Original Article

Expression of Estrogen Receptor Alpha in Malignant Melanoma

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Abstract

Background: Features of malignant melanoma (MM) vary in the different geographic regions of the world. This may be attributable to environmental, ethnic, and genetic factors. The aim of this study was to determine the expression of estrogen receptor alpha (ER-α) in MM in Isfahan, Iran. **Materials and Methods:** This study was planned as a descriptive, analytical, cross-sectional investigation. During this study, paraffin-embedded tissue blocks of patients with a histopathologic diagnosis of MM was studied for ER-α using immunohistochemistry (IHC). **Results:** In this study, 38 patients (female/male; 20/18) with a definite diagnosis of malignant cutaneous melanoma and mean age of 52.4 ± 11.2 years were investigated. Using envision IHC staining, there were not any cases with ER-α expression. **Conclusion:** In confirmation to the most previous studies, expression of ER-α was negative in MM. It is recommended to investigate the expression of estrogen receptor beta and other markers in MM.

Keywords: *Estrogen receptor alpha, immunohistochemistry, melanoma*

Introduction

Skin cancer is considered as one of the most common cancers worldwide.[1] Although malignant melanoma (MM) comprises only 5% of all skin cancers, it has the most malignant behavior and the highest mortality rate among these tumors.[2] The incidence rate of MM has great variability worldwide, with the higher rate in Auckland and New Zealand and the lower rate in Asia. The incidence rate of melanoma in Iran is lower than the other developed countries.[3,4] Difference reported in the rate of melanoma is related to genetic factors, skin type, societal customs, cultural issues, and habits.[5]

Several studies have investigated the role of different factors related to the pathophysiology of MM.[6] The role of sex steroid hormones in MM has been investigated in many studies, but the results are controversial. There is evidence which support the role of sex hormones in the pathophysiology of MM. Worse prognosis of MM in prepuberty, during pregnancy, and in male patients support the role of sex hormones in this field.[7-9]

It is suggested that estrogen have a genotropic effect which could directly or indirectly regulate the transcription of genes. Moreover, it is supposed to affect cell survival and/or modulate other growth factor, signaling through a cytoplasmic effect, which is activating the signaling pathways.[10]

The expression of estrogen receptors (alpha and beta) in different benign and malignant melanocytic lesions has been studied. Findings of the mentioned studies regarding the role of different ER expression in these lesions are controversial.[11,12]

Features of MM vary in different geographic regions of the world. This may be attributable to environmental, ethnic, and genetic factors.[5] Moreover, previous studies have reported different sex distributions of MM in Iran.[4] The aim of this study was to determine the expression of ER-alpha (ER-α) in MM in Isfahan, Iran.

Materials and Methods

This study was planned as a descriptive, analytical, cross-sectional investigation. During this study, paraffin-embedded tissue blocks of patients with histopathologic diagnosis of MM prepared from the samples of their lesion excisional biopsies were retrieved from the archives of the Department of Pathology of Al-Zahra Hospital, Isfahan, from 2004 to

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2011. The section was selected using the simple sampling method.

The protocol of this study was approved by the regional Medical Ethics Committee of the Isfahan University of Medical Sciences (research project number: 390070).

Sections from the patients with tumor recurrence, anti-cancer treatment, and those with familial dysplastic nevus were excluded from the study.

The histopathologic diagnosis of the sections was confirmed by two pathologists. Demographic, clinical, and pathologic characteristics of finally selected sections were recorded from the patient's medical files.

Prognostic factors of the tumor, including age, Breslow depth of the tumor (I, II, III), necrosis, tumor infiltrating lymphocytes (TIL; absent, nonbrisk, brisk), and mitotic rate (0, I, II), were determined in each section. Expression of ER-α in all the sections was determined using immunohistochemistry (IHC) method.

Breslow depth separating stage as follows: Stage I - ≤0.75 mm, stage II - 0.75–1.5 mm, stage III - 1.51–3 mm, and stage IV - >3.0 mm.[13]

The criteria formulated by Clark *et al*. were used to classify the lymphocytic infiltrate in H and E stained sections. Briefly, lymphocytes were needed to disrupt and surround the tumor cells of the vertical growth phase (VGP) to qualify as TILs, if TILs diffusely infiltrated the entire invasive component or were present and infiltrating across the entire base of the VGP, they are qualified as brisk. TILs were absent if no lymphocytes were present or if lymphocytes were present but did not infiltrate the tumor at all.

