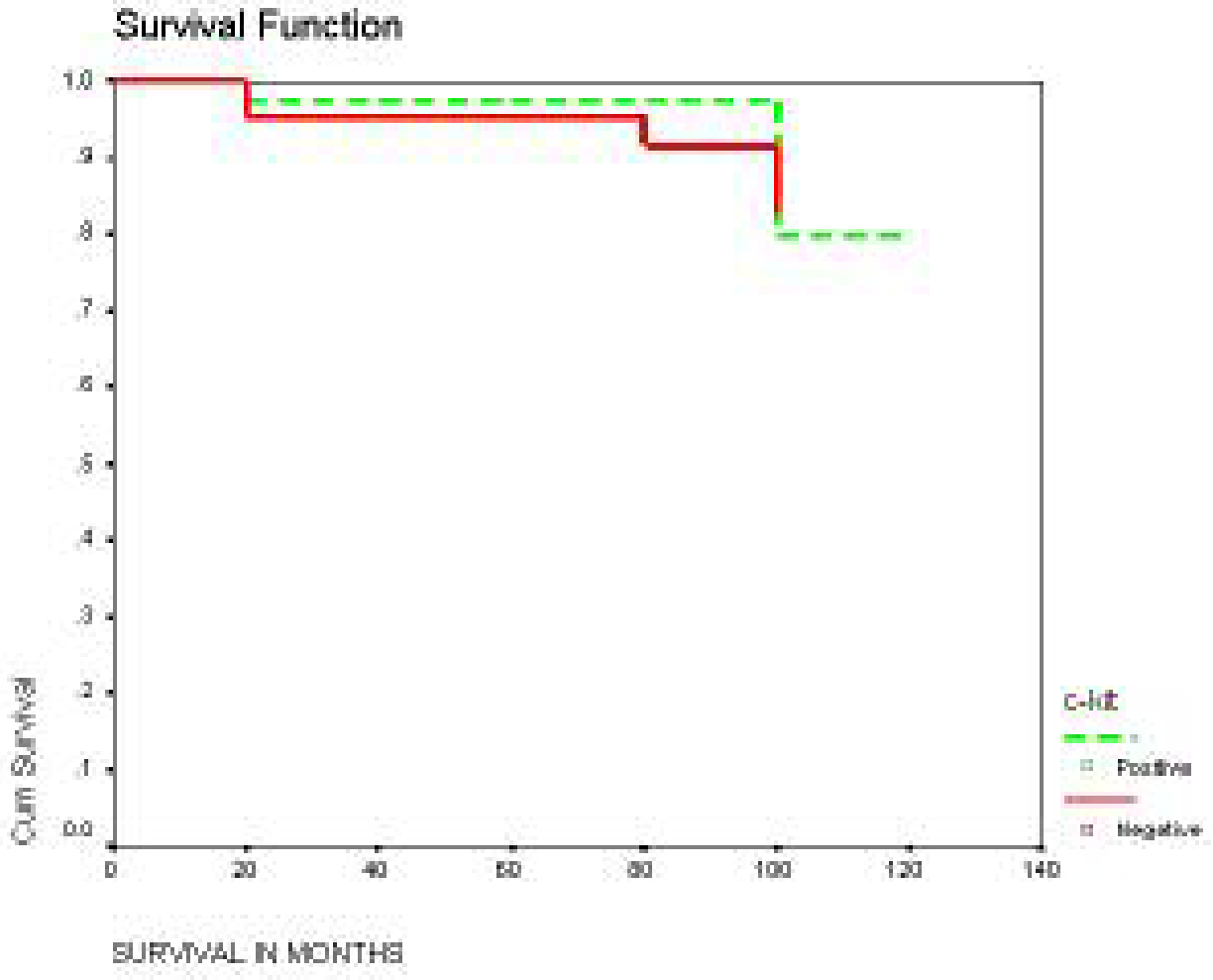

Figure 1
Kaplan-Meier survival analysis by c-Kit status determined by immunohistochemistry.

Discussion

Despite recent advances in therapy, the morbidity and mortality associated with malignant melanoma is substantial. At present there are few useful diagnostic/prognostic markers that are also potential targets for therapy in patients with malignant melanoma. Overexpression of HER-2/neu has been established as a poor prognostic indicator and a target for therapy in a variety of malignancies [4–8]. However, its role in malignant melanoma has not been defined. Also, several small studies have linked the loss of expression of proto-oncogene c-Kit with tumor progression in malignant melanoma [15,16], but its role as a molecular target has not been explored.

In an attempt to elucidate the role of HER-2/neu and c-Kit overexpression in malignant melanoma, we analyzed archival tissue specimens of 202 melanoma cases identified between 1991 and 2001. IHC was preferred over fluorescent in situ hybridization (FISH) since it is a routine, relatively inexpensive analytic method. It can be performed on the smallest of specimens, including cytological specimens, provided tumor cells are present. Also, interpretation is not influenced by non-tumor material present in the specimen and this method will not detect normal low levels of the protein on pathological tissue. Moreover, IHC is the technique that has been more extensively studied in large clinical trials.

