A comparative study of estrogen receptor beta expression in melanoma and benign melanocytic lesions

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Background: Malignant melanoma is the most aggressive form of skin cancer. In contrast to other tumors, the role of estrogen in the initiation and progression of melanoma remains unclear. The aim of this study was to evaluate estrogen receptor beta protein expression in human melanoma tissues and in the benign melanocytic lesions.

Method: Twenty-one patients, 11 with cutaneous melanoma and 10 with benign melanocytic lesions were enrolled in this study. Estrogen receptor beta expression in benign melanocytic lesions and melanoma was analyzed by using immunohistochemical staining.

Result: All melanocytic lesions expressed estrogen receptor beta protein. We found lower estrogen receptor beta protein levels in melanoma.

Conclusion: These initial observations, to be confirmed by further comprehensive studies, could suggest a role for estrogen receptor beta in melanoma, pointing at the possibility of using estrogen receptor beta expression for differentiating between malignant and benign lesions.

Keywords: benign melanocytic lesions, estrogen receptor beta, melanoma

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INTRODUCTION

Malignant melanoma is the most serious form of skin cancer with a dismal prognosis. There has always been suspicion that a relationship exists between estrogens and this cancer [1]. Some findings that support this hypothesis include better survival rate for female patients with metastatic melanoma; rare incidence of melanoma prior to puberty and the peak incidence in the late childbearing years and the beginning of menopause in female patients [2].

Most of the effects of estrogen are mediated through estrogen receptors which belong to the nuclear hormone receptor family. Two forms of estrogen receptors exist: alpha and beta [3]. Estrogen receptors classically mediate their action by ligand-dependent binding to the estrogen-response element, leading to transcriptional regulation of target genes [4]. Both of these proteins have a high degree of homology in the DNA-binding domain but differ considerably in the *N*-terminal domain and, to a lesser extent, in the ligand-binding domain (E domain) [5]. Some differences exist between the effects of these two receptors; while estrogen receptor alpha is associated with stimulation of growth; estrogen receptor beta is associated with suppression of stimulation or inhibition of cells from multiplying [2]. Several reports have shown either decreased expression of estrogen receptor beta messenger RNA and estrogen receptor beta protein or an increased estrogen receptor beta: alpha mRNA ratio in the tumor versus normal tissues in several cancers, like breast, ovary, colon, and prostate cancers [6,7].

The question of whether melanoma tumors express the two estrogen receptors is intensely

debated; for this reason, we embarked on a project of evaluating estrogen receptor beta expression in benign melanocytic lesions and melanoma using immunohistochemical staining.

PATIENTS AND METHODS

The expression of estrogen receptor beta was investigated in 11 patients with cutaneous melanoma and 10 patients with melanocytic nevi at the department of dermatology of Shahid Beheshti University of Medical Sciences. All melanoma patients had Breslow thickness more than one millimeter. The characteristics of the patients are listed in Table 1.

All cases underwent immunohistochemical analysis according to the method described by de Giorgi et al [2]. A representative specimen, 4µm thick, from each formalin-fixed and paraffin-embedded skin lesion was evaluated. The slides were deparaffinized in Bio-Clear and hydrated with a series of decreasing ethanol concentrations and finally distilled water. Antigen retrieval was routinely performed by immersing the slides in a thermostat bath containing preheated 10mM citrate buffer (pH=6.0) for 40 minutes at 97°C followed by cooling for 20 minutes at room temperature. To block endogenous peroxidase activity, slides were treated with 3.0% hydrogen peroxidase in distilled water for 10 minutes. After blocking nonspecific antigens with normal horse serum, sections were incubated for 30 minutes at room temperature with mouse monoclonal antibody against the C-terminus epitope of estrogen receptor beta, clone EMR02, diluted 1:50 in antibody diluent. Staining was achieved using a biotin conjugated anti-mouse and anti-rabbit secondary antibody and streptavidin-peroxidase. The bound antibody was detected using 3, 3′ diaminobenzidine as chromogen. Nuclei were slightly counterstained with Mayer hematoxylin. The negative control procedure was performed by substituting a non-immune serum for the primary antibody at the same concentration. As a positive control, we used a fibromatosis sample certainly positive for estrogen receptor beta. Definite nuclear staining was regarded as positive and cases were scored by the percentage of tumor cells that stained (I [<20%],II [21-50%],III [>50%]).

