THE ROLE OF ESTROGENS AND ESTROGEN RECEPTORS IN MELANOMA DEVELOPMENT AND PROGRESSION

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Abstract

Melanoma has a significant mortality and its growing incidence is associated with important social and health care costs. Thus, investigation of the complex mechanisms contributing to emergence and development of melanoma are of real interest both in scientific research and clinical practice. Estrogens play an important role in the emergence and development of certain types of cancer, such as breast cancer, endometrial cancer and ovarian cancer, but their role in development of cutaneous melanoma is still a matter of debate. Various data suggest that increased levels of endogenous estrogens during pregnancy or exposure to exogenous estrogens by use of oral contraceptives (OCs) and hormone replacement therapy (HRT) may have a potential role in melanoma development and progression. Moreover, there were revealed several intracellular pathways which can support the connection between estrogens, estrogen receptors (ER) and melanoma. While ER- β plays an antiproliferative role, ER- α promotes cell growth and cellular atypia. Thus, inhibition of ER- β activity in the skin can increase the risk for development of cutaneous melanoma and spread of metastatic cells. However, despite recent advances in this area, the exact role and clinical implications of estrogens and estrogen receptors in melanoma are still not entirely understood and require further investigations.

Key words: estrogens, estrogen receptors, melanoma, oral contraceptives, hormone replacement therapy, pregnancy.

INTRODUCTION

One of the most aggressive cancers, melanoma may occur anywhere melanocytic cells are present: skin, mucosa (oral, genital, nasal, ano-rectal, esophageal),

eye, meninges, nervous central system. With a significant morbidity and mortality, the incidence of melanoma has increased faster than any other form of cancer in the last decades (1-5). Cutaneous melanoma can occur at any age, usually between 20 and 60 years. It seldom occurs before the onset of puberty, usually by malignant transformation of giant congenital nevi. Exceptionally, melanoma can develop prenatally, as a primary tumour or by metastatic transplacental transfer. Melanoma occurs more often in women, usually on their shins, in men being more common the upper thoracic location (6, 7).

Although melanoma is a disease with a strong genetic component, there are various other factors, such as extensive exposure to UV radiation, chronic inflammation induced by mechanical or chemical injuries, endogenous or exogenous hormones that are strongly involved in the development and progression of the disease (1-3, 8).

The scientific interest was drawn towards the estrogens and estrogen receptors, which proved to be involved in the emergence and progression of certain types of tumors, such as breast, ovarian, endometrial, bladder, kidney, adrenal, prostate, testis, lung, colon, thyroid, brain, pancreas, bone and skin cancers (see Fig. 1) (9-16). However, their role in the development of cutaneous melanoma still remains unclear (17). The hypothesis of a certain role of estrogens in melanoma derived from the observations of gender-related differences in melanoma progression and studies concerning the evolution of melanomas during pregnancy (17-19).

Epidemiological studies revealed a higher survival rate in women with metastatic melanoma

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compared to men of the same age, and also a higher survival rate observed in premenopausal women than postmenopausal women. Possible roles of estrogens were suggested by the low incidence of cutaneous melanoma before puberty, followed by a dramatic increase during the childbearing period and a reduction after the menopause (18-20).

The human skin has the capacity to synthesize estrogens, suggesting their important role in cutaneous physiology. They influence the activity of keratinocytes, melanocytes and fibroblasts. It has been emphasized a significant variation of skin thickness during the normal menstrual cycle, the cutaneous tissue being thinner at the beginning of the cycle, when estrogen levels are reduced. Subsequently, as estrogen levels rise, the thickness of the skin increases (21). In menopause, when estrogens concentrations diminish, skin loses its tonicity and elasticity. Estrogen administration can delay the development of aging-related skin changes (22). Also, skin healing process is stimulated by estrogens (23).

Estrogens can stimulate proliferation of melanocytes and increase the melanin content in the skin, with consecutive hyperpigmentation. Estrogen and estrogen-progestin combinations induce melanogenesis, stimulating the increase of melanin content both intracellularly and extracellularly. This is a physiological effect of estrogens action on melanocytes which can occur both in women using oral contraceptives (OCs) or hormone replacement therapy (HRT), and also in pregnancy (17).