HER-2/neu overexpression

In our series, only 2 of the 202 patients (0.9%) revealed HER-2/neu overexpression. Previously, Persons et al [23] reported that HER-2/neu is amplified and overexpressed in an estimated 0.06% to 13.16% of melanomas. In contrast, variable levels of HER-2/neu mRNA were reported by Easty et al in 19 melanoma cell lines [9] and Cheneuix-Trench et al reported a low level of HER-2/neu mRNA in 16 additional melanoma cell lines [10]. HER-2/neu was detected in 5 out of 8 melanoma cell lines as analyzed by flow cytometry by Rongcum and co-workers [11]. Bodey et al performed the largest previous study using IHC and reported that HER-2/neu was expressed in 12 out of 30 primary cutaneous malignant melanomas and 8 out of 10 metastatic malignant melanomas [24]. There are various reasons for the discordant results between these studies. The use of established cell lines causes induction of expression of HER-2/neu during culture. Therefore, measuring the presence of HER-2/neu mRNA may not directly correspond to overexpression of HER-2/neu protein. Also, differences in fixation techniques, differences in epitope and antigen retrieval methods employed and differences in the sensitivity of the particular antibody used in detecting the HER-2/neu onco-protein can account for the discordant results between the various smaller studies [25]. Our study, using the semi quantitative immunohistochemical assay (DAKO Hercep kit), is the largest study suggesting that overexpression of HER-2/neu onco-protein does not commonly occur in malignant melanoma. Therefore, our findings show HER-2/neu has no value as a biomarker or as a molecular target for immunotherapy in patients with melanoma.

CD117 (c-Kit) overexpression

The c-Kit proto-oncogene is a transmembrane tyrosine kinase type receptor that is crucial for melanocyte development and proliferation [17,18,26,27]. Several smaller studies have addressed c-Kit expression in malignant melanomas. Larue et al [27] observed that mutation of the c-Kit gene in murine models could result in malignant transformation with production of tumors similar to amelanotic melanoma. Lassam and Bickford [17], Natali et al [18] and Zakut et al [19] suggested that c-Kit expression progressively decreases during local tumor growth and invasion of human melanomas. As opposed to recent findings in gastrointestinal stromal tumors, lack of c-Kit expression directly correlates with the metastatic potential of human melanoma cells in nude mice [28]. In our study, we analyzed c-Kit overexpression in all 202 melanoma cases using IHC. C-Kit was overexpressed in 46 (22.8%) cases. There was no statistically significant difference in survival between c-Kit positive and negative groups and hence no prognostic significance of c-Kit overexpression was identified. However, c-Kit was overexpressed in 53.7% cases of superficial spreading melanoma (early stage) indicating that c-Kit is overexpressed primarily in the early stage of the disease and downregulation of c-Kit is likely in metastatic/advanced disease. The mechanism by which malignant melanoma cells lose c-Kit expression is unclear. The c-Kit gene is neither deleted nor rearranged in c-Kit negative melanoma cell lines, suggesting that lack of c-Kit overexpression is most likely due to altered expression of transcription factors [17,19,29]. Huang et al [20] suggested that in metastatic melanoma cells, lack of c-Kit expression correlates with lack of expression of the AP-2 transcription factor, and that in melanoma cells, AP-2 serves as a positive regulator of c-Kit expression. Our study results indicate that c-Kit overexpression has no prognostic value in patients with malignant melanoma. However, the identification of c-Kit in a significant proportion of our patient population could possibly have important therapeutic implications for future clinical trials evaluating the role of site-specific therapies in melanoma. As previously discussed, c-Kit was overexpressed in only 26.6% (n = 41) of the melanoma patients (n = 154) without associated second primary malignancies ($p = 0.01$). Thus, the absence of c-Kit overexpression is also clinically related to the absence of other associated primary malignancies in patients with malignant melanoma.

From our results, it is now clear that some therapeutic value but no prognostic value could be potentially found in the testing of patients with malignant melanoma for c-Kit overexpression. No value could be found in testing for HER-2/neu in patients with malignant melanoma. However, it is important to mention that a major limitation of our study is the fact that our cohort of patients with malignant melanoma did not include more patients with diminished survival or early death (Figure 1). More patients with diminished survivals in the study may have defined the possible prognostic values better. Future studies on the role of bio-markers in malignant states would benefit by evaluating selected matched subgroups of patients (with and without adverse outcomes). This would be more likely to define the prognostic association if any between the individual molecular marker and the malignant condition.

Conclusions

Despite recent advances in the early detection and treatment of malignant melanoma, the diagnosis of melanoma is often made after the cancer has already metastasized to the regional and/or distant lymph nodes, liver, lung or central nervous system. Since current clinicopathologic staging systems enable us to identify only a proportion of patients with adverse survival characteristics, better clinical/molecular prognostic indicators need to be identified. Our study, which is the largest study to date, found that (i) HER-2/neu, although a poor prognostic indicator in a variety of solid tumors has no role in melanoma patients; (ii) the presence of c-Kit overexpression (detected in 22.8% specimens), although not predictive of survival, is associated with the presence of second primary tumors in patients with melanoma; (iii) overexpression of c-Kit is more likely to be seen in the superficial spreading type; (iv) in a proportion of patients, the efficacy of target-specific therapies against c-Kit in malignant melanoma warrants further investigation.

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