Data analysis was performed with SPSS software. *P* values less than 0.05 indicated a significant difference.

RESULTS

Eleven patients with malignant melanoma and ten patients with benign melanocytic lesions were enrolled in this study. The mean age of the melanoma and benign melanocytic lesion patients was 54 and 43 year, respectively. All melanoma patients had Clark level more than two and benign melanocytic lesions were either congenital nevus or intradermal nevus (Table 1).

Among melanoma patients, only one had estrogen receptor beta expression of grade III but among patients with benign melanocytic lesions, 70% had grade III staining (Table 1). Comparison of the staining levels of melanocytes with the Mann-Whitney U-test showed a significant difference between estrogen receptor beta staining in the two groups (P-value=0.0002).

Table 1. Patients characteristic and immunohistochemical expression for estrogen receptor beta

GROUPS					
Melanoma			Benign lesions		
Patients nom/age/sex	Clark level	ERbeta expression*	Patients nom/age/sex	type	ERbeta expression
1/51/F	V	I	1/61/M	Intradermal nevus	III
2/42/F	IV	II	2/39/M	Congenital nevus	III
3/51/F	V	I	3/31/M	Intradermal nevus	II
4/40/F	IV	I	4/50/M	Intradermal nevus	III
5/7/F	IV	II	5/64/F	Congenital nevus	II
6/77/M	V	I	6/22/M	Intradermal nevus	III
7/50/M	II	III	7/41/F	Congenital nevus	II
8/82/F	V	I	8/40/M	Intradermal nevus	III
9/73/M	IV	II	9/50/F	Intradermal nevus	III
10/63/F	III	I	10/33/F	Intradermal nevus	III
11/64/F	III	II			

ER:estrogen receptor, F: female, M:male

DISCUSSION

Malignant melanoma is the most serious form of skin cancer with a rapidly increasing incidence rate. Despite the wide variety of therapeutic options, metastatic disease is still associated with a dismal prognosis [2].

The role of estrogens in the initiation and progression of melanoma is unclear. The better survival rate of the female patients with metastatic disease and the peak incidence in the late childbearing years and the beginning of menopause all point to the possible role of estrogens in melanoma.

The epidemiologic data are supported by researches using melanoma cell lines grown in experimental animals [8]. According to these data, it could be hypothesized that melanoma acts as an estrogen-responsive tumor and estrogen receptors could mediate molecular responses associated with the neoplastic transformation of normal benign nevi.

Tamoxifen, an ER antagonist, was used in the past in the treatment of patients with metastatic melanoma either as a single agent or in combination with other agents [9] but the results of recent studies do not support the use of tamoxifen in the treatment of metastatic melanoma.

The aim of our study was to investigate whether estrogen receptors were present in melanocytic lesions, and if their levels were different in benign lesions compared to those of melanoma. Estrogen receptor beta expression was higher in patients with benign melanocytic lesions as compared to melanoma patients. Loss of estrogen receptor beta expression with increased melanoma thickness was also reported by Schmidt et al [10] and de Giorgi et al [2]. These primary observations suggest a probable role for estrogen receptors in melanoma, particularly in more advanced and metastatic processes. Moreover, estrogen receptors could be used as a marker for differentiation between benign melanocytic lesions and melanoma; but due to the relatively small number of patients, our findings require further confirmation by larger comprehensive studies.

In conclusion, despite the relatively small number of patients, it could be hypothesized that the expression of estrogen receptor beta is markedly decreased in melanoma.

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