Estrogen receptors and melanoma

Estrogens exert their physiological roles acting on two types of receptors: estrogen receptor α (ER- α) and estrogen receptor β (ER- β). ER- α and ER- β are proteins encoded by two different genes located on different chromosomes. ER- α was the first discovered in the 1960s and it is known for its importance in breast cancer. ER- β was discovered 30 years later and its exact role is still under study (16, 18, 24).

ER- β is the most prevalent estrogen receptor in the skin and, besides the well known estrogen responsive tissues such as uterus and mammary glands it was also found in other tissues including the brain, colon and prostate (18, 22). Activation of ER- β induces antiproliferative effects which appear to be balanced by the opposite action of ER- α . Recent studies concluded that estrogen signaling depends mainly on the balance between expression of ER- α and ER- β . Depending on the predominant estrogen receptor, in one specific

tissue the global effect will be stimulation of cellular growth and proliferation or, on the contrary, their inhibition (25). In the skin, perturbation of this balance and a predominance of pro-proliferative ER- α over antiproliferative ER- β may lead to melanoma formation (26; 27) (see Fig. 2).

Interaction of estrogens with ERs generates intracellular effects through genomic and nongenomic pathways involving signaling factors which can be altered in melanoma cells (28) (see Fig. 3). The genomic pathway involves the interaction of ERs with different transcription coactivators such as cAMP response element-binding protein (CREB), modulating transcription of various genes and leading to alteration of tumor cells proliferation. Generally, is considered that ER β induces an inhibitory effect on gene transcription and cell proliferation while ER α has an activation effect.

The non genomic pathway effects are induced by ERs interaction with several intracellular pathways in which a key role is played by MAPK/ERK pathway, a signaling system involved in carcinogenesis, cell proliferation, and with a strong connection with melanoma. Another signaling pathway potentially involved is PI3 kinase (PI3K) pathway, which can be activated in melanoma and other skin tumors (28-32).

Clinical studies using immunohistochemical staining to assess the expression of estrogen receptors emphasized the presence of both ER- α and ER- β in normal sebaceous glands and hair follicles. ER- α was reduced in melanocytic nevi and melanomas but ER- β was present in both benign and malignant melanocytic lesions, suggesting an important role of ER- β in normal skin physiology as well as in modulation of tumor cell responses (24). In addition, ER- β expression diminishes in thicker tumors (20, 26, 27, 33, 34). Furthermore, it was found that patients with cutaneous melanoma who developed lymph node metastases, showed a lower level of ER- β in the tumor compared to normal skin suggesting a possible involvement of ER- β in development of metastases (26, 27, 33). Thus, several studies suggested that ER β tumor tissue expression may constitute a possible prognostic marker in melanoma (20, 34, 35).

Studies results performed in melanoma animal models also suggest a link between ER- β signaling and tumor growth which can be mediated by immune pathways. Tumour growth and proliferation of B16/F10 melanoma cells were significantly increased in ER- β deficient mice than in wild type, C57BL/6 control mice (36).

Thus, it is assumed that reduction of ER- β expression and a decrease on its possible antiproliferative effect can lead to melanoma development. The fine adjustment of ER- β expression may represent a possible protective mechanism against malignant transformation of melanocytic lesions. Thus, in conditions of estrogen exposure or cellular atypia, up-regulation of ER- β seems to be the key for maintaining the balance (25, 37).

A recent *in vitro* research opened new perspectives in the study of the relationship between ERs and melanoma (38). The study showed that ER- β is expressed in several human melanoma cell lines, such as BLM, WM115, A375 and WM1552. However, the expression of ER β isoforms considerably varied between different cell lines, offering a plausible explanation for the variable effects of ER- β activation on melanoma cell growth. While in BLM and WM115 melanoma cells, activation of this receptor reduced cell proliferation, suggesting a tumor suppressor activity of ER- β , in A375 and WM1552 melanoma cells ER- β agonists failed to induce a similar antiproliferative effect.

It has been recently reported another estrogen receptor named G protein-coupled estrogen receptor (GPER), an integral membrane protein expressed by melanoma cells, which can mediate both tumor-promoting and antitumor actions (16, 39).

Several studies regarding tamoxifen suggested another link between estrogen receptors and cutaneous melanoma. Tamoxifen has a dual effect, acting as an antagonist of ER in breast tissue, and as an agonist of ER in other tissues such as endometrium. Thus, tamoxifen may be regarded as a selective estrogen-receptor modulator (40). Early studies have shown conflicting results. Thus, in hamster melanoma cell line HM-1, tamoxifen significantly reduced melanoma growth (41), but another *in vitro* research performed on three human melanoma cell lines, UIISO-MEL-1, UIISO-MEL-2 and UIISO-MEL-4 was not able to reveal an effect of tamoxifen on cell growth in any melanoma cell line (42). In a more recent study performed on murine melanoma cell line B16BL6 tamoxifen significantly reduced cell migration, invasion and metastasis (43). These effects may be explained by the decreased expression and activity of matrix metalloproteinases and inhibition of protein kinase C (PKC) signaling, which appear to play major roles in melanoma progression. These results suggest that tamoxifen could have potential therapeutical applications in metastatic melanoma. The anti-metastatic effects of anti-estrogens

could also be explained by reduction of interactions between melanoma cells and matrix proteins. Thus, an *in vitro* study on murine B16 melanoma cells showed that tamoxifen and droloxifene reduce the attachment of melanoma cells to extracellular matrix proteins. Moreover, tamoxifen decreases the interactions between human ocular melanoma cells and type I collagen (44) and reduces the formation and proliferation of neoplastic cells, diminishing the occurrence of metastasis in mouse melanoma (43).

A recent experimental study on K1735-M2 mouse melanoma cells showed a more intense cytostatic action induced by endoxifen, the active metabolite of tamoxifen (45).

However, the use of estrogen-receptor modulators in treatment of melanoma still remains controversial and deserves further investigations (22).

Effect of estrogens in relation to melanoma

Various *in vivo* and *in vitro* experimental studies that have examined the effects of estrogen on melanoma in several species have often led to conflicting results.

Estradiol did not significantly influence the *in vitro* growth and plating efficiency of S91 mouse melanoma B line cells, but slightly increased their pigment production (46). Estrogen enhanced tumor growth and stimulated metastasis of B16 melanoma in mice (47). Also, estrogen treatment increased proliferation of B16 mouse melanoma cells inoculated intramuscularly in normal female mice or in male C57BL6J syngenic mice and was associated with a higher number of metastases (48).

Conversely, estrogen inhibited growth of hamster melanoma cell line HM-1 *in vitro* and reduced growth of hamster HM-1 melanoma in athymic mice (41).

An *in vitro* study conducted on three human melanoma cell lines, UIISO-MEL-1, UIISO-MEL-2 and UIISO-MEL-4 showed that estradiol exposure did not influence the plating efficiency or growth of melanoma cells (42). Other *in vitro* results revealed that estradiol exposure induces growth inhibition of human metastatic melanoma cell lines by reducing the interleukin-8 production (49).

Chronic estradiol exposure enhanced tumor latency and reduced tumor progression in an *in vivo* athymic mice model bearing UIISO-MEL-2 human melanoma cells (42). On the other hand, experimental results on human melanoma cell lines grown in young athymic mice showed that the incidence of

metastasis slightly increases after estradiol exposure. This effect was attributed to the strong inhibition of NK cell activity induced by estradiol (50). A similar immunosuppressive effect of estradiol was associated with an increased number of pulmonary metastasis in mice bearing B16 melanoma tumors (51).

Conflicting results resulting from experimental studies may be explained by species differences, various experimental designs, different expression of estrogen receptors in various cell lines, as well as by indirect actions of estrogens.

Various clinical studies suggested a potential role of estrogens in the pathogenesis of melanoma (17; 35; 52). A retrospective study stated that ever use of estrogens is associated with a higher risk of melanoma (53). Other researches have confirmed the existence of a correlation between exposure to exogenous estrogens and the appearance of cutaneous melanoma (54; 55). However, no statistically significant association between the use of estrogens and tumor thickness was observed (53).

It is known that some cancers are estrogen dependent, such as breast cancer. The incidence rates of cutaneous melanoma in women resemble to those of breast cancer, suggesting that female sex hormones can be involved in the development of melanoma in women (56). At the same time, another study showed that women previously diagnosed with breast cancer have a higher risk of melanoma and patients with melanoma are at risk for breast cancer (57).

In addition, some studies have linked obesity with increased risk for melanoma, in the context of which the adipose tissue represents an endogenous estrogen production (54, 58).

Other reports showed that exogenous estrogens do not increase the risk for developing cutaneous melanoma (59) and various data even suggested a protective role of estrogens. First is the important advantage regarding progression of cutaneous melanoma found in the female population. Furthermore, women have a longer delay before relapse and a higher cure rate compared to men (60). A recent study pointed out that menstrual irregularity - reflecting anovulatory cycles and implying a reduced exposure to hormonal factors including endogenous estrogens, can be associated with an increased risk of lentigo maligna and lentigo maligna melanoma (61).

Conflicting results have been found by the numerous studies that have taken into consideration aspects that may indicate endogenous variations of estrogen levels, such as age at menarche, age at first

full-term pregnancy, number of pregnancies or age at natural menopause (58, 62-64). A case-control study highlighted a positive association between nodular melanoma and age at menarche ≥ 15 years, in contrast with menarche < 13 years (64). Conversely, a large French prospective analysis, stated that risk of melanoma is decreased in women with age at menarche ≥ 15 years and age at natural menopause < 48 years, suggesting an apparent protective effect induced by a shorter ovulatory life (61).

The above overview highlighted the major differences between various clinical situations linked with modified plasma estrogen levels. The following sections will present a detailed review of the main clinical research concerning the relationship between cutaneous melanoma and both exposure to exogenous estrogens (OC and HRT) and modifications of the endogenous estrogen levels, as seen for instance in pregnancy; the results of the most relevant clinical studies are summarized in Table 1.

Oral contraceptives (OCs) and risk of melanoma

Starting with the late 1970s, few cohort studies found a higher incidence of cutaneous melanoma among women using OCs compared to women who never used OCs (65), but it must be taken into account that doses of estrogens used in OC pills have declined since the 1970s. At the same time, large studies with a long-term follow-up of the patients showed that risk of developing cutaneous melanoma is higher among women using OCs, but without demonstrating a dose-effect relationship (66).

A large prospective study showed a two fold increase of melanoma risk in women who use OCs compared to women who never used OCs, with a supplementary risk in women who used OCs for over 10 years. However, the same study showed a rapid risk reduction after discontinuation of OCs, two years after cessation the treatment the risk of melanoma being similar with women who never used OCs (67). It is interesting that the same effect of OCs was observed on breast cancer.

It was assumed, that use of OCs may not be an independent risk factor for melanoma, but associated with other significant risk factors may contribute to melanoma induction. It was estimated that the risk for melanoma induced by current use of OCs is three times higher in patients with other associated risk factors and it is minimal in patients without other risk factors (67). However, other numerous studies showed opposite

findings, so it has not been still confirmed whether OCs use increase the risk of cutaneous melanoma (54, 58, 62, 64, 68).

Influence of hormone replacement therapy (HRT) on melanoma

Menopause is the permanent cessation of menstruation, stage in which the ovaries follicular reserve is exhausted and endogenous estrogen production decreases. After menopause, women are deficient in their main estrogenic hormone, 17 β -estradiol. Estrogen deprivation exposes women to risks such as osteoporosis, stroke and other neurological pathology. HRT in menopausal women requires long-term administration of synthetic estrogen. The product 17 β -estradiol has the same action on ER- α and ER- β and is present in most therapies for HRT.

The mean age of melanoma onset in women is the early fifties, which is the same with onset of menopause (69). The risk of melanoma in post-menopausal women could be influenced by exogenous administration of estrogens, particularly for women that undergo menopause at younger ages (premature menopause). Therefore, several studies tried to unravel the possible relationship between HRT use and risk of melanoma and provided conflicting results (70). Even though most of these studies identified no connection between HRT use and risk of cutaneous melanoma (53, 54, 58, 63, 64, 68), the others indicated an elevated risk (62). Conversely, data from one prospective observational study suggested that HRT may induce a survival advantage in melanoma patients (71).

Evolution and prognosis of cutaneous melanoma in pregnancy

During pregnancy, the increased levels of melanocyte-stimulating hormone and estrogens can induce cutaneous hyperpigmentation. Characteristic are brown macules and patches on skin creases (especially of the hands), nipple and buccal mucosa. A significant increase of melanocyte-stimulating hormone synthesis occurs during the first trimester and may promote cell proliferation, which can be further intensified by growth hormone activation. Pregnancy-related skin hyperpigmentation occurs because of an increased synthesis of melanin resulting from enlarged melanocytes, whose number, however, does not change (72).

