Original Article

Intratumoral and Peritumoral Mast Cells in Malignant Melanoma: An Immunohistochemical Study

Abstract

Background: The aim of the current study was to determine mast cell infiltration in malignant melanoma by immunohistochemistry method and its relationship with some of the cancer prognostic factors, including age, sex, and depth of the tumor. **Materials and Methods:** In this retrospective analytic cross-sectional study, paraffin-embedded tissue blocks of patients with cutaneous malignant melanoma who had undergone excisional biopsy were studied. Mast cells count in studied cases in different stages of the tumor depth was evaluated by mast cell tryptase immunohistochemistry method. Mast cells infiltration was evaluated both inside the tumor and peritumoral area. Tumor infiltrating lymphocytes (TILs) was also determined. Distribution of intratumoral and peritumoral mast cells and TILs was compared in different stages tof tumor depth. **Results:** In this study, 51 cases with melanoma were studied. Mean ± standard deviation (SD) of intratumoral mast cells in stages 1, 2, and 3 was 9.4 ± 4.2, 10.8 ± 5.1, and 2.1 ± 2.3, respectively (P = 0.000). Mean ± SD of peritumoral mast cells in stages 1, 2 and 3 was 13.4 ± 2.4, 16.6 ± 2.4 and 8.2 ± 4.6, respectively (P = 0.000). There was a significant direct relationship between depth of the tumor and TIL (P = 0.000) and distribution of intratumoral (P = 0.000) and peritumoral mast cells (P = 0.000). **Conclusion:** Lower distribution of intratumoral and peritumoral mast cells and TILs in higher stages of tumor depth in malignant melanoma suggests a possible inhibitory effect of infiltrating mast cells and lymphocytes on the progression of this tumor.

Keywords: *Immunohistochemistry, mast cell, melanoma*

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Introduction

Melanoma comprises a small proportion of cutaneous malignancies (4%), but due to its high mortality rate, it is known as the most invasive form of this group of cancers.[1,2]

Many studies have investigated the pathogenesis of malignant melanoma, however, it is not clearly understood yet.[3,4] Considering that the interactions between the malignant cells and their environment such as surrounding connective tissue, inflammatory factors as well as newly forming vessels, have an important role in the growth and development of the cancers, and the concept of inflammatory origin of the cancers was developed.[5] The concept was first reported by Virchow in 1863 and expanded by many studies.[6,7] Nowadays, the causal relationship between inflammation, innate immunity, and different cancers is widely demonstrated.[8] According to that theory inflammation could be a mediator for cancer growth and cancer growth is controlled by inflammatory mechanisms.[9-11]

Current evidence suggests that chronic inflammation and its related components such as mast cells have an important role in neoangiogenesis of cancers, which is crucial for its continued growth.[9] Coexistence of cutaneous cancers and elevated mast cells that are one of the components of chronic inflammation has been reported for many years.[10] Studies indicated that four probable mechanisms of mast cells that contribute to tumorogenesis of cutaneous malignancies are immunosuppression, angiogenesis, degradation of extracellular matrix, and mitogenesis. However, mast cells are not studied extensively in this regard due to some of their specific characteristics such as difficulty in detection in the tissue sections by routine pathologic staining methods.[11] In spite of the presence of evidences regarding accumulation of mast cells around tumors, their role as a modulator or inhibitor for tumor growth remains controversial.[12]

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Factors such as tumor thickness, ulceration, location, and gender could affect the prognosis of the malignancy.[13]

The aim of the current study was to determine mast cell infiltration in patients with malignant melanoma by immunohistochemistry method and its relationship with some of the cancer prognostic factors, including age, sex, Tumor infiltrating lymphocytes (TILs), and depth of the tumor.

Materials and Methods

In this retrospective analytic cross-sectional study, paraffin-embedded tissue blocks of patients with definitive pathologic diagnosis of cutaneous malignant melanoma who had undergone excisional biopsy were retrieved from the archives of the department of pathology of Al-Zahra hospital affiliated with Isfahan University of Medical Sciences, from 2003 to 2010. All sections were included in the study by the simple sampling method.

The Medical Ethics Committee of the Isfahan University of Medical Sciences approved the study protocol (research project number: 390069).

Cases with incompletely excised tumor and unknown tumoral depth, recurrent lesions, familial cases of melanoma such as familial dysplastic nevus, melanoma in mucosal areas, and those who had initiated anti-neoplastic treatment, were excluded.

Diagnosis of studied cases was re-evaluated by two pathologists. The clinical and histopathological characteristics of selected cases, including age, gender, and pathologic characteristics (necrosis, Breslow's depth), were recorded from the pathology reports of the patients and verified by a pathologist also.

Mast cell count at high power field (×400) in studied cases in different stages of the tumor depth (at least for ten field) was evaluated by mast cell tryptase immunohistochemistry method using Breslow's depth separating stage as follows: Stage 1: Less or equal to 0.75 mm, Stage 2: 0.75–1.5 mm, Stage 3: 1.51–3 mm, and Stage 4: >3.0 mm.[14] Mast cells infiltration was evaluated both inside the tumor and peritumoral area.

TILs (absent, nonbrisk, and brisk) in studied cases in different stages of the tumor depth was also evaluated. The criteria formulated by Clark WH Jr *et al.* were used to classify the lymphocytic infiltrate in hematoxylin and eosin stained sections. Briefly, lymphocytes that disrupted and surrounded tumor cells of the vertical growth phase (VGP) were qualified as TILs. If TILs infiltrated the entire invasive component diffusely or were present across the entire base of the VGP, they were categorized as brisk. TILs were absent if no lymphocytes were present or if lymphocytes were present but did not infiltrate the tumor at all. If lymphocytes infiltrated melanoma only focally or not along the entire base of the VGP, the term nonbrisk was used.[15]

Distribution of intratumoral and peritumoral mast cell and TIL was compared in different stages of tumor depth.

Immunohistochemical staining

Two 4 μm-thick slides of selected formalin-fixed, paraffin-embedded tissues were prepared for immunohistochemistry. The sections were deparaffinized, rehydrated by immersion in xylene, graded in ethanol, and rinsed in tap water. Endogenous peroxidase activity was blocked by incubation of the slides in 1% H_2O_2 with methanol for 30 min.

For antigen retrieval, sections were immersed in citrate solution (pH: 6.0) with microwaving for 5 min and then the sections were retained in the same solution for 15 min and finally the slides were washed in distilled water for 5 min.

Primary anti-tryptase antibody (clone AA1, Novocastra, UK) at 1:100 dilution was used. The EnVision + System kit (DAKO, Denmark) detection system was used. 3-amino-9-ethylcarbazole (DAKO, Denmark) was used as the chromogen. The immunostained slides were examined under Olympus lens × microscope (Olympus Microscope Services Limited, UK) equipped with an objective lens 40 (field of vision diameter: 0.45 mm). Immunohistochemical (IHC) evaluation of tryptase expression was performed by two independent pathologists. For the intratumoral count, the entire area of the melanocytic lesion was scanned, and the immunopositive cells, as well as the number of fields, were recorded. For the peritumoral count, only the fields of view along the interface between the lesion and its neighborhood were chosen, and then the number of positive cells and fields were recorded. The results were expressed as the average number of positive cells per high power field [Figure 1]. Positive controls were performed using sections from patients with skin mastocytosis. Negative controls were performed using sections from patients referred for diagnosis of skin disease and have normal pathology.

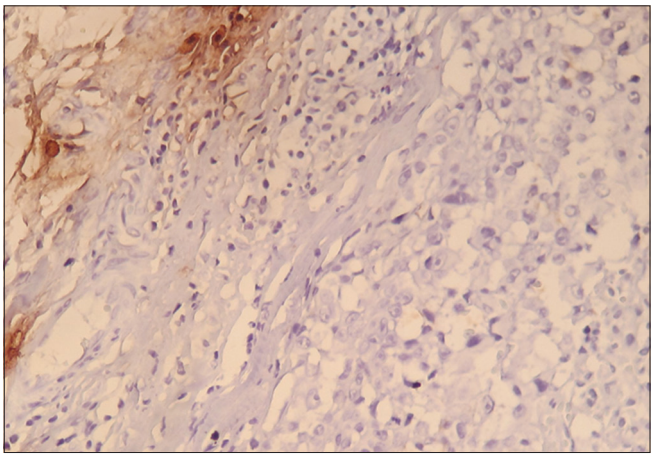

Figure 1: Immunohistochemical staining (tryptase) of mast cells in malignant melanoma

Statistical analysis

Obtained data were analyzed using IBM SPSS 18 (A Guide for Social Scientists. New York: Routledge) and descriptive, one-way analysis of variance, and *post-hoc* tests. $P < 0.05$ was considered statistically significant.

Results

In this study, 51 cases with melanoma were studied. Characteristics of studied cases are presented in Table 1.

Regarding age groups, frequency of studied population in 35–45, 46–55, 56–65, and 66–75 years old was 25.5%, 35.3%, 17.6%, and 21.6%, respectively.

Frequency of different stages of tumor depth including 1, 2, and 3 according to Breslow's classification was 13.7%, 7.8%, and 78.4%, respectively.

Lymphocytic infiltration was significantly absent (31/51; 60.8%) in stage 3 of the tumor depth.

Mean ± standard deviation of intratumoral and peritumoral mast cells in different stages of tumor depth is presented in Table 2.

Distribution of intratumoral and peritumoral mast cells according to the depth of the tumor in studied age groups is represented in Figure 2.

Table 1: Basal characteristics (mean±SD) of studied cases with melanoma

Variable	Rate
Sex	
Female/male	25/26
Age (year)	53.5±10.7
Pathologic characteristics of the tumor	
Depth (cm)	5.1±4.1
Necrosis (%)	4 (7.8)
Lymphocytic infiltration (%)	
Absent	41 (80.4)
Nonbrisk	8 (15.7)
Brisk	2 (3.9)
Peritumoral mast cells (/hpf)	9.7±5.0
Intratumoral mast cells (/hpf)	3.9±4.5

SD: Standard deviation

Table 2: Mean±SD of intratumoral and peritumoral mast cells and TILs in different stages of tumor depth

Variable	Stage 1	Stage 2	Stage 3	*P*
Intratumoral mast cells (/hpf)	9.4±4.2	10.8±5.1	2.1±2.3	0.000*
Peritumoral mast cells (/hpf)	13.4±2.4	16.6±2.4	8.2±4.6	0.000*
TILs				
Absent	7	3	31	0.007
Nonbrisk	0	0	8	
Brisk	0	2	0	

*P value was significant between stages 1 and 3 and 2 and 3. TILs: Tumor infiltrating lymphocytes, SD: Standard deviation

There was no significant direct relationship between depth of the tumor and age ($P = 0.8$) and necrosis ($P = 0.3$).

There was a significant direct relationship between depth of the tumor and lymphocytic infiltration ($P = 0.000$), and distribution of intratumoral ($P = 0.000$) and peritumoral mast cells ($P = 0.000$).

Discussion

In this study, the mean distribution of peritumoral and intratumoral mast cells in malignant melanoma and their relationship with prognostic factors of the tumor was investigated. The findings of the current study indicated that both peritumoral and intratumoral mast cells were significantly lower in stage 3 of tumor depth. There was a significant relationship between peritumoral and intratumoral mast cells and tumor depth in malignant melanoma.

Though the role of mast cells in different tumors has been studied widely,[14,16] it seems that the role of this inflammatory factor has not been studied extensively in melanoma. Based on their expression, mast cells were described in two different population of chymase and tryptase and sole tryptase expression.[17] It is thought that tryptase if assessed by IHC, could detect total mast cells with higher specificity and sensitivity.[18] In this study, distribution of peritumoral and intratumoral mast cells in malignant melanoma was detected using IHC mast cell tryptase staining method.

Reports regarding the role of mast cells in the pathogenesis of melanoma are controversial. They could have modulatory or inhibitory role in tumor growth.