Nonbrisk term was used for cases with focal lymphocytic infiltration or infiltrations which are not along the entire base of the VGP.[14]

Mitotic rate was considered as grade 1, 2, and 3 by counting in mm^2, if there was 0–1 mitosis mm^2 grading as 0, 1–10 mitosis mm^2 grading as 1, and >10 mitosis mm^2 grading as 2. The method for mitosis enumeration was the hot spot.[15]

Immunohistochemical staining

Three to four micrometer slides from selected formalin-fixed, paraffin-embedded tissues were prepared for envision IHC staining.

After preparation of 4 μm thin slides, sections were placed on the poly-l-lysine slides, and then they were deparaffinized and dried in an oven at 60°C for 60 min. After rehydrate, their antigens were retrieved by boiling them in Tris-buffered saline by microwave heat-induced epitope retrieval method. After inactivation of endogenous catalase by using 3% hydrogen peroxide, the slides were incubated with antibodies to ER clone 1D5 (Dako, Carpinteria, CA) for 1 h and secondary antibody for 30 min, and then antibodies was localized and made apparent by streptavidin-biotin method and diaminobenzidine as chromogen. Stained slide was considered as positive if there was nuclear staining: 1 (upto 10% of cells positive), 2 (11–50% positive), and 3 (>50% positive).

Statistical analysis

Obtained data were analyzed using IBM SPSS software (version 18, Chicago: SPSS Inc). Chi-square and *t*-test were used. $P < 0.05$ was considered as statistically significant.

Results

In this study, 38 patients with definite diagnosis of malignant cutaneous melanoma were studied. Demographic and histopathologic characteristics of the studied population are presented in Table 1.

Using envision IHC staining, there were not any cases with ERα expression [Figure 1].

Discussion

The current study was designed to investigate the expression of ER-α in MM. The results indicated no expression of ER-α in MM.

The effects of estradiol in cell are activation in the mitogen-activated protein kinase signaling cascades, calcium flux, the generation of cyclic adenosine monophosphate (cAMP) and IP3, the activation of

Table 1: Demographic and histopathologic characteristics of patients with malignant cutaneous melanoma

Variable	Rate
Sex (female/male)	20/18
Age (years)*	52.4±11.2
Pathologic characteristics of the tumor	
Breslow depth (%)	
Stage I	5 (13.2)
Stage II	3 (7.9)
Stage III	30 (78.9)
Necrosis	4 (10.5)
Mitosis (%)	
0	29 (76.3)
I	5 (13.2)
II	4 (10.5)
Lymphocytic infiltration (%)	
Absent	32 (84.2)
Nonbrisk	5 (13.2)
Brisk	1 (2.6)

*Mean±SD. SD: Standard deviation

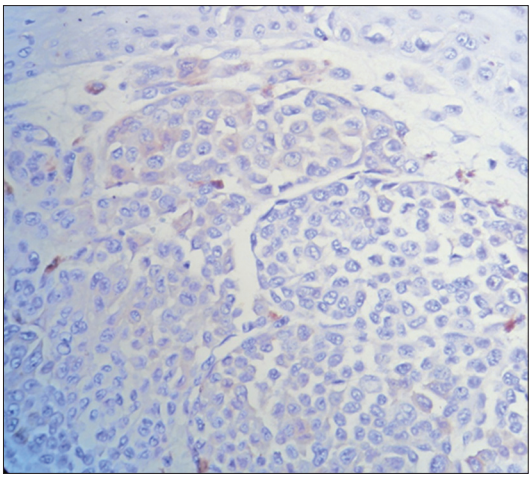

Figure 1: The absence of immunohistochemical staining of estrogen receptor alpha in malignant melanoma

phospholipase C, and interacting with growth factors. The ER pathway could be indirectly regulated by other modulatory pathways such as insulin-like growth factor 1, epidermal growth factor, the second messengers' cAMP, and dopamine.[16-18]

Estrogen has some direct influence on animal and human skin such as synthesis, maturation and turnover of collagen, increased synthesis of hyaluronic acid, increased mitotic activity in the epidermis, modulation of epidermal carcinogenesis, and enhanced vascularization. Estrogen directly induces melanocyte enlargement and increases their number and melanin content. Some studies have shown that estrogen deprivation causes a decrease in the size of melanocytes and shortening of their dendritic processes. Increase in cutaneous pigmentation during pregnancy and chloasma are another example of estrogen's effect on melanocyte.[16]

In one multivariate analysis, which included age, sex, site of primary tumor, thickness of primary tumor, level of invasion, nodal status, and ER status, the most significant predictors of survival and disease-free survival were nodal status, level of invasion, and ER status.[17]

There are sufficient evidence that suggest a relationship between melanoma and estrogen, but studies have shown contradictory results.[1,19,20] Several studies have evaluated the role of ER-α in MM. Though almost all studies failed to demonstrate the expression of ER-α in this group of skin tumors, it seems that the role of steroid hormone related receptors is controversial.[19,20]

ER-α is from a superfamily of transcription activators, which have many physiologic properties such as tumor progression.

Several studies have reported the presence of ER in melanoma cell lines, but the analysis of human melanomas has shown variable ER-α expression.[19,20]

In this study, we examined the expression of ER-α in MM using IHC method.

Recent studies indicated that the presence of estrogen-binding proteins in MM, reported by biochemical assays have been false positives.[21,22] Thus, immunohistochemical studies using specific antibodies to the ER have been developed. The method for detection of ER has been identified for the 1st time in 1986.[21] Almost all of the studies using IHC for the determination of ER in MM have demonstrated no expression of ER-α in MM.[23-26] This study was designed as a confirmatory study in this field, in our region.

However, some studies in Iran have reported that the sex distribution of MM is different from other studies worldwide, with a male: female ratio of 1.5.[4] So, it is suggested that other features of MM would be different in our region, which need more studies.

Our result showed no ER-α positivity in melanoma cells with anti-ER-a clone 1D5 (Dako). There was no nuclear staining not only in malignant nuclear cells but also neither in melanocytes nor in other normal elements of the skin.

Many researchers have failed to determine ER-α in melanoma by IHC method. Despite the disability to detect ER-α receptor by IHC method, some studies have shown that all melanocytic lesions express ER-α and ER-beta (ER-β) mRNA which could be detected by polymerase chain reaction (PCR) method.[27] So, it seems that our results should be re-evaluated by more accurate methods such as PCR.

Another explanation for our findings is that some tumor that initially express ER-α loss their receptor during tumor progression, this is due to the aberrant methylation of CpG region of ER gene, which is a cytosine–guanine-rich area and is located in the 5 regulatory regions of this gene.[28-30] The ER-α loss means that the tumor does not control ER-α growth hormone modulatory activation which causes tumor aggressive behavior.[31]

Schmidt and colleagues in the USA have demonstrated the ER-α and ER-β immunostaining patterns in melanomas, benign nevocytic nevi, dysplastic nevi with different grade of cytological atypia, and lentigo malignancies.

According to their report, ER-β and not ER-α, is the predominant ER receptor in melanocytic lesions. The distribution and degree of ER-β immunoreactivity were markedly different among the various classifications of lesions.[25]

Ohata *et al*. in Japan used immunohistochemical staining to characterize the expression of ER-α and ER-β in normal skin and in melanocytic lesions in 40 patients. They showed that melanocytic nevi and MMs were negative for ER-α, but both were positive for ER-β. The ubiquitous expression of ER-β may play a fundamental role in various normal skin cells and melanocytic tumors.[26]

The limitations of the current study were as follows; we did not study the expression of ER-α in benign melanocytic lesions and normal skin. Our results would be more conclusive if the role of expression of ER-β has been studied also. It is recommended to study the role of ER-β in different malignant and benign skin lesions in a larger sample size of patients in future studies.

Conclusion

In confirmation to most previous studies, expression of ER-α was negative in MM. It is recommended to investigate the expression of ER-β and other markers in MM.

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Conflicts of interest

There are no conflicts of interest.

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