Pregnancy is also associated with darkening and enlargement of cutaneous nevi, but usually these physiological changes tend to disappear after delivery

(22, 25). Also, melanocytic conjunctival lesions may change during pregnancy (73). The most common changes found in the nevi during pregnancy are structural disorganization, increased vascularization and increased pigmentation (25). Immunohistochemical evaluation of congenital nevi during pregnancy revealed a reduced expression of ER- β (25), but other research has not identified a significant difference between pregnant and non-pregnant women (74).

In addition to endogenous estrogen exposure, the level of growth hormone is also elevated during pregnancy and can stimulate the melanocyte-stimulating hormone signaling pathway, accelerating the growth of various skin lesions (63). Moreover, typical physiological immunosuppression during pregnancy may stimulate the development and the clinical expression of the preexisting melanocytic lesions (63).

Melanoma is the most common malignancy developing during pregnancy (75) with a rate almost double compared to general population (76). The risk of pregnancy-associated melanoma is even higher with increasing maternal age (68, 77). However, the impact of pregnancy on the clinical course of melanoma is also a source of controversy in the literature.

First studies have documented an accelerated course of melanoma during pregnancy and an enhanced risk of malignant transformation of nevi. In addition, it was suggested a worse prognosis for pregnant women diagnosed with melanoma, hence it was recommended the termination of pregnancy (78, 79).

Later studies highlighted that melanomas diagnosed during pregnancy are thicker and often localized on head, neck and torso, areas encumbered with a worse prognosis (77, 80, 81). Also, it has been revealed that pregnancy is associated with an increased incidence of lymphatic metastasis and a higher mortality rate (75, 80, 82, 83). It was also suggested that pregnancy may affect the course of the disease by inhibition of cellular immunity and stimulation of lymphangiogenesis (17, 82, 83).

Other research showed a more complex perspective, revealing that a single pregnancy is associated with an increased risk of melanoma, while multiparity and younger age at first birth are associated with risk reduction (54, 84). Other studies have also shown that women with multiple pregnancies (five or more live births) have a lower risk of developing melanoma as compared with nulliparous women (62-65, 84-86). At the same time, other reports have shown no effect of parity on the risk of developing cutaneous

melanoma (54, 62).

Data from more recent studies suggest that there is no correlation between present or subsequent pregnancy and development of cutaneous melanoma. Also, in women with cutaneous melanoma there is no correlation between pregnancy and a worse prognosis, more frequent metastasis or a reduced survival. Thus, there is no scientific support for the idea that patients diagnosed with melanoma should avoid future pregnancy (7, 59, 80, 85, 86). Moreover, since the risk of transplacental metastases is low, abortion in pregnant melanoma patients is no longer recommended (85). On the other hand, considering the fact that most relapses of melanoma occur in the first 2-3 years, it is a cautious approach to avoid or postpone pregnancy for at least 3 years after melanoma treatment. However, planning future pregnancies after diagnosis of melanoma must be adapted to each particular case (70, 80-83).

There is also a controversy in the literature regarding the treatment of cutaneous melanoma during pregnancy. If the primary tumor is identified, it is necessary to excise it, because minor surgery constitutes a minimal risk for the fetus. Conversely, there are major problems for a pregnant patient with metastatic melanoma, because the fetus can be threatened by major surgery or systemic treatment (81).

Considering that early diagnosis of melanoma is the best way to increase melanoma cure rates, it is essential that the family doctor or the obstetrician perform a complete clinical examination, with careful investigation of the skin all over the body, in order to identify any suspect lesions (81, 86).

In conclusion, even if the relationship between estrogens, ERs and melanoma was extensively studied, their role in the occurrence and development of this aggressive skin malignancy has not been fully clarified. Although it has been suggested a possible link between melanoma and OC or HRT use, recent data do not confirm entirely this association. Thus, the use of OC or HRT should not be forbidden in women with a history of localized cutaneous melanoma. However, it must be taken into account that use of OC or HRT may be associated with a higher risk of melanoma in patients with other associated risk factors.

Regarding the association between melanoma and pregnancy, the current opinion is that a future pregnancy is not contraindicated in women diagnosed with localized cutaneous melanoma. However, counseling women about future pregnancies should be based on prognostic factors and must be adapted to each particular case. A closer attention should be given

to each new or preexisting skin lesion during the entire pregnancy period.

Future research is required and may reveal new pathophysiological mechanisms and new therapeutic targets in cutaneous melanoma.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

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