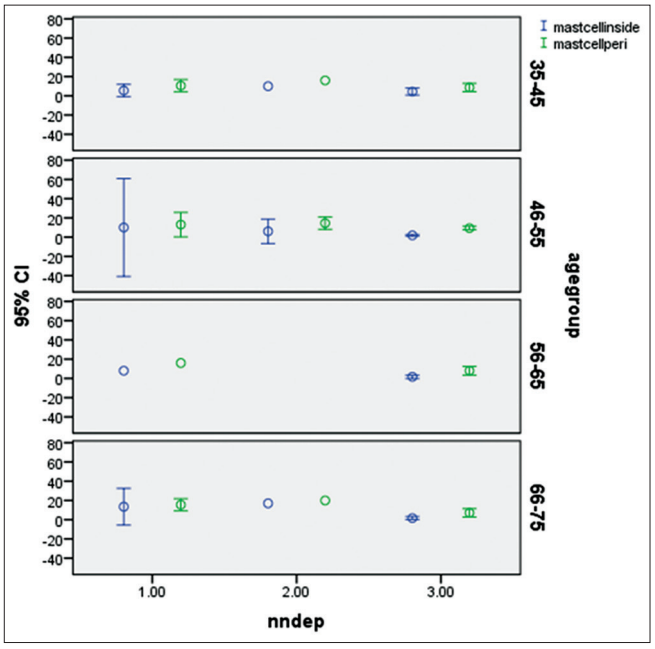

Figure 2: Distribution of intratumoral and peritumoral mast cells according to the depth of the tumor in studied age groups

Ch'ng S *et al.* in New Zealand reviewed the role of mast cells in the development and progression of cutaneous malignancies, including malignant melanoma. They concluded that mast cells could have an accessory role in this field. Accordingly they may have an opposing role in a way that the tumor's microenvironment could lead mast cells to have either promoting or inhibitory effects on this group of tumors.[12]

Though most of the studies suggest a tumorogenic effect for mast cell, some of them reported the anticancer effect of mast cells in cutaneous tumors.[19-21] Mediators such as tumor necrosis factor-α, interleukin 1 (IL-1), and IL-6 have been reported to have an inhibitory effect on tumor growth and angiogenesis.[22] Mast cells could have a direct cytotoxic effect on tumor cells growth by activating mediators such as toll-like receptors 2 which subsequently increases IL-6 and inhibits tumor cells growth.[23]

Artuc *et al*. have shown that interaction of mast cells and cutaneous tumor cells could have an important role in tumor growth, invasion, and neovascularization through altered cytokine release.[19]

In another study in Japan, Tóth-Jakatics *et al*. have reported that peritumoral mast cells accumulation and tumor-host interaction could promote the progression of cutaneous malignant melanoma due to the release of angiogenic factor such as VEGF.[20]

The results of this study demonstrated that both peritumoral and intratumoral mast cells were significantly lower in stage 3 of tumor depth.

Dyduch *et al*. have investigated the distribution of mast cells in pigmented nevi, dysplastic nevi, and melanomas. Their results indicated that both intralesional and perilesional tryptase count was lower in melanoma than two other benign lesions.[24]

Different results obtained from various studies in this field could be explained by differences in the methods of study. Another explanation is that mast cells have promoting role in early stages of the tumor and overcoming of host defenses will reflect by lower mast cell count. It is assumed that by the progression of tumor growth and reducing immune reaction, mast cell count will increase.

In this study, mean of peritumoral mast cells were significantly higher than intratumoral mast cells. It was in line with previous reports. Most studies suggest that peritumoral mast cells count is higher than intratumoral ones.[25]

In this study, TIL in higher stages of malignancy was significantly absent. Our results were in accordance with other studies in this field.[26,27] Clinicopathologic evidences indicated that lymphocyte infiltration in some tumors such as malignant melanoma is associated with better prognosis of the tumor. The role of TIL as a better prognostic factor was first described by Clark *et al*.[15] Thereafter some studies confirm the findings. Tuthill *et al*. indicated that in patients with malignant melanoma brisk host response of TILs has significant protective effect in the survival of the patients.[28] Piras *et al*. have reported similar results. Our findings confirm the work of mentioned studies.[29]

Though necrosis and age are considered as prognostic factors for malignant melanoma,[30] there was not a significant relationship between necrosis and age and depth of the tumor in this study. It may be due to the small sample size.

The limitation of this study was that we did not compare our findings with benign melanocytic skin lesions such as pigmented and dysplastic nevi or normal tissue.

Conclusion

Lower distribution of intratumoral and peritumoral mast cells and TIL in higher stages of tumor depth in malignant melanoma suggests a possible inhibitory effect of infiltrating mast cells and lymphocytes on the progression of this tumor. For more conclusive results, further studies with larger sample size and consideration of mentioned limitations of the current study are recommended.

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Conflicts of interest

There are no conflicts of interest.

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