Chapter

Hormonal Regulation of Cutaneous Melanoma: A Brief Review of In Vivo and In Vitro Studies and Its Clinical Implication

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Abstract

Skin is an endocrine organ. Skin produces various hypothalamic, pituitary, adrenal and sex steroid hormones. This raises the question whether skin cancer melanoma is a hormone dependent cancer. But, a review of in-vivo and in-vitro studies suggested that melanoma could be a hormone responsive cancer or hormone sensitive cancer. In fact, previous clinical study showed that menstruating females were better protected in melanoma than post-menopausal women and men of any age. However, the study did not show any direct effect of steroid hormone on melanoma cells. Our in-vitro study showed that progesterone, a female sex hormone significantly inhibited human melanoma (BLM) cell growth. Progesterone inhibitory effect on other melanoma cell lines was also reported by Fang et al., Moroni et al. and Kanda and Watanbe. So, it was hypothesized that progesterone could be protecting menstruating females in melanoma. Our further research showed that progesterone action was mediated by a specific suppression of pro-inflammatory cytokine IL-8. Several in-vivo and in-vitro studies showed the importance of IL-8 in the regulation of melanoma growth. Hence, IL-8 could be considered as a potential target for melanoma treatment.

Keywords: skin, steroid hormones, melanoma, in vivo and in vitro studies, progesterone, IL-8

1. Introduction

The skin is not only a target organ for sex hormones [1] but also an endocrine organ. The skin produces sex hormones, viz., androgens, estrogen, and progestins, which function locally [2, 3]. Weak androgens such as dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), and androstenedione are converted to more potent testosterone and 5-α-dihydrotestosterone in the skin [4]. In addition, the skin has all the elements of neuroendocrine axis with the expression of corticotrophin-releasing hormone (CRH), pro-opiomelanocortin (POMC), and associated peptides ACTH, α-melanocyte-stimulating hormone (MSH), β-endorphin, and corticotrophin-releasing hormone receptor-1 [5, 6]. The presence of receptor and the peptides in the same cell suggests auto-, para-, and intracrine functions of these axes. The skin has nervous and hormonal pathways not only to regulate itself but also to regulate systemic homeostasis. Imbalances in hormones affect skin texture

and cause skin diseases such as rosacea, atopic dermatitis, and psoriasis [7, 8]. Melanoma is one such fatal disorder or disease of the skin [9], which is believed to be caused by UV rays [10]. According to the Cancer Society Report, melanoma is on the rise. In 2018 alone 91,720 new cases would be diagnosed in the United States with an estimated 9000 deaths in the United States alone [11]. It has been shown that sex steroids are essential for a healthy skin. Since melanoma is a serious skin disease, the question, whether melanoma is a hormone dependent cancer or not is relevant here. Literature survey showed possible dependence of melanoma on endocrine influences [12–14]. Several in vivo and in vitro studies showed the involvement of steroids in the regulation of melanoma growth.

2. Brief review of in vivo studies

2.1 Animal studies

Animal studies showed the involvement of sex steroid hormones in the regulation of melanoma growth, and there were also differences in the regulation of growth between male and female mice:

a. In one study, estrogen receptor-positive human melanoma cells grew more slowly in female than in male mice [15].

b. Female survival benefit with metastatic melanoma was observed, when melanoma cells produced liver metastases preferentially in male compared to female mice [16].

c. In another study, dihydrotestosterone was shown to stimulate proliferation, whereas anti-androgen receptor hydroxyflutamide [17] showed anticancer action in a male mouse transplanted with melanoma.

In the following two studies, it was shown that male mice were more prone to cancer than female mice:

d. When induced with carcinogen [18].

e. When exposed to UV-B [19].

2.2 Clinical studies

Overall survival outcome for young women (45 years of age and under) was far superior to older women (55 years of age and older) and men of any age group [20]. A 22% survival advantage and 17% 5 year disease-free interval advantage were observed in females [21]. In addition, women were found to survive longer than men after the development of stage III disease [22]. Clinical studies also suggested the involvement of hormones in the regulation of melanoma growth. So, clinical studies underlined the involvement of female sex steroid hormones in protecting menstruating females in melanoma. But, these clinical studies did not identify the exact female hormone involved in the protection. In addition, there was no statistically significant difference observed in the survival rates between controls and women diagnosed with melanoma stage I or stage II during pregnancy [23–25]. Data also showed no correlation between melanoma and oral contraceptives

[26, 27]. Available data suggested no connection between exogenous hormones and the risk for malignant melanoma [28, 29].

3. Brief review of in vitro studies

The following in vitro studies showed inhibitory effect of steroid hormones on a variety of melanoma cell lines, suggesting melanoma could be a hormone-sensitive cancer:

a. 2-Methoxyestradiol (2-ME), an estrogenic metabolite, inhibited all tested melanoma cell line growth, without affecting the growth of non-tumorigenic cells [30].

b. Kanda and Watanbe showed that 17-β-estradiol, progesterone, and dihydrotestosterone inhibited melanoma cell growth in a receptor-dependent manner by suppressing IL-8 transcription [31].

c. Amelanotic strain cells grew faster in vivo in female hamsters [32], whereas testosterone inhibited the cell growth in vitro.

d. Glucocorticoids also showed their effect on melanoma cell growth in a receptor-dependent manner [33].

e. Another in vitro study showed that melatonin at physiological concentrations (1 nM to 10 pM) inhibited metastatic mouse melanoma (B16BL6) cell growth [34].

4. In vitro studies from our lab

Our lab in vitro studies showed involvement of progesterone in the regulation of mouse and human melanoma cell growth.

4.1 Dose-response studies of progesterone with mouse (B16F10) and human melanoma (BLM) cell line

Initially four sex steroids, viz., dehydroepiandrosterone (DHEA), androstenedione (AD), testosterone (T), and progesterone (P4), were checked for their effect on mouse melanoma (B16F10) cell growth [35]. Though all four steroids showed a dose-dependent effect, progesterone showed a significant effect on the inhibition of mouse melanoma cell growth (**Figure 1**). As the initial study was carried out at high concentrations (100, 150, and 200 μM), dose-response study was carried out to rule out toxic effect of high concentrations of steroids on melanoma cell growth inhibition. Mouse (B16F10) and human melanoma (BLM) cells showed a dose-dependent cell growth inhibition [35, 36], suggesting the inhibition was not due to toxic effect at high concentration of steroids (**Figure 1**).

4.2 Mechanism of inhibition of human melanoma (BLM) cell growth

After having ruled out necrosis and apoptosis as the cause of cell growth inhibition, it was found out that autophagy was the mechanism of cell growth inhibition (**Figure 2**), using a known inducer of autophagy (spermidine) in a control experiment [36].

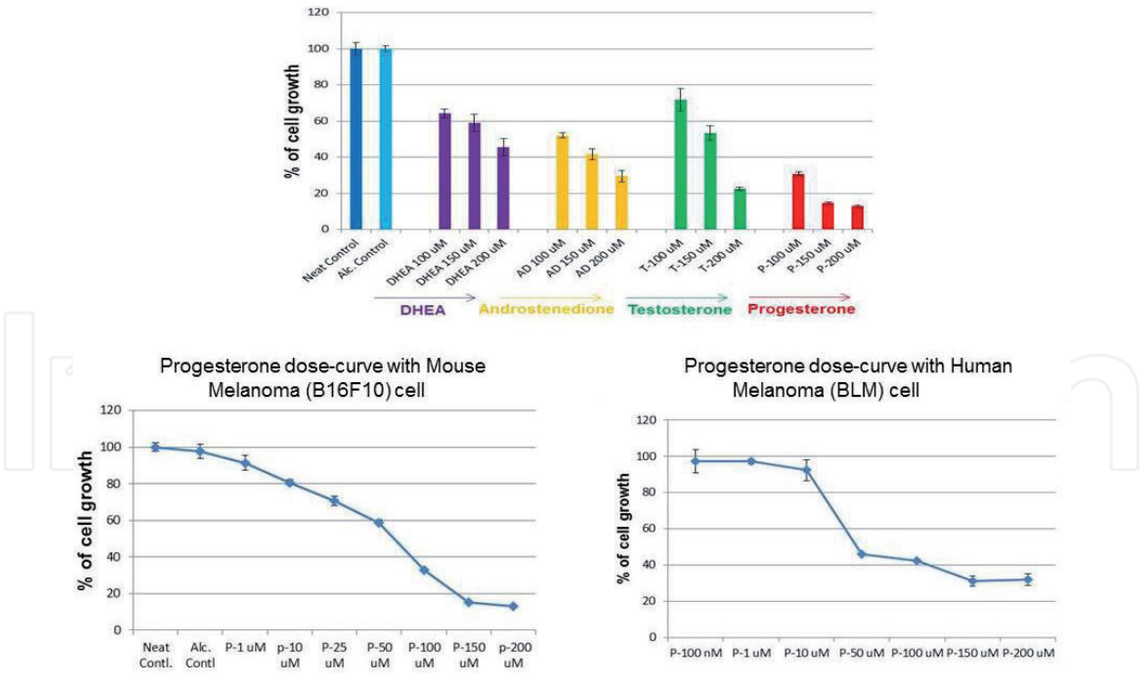

Figure 1.
Dose-response studies of progesterone with mouse (B16F10) and human melanoma (BLM) cell lines. Initially dehydroepiandrosterone, androstenedione, testosterone, and progesterone were tested for their effects on mouse melanoma (B16F10) cell growth by MTT assay. Progesterone, a female sex hormone, showed significant inhibition at 150 and 200 μM concentrations. As steroids were tested initially at high concentrations (100, 150, and 200 μM), dose-response study was carried out to rule out toxic effect of steroid at high concentrations. Dose-response studies of mouse (B16F10) and human melanoma (BLM) cell lines showed a sigmoidal dose-response curve, ruling out toxic effect of steroids due to high concentrations.

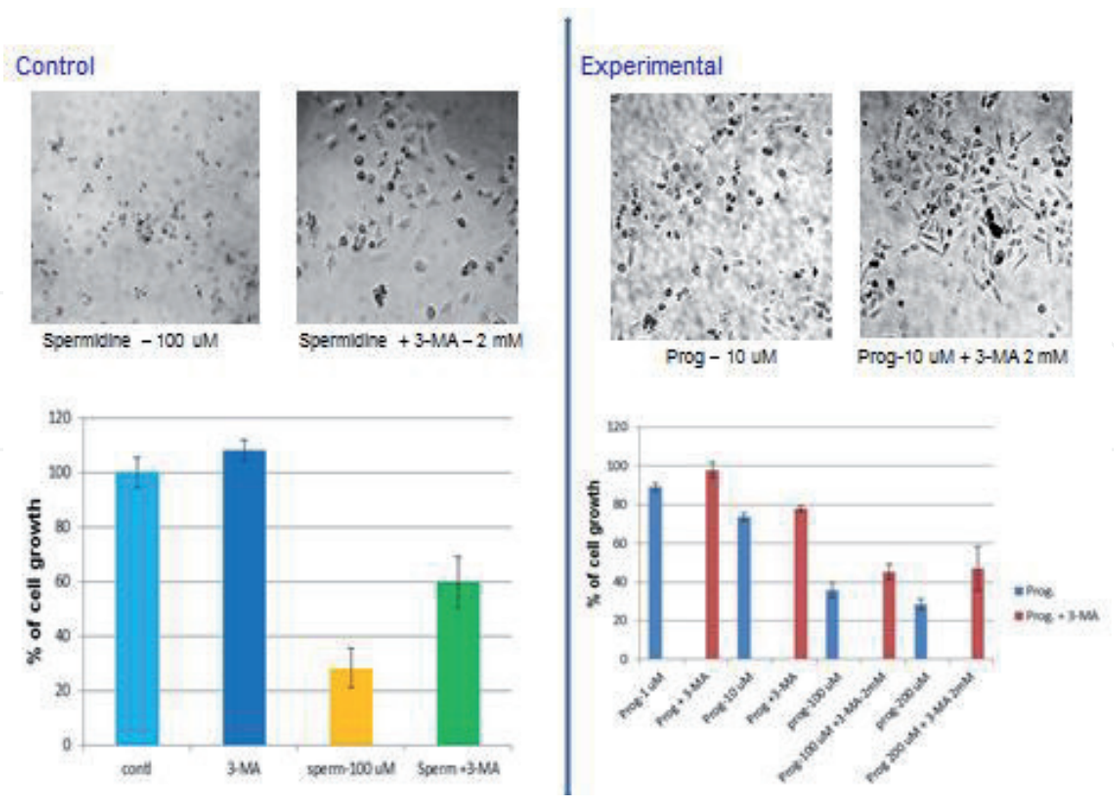

Figure 2.
Mechanism of cell death by progesterone. After having ruled out necrosis and apoptosis, autophagy was checked by adding 3-methyladenine (3-MA). Control experiment with 100 μM of spermidine-induced autophagy was partially rescued by the addition of 2 mM of 3-methyladenine (as 3-MA inhibited the assembly of autophagosome formation [37, 38]). Similar partial rescue of cell growth was observed at various concentrations of progesterone, suggesting the mechanism of inhibition of cell growth was due to autophagy.

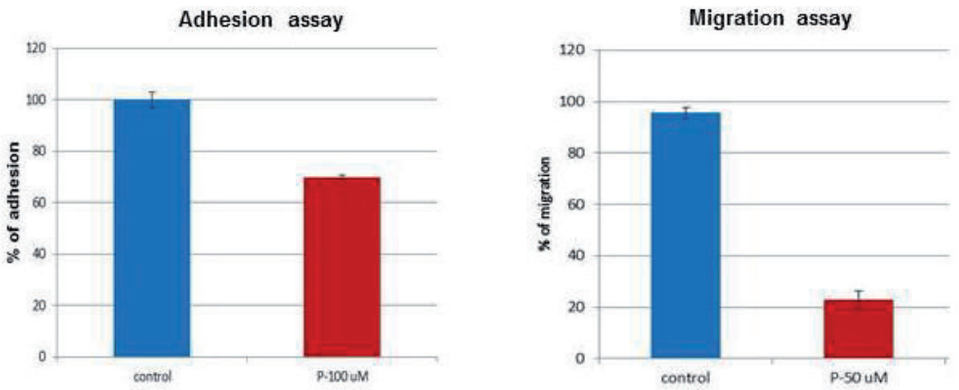

Figure 3.
In vitro adhesion and migration functions of human melanoma cell line. Human melanoma cells were treated with progesterone at 100 μM for 48 h in petri dish. After 48 h, both control and progesterone-treated cells were harvested, and adhesion assay was carried out as per the protocol in Ref. [28]. For migration assay, control and progesterone (50 μM)-treated cells were harvested after 48 h of treatment. Adhesion experiment showed partial inhibition of adhesion in progesterone-treated cells compared to untreated control cells. Similarly, progesterone-treated cells showed a significant decrease in migration function in progesterone-treated cells compared to untreated control cells.

4.3 Effect of progesterone on adhesion and migration functions of human melanoma cells

Effects on adhesion and migration functions were checked after 48 h incubation of human melanoma cells with progesterone. Progesterone at 100 μM concentration partially inhibited adhesion function (**Figure 3**). Similarly, progesterone (50 μM) treatment significantly decreased migration function of human melanoma cells (**Figure 3**). This study indicated that progesterone treatment decreased adhesion and migration functions [39] which were essential for metastasis of melanoma.

5. In vitro studies from other labs

In addition, in vitro inhibition of melanoma cell growth by progesterone was also shown by other labs:

a. Fang et al. showed inhibition of human melanoma cell lines (A375, A875) by progesterone and RU-486, which were not mediated through progesterone receptor [40].

b. Moroni et al. repeated the studies with A375 cell line and used progesterone concentration up to 1000 μM, which also showed inhibition of human melanoma cell growth [41].

c. Kanda and Watanbe used progesterone along with dihydrotestosterone and estrogen and showed that all the three steroids inhibited human melanoma cell growth by decreasing IL-8 transcription [31].

6. Biochemical basis of progesterone action

Further research [42] involving ELISArray of supernatants of the cells treated with progesterone along with untreated control cells showed that progesterone action was mediated by a specific suppression pro-inflammatory cytokine IL8 (**Figure 4**).

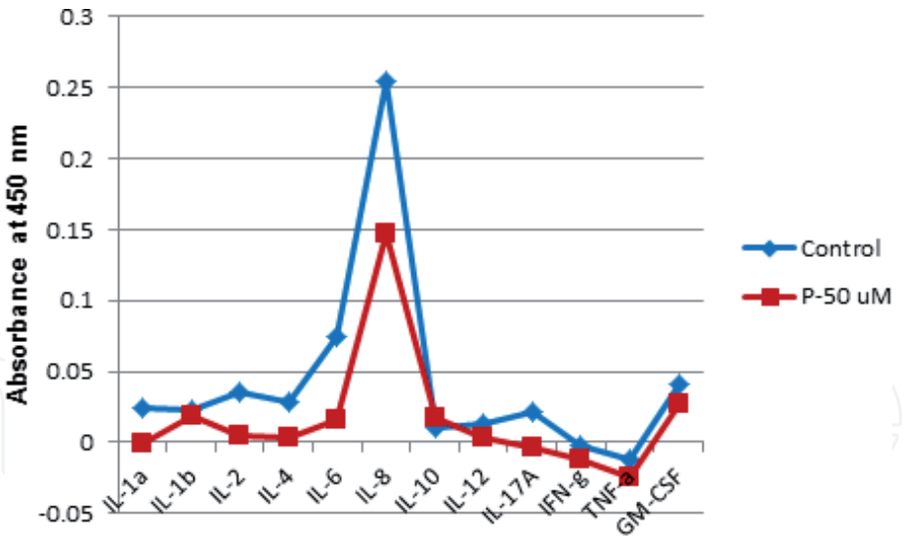

Figure 4.
Biochemical basis of progesterone action. An ELISArray, containing pro- and anti-inflammatory cytokine antibodies coated in different wells, showed a specific suppression of IL-8 cytokine alone in the supernatant of cells treated with progesterone (50 μM) compared to untreated control cell supernatant.

6.1 Involvement of IL-8 in melanoma growth

In vivo and in vitro studies from other labs showed the involvement of IL-8 in melanoma growth:

1. IL-8 cytokine produced in vitro was an essential autocrine growth factor for melanoma cells [43].

2. Expression of IL-8 in human melanoma cells upregulated the activity of matrix metalloproteinase (MMP) and increased tumor growth and metastasis [44].

3. Expression of IL-8 correlated with metastatic potential of human melanoma cell in nude mouse [45].

7. Summary

In vivo and in vitro studies showed the inhibition of melanoma growth by various hormones. This inhibition of cell growth by various hormones suggested that melanoma could be a hormone-responsive cancer, where hormones were essential for survival in melanoma. This was supported by the clinical studies carried out in the 1950s and 1960s. One clinical study reported that menstruating females were better protected in melanoma than postmenopausal women and men of any age [20]. But, the study did not correlate with steroid status of females. Literature showed that progesterone level peaked in menstruating females between 1000 and 1500 ng/dl, whereas progesterone level ranged between 20 and 100 ng/dl in postmenopausal women [46]. Our research also showed that progesterone inhibited human melanoma (BLM) cell growth in vitro significantly. In addition, progesterone inhibitory action was also shown by Fang et al., Moroni et al., and Kanda and Watanbe. So, it was hypothesized that progesterone could be protecting menstruating females. Recently, it was shown that the protective function of progesterone was mediated by a specific suppression of pro-inflammatory cytokine IL-8. Various in vitro and in vivo studies already showed the importance of IL-8 in melanoma cell growth.

8. Conclusion

Several studies showed the involvement of progesterone in the regulation of in vitro melanoma cell growth and also in the regulation of in vivo melanoma growth. Further in vitro research showed that the progesterone inhibitory action was mediated by a specific suppression of pro-inflammatory cytokine IL-8. The connection between IL-8 and melanoma growth was already established by other investigators. This brought IL-8 into focus in melanoma and suggested that IL-8 could be considered as a potential target for melanoma treatment.

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