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Is Melanoma a Hormone-Dependent Cancer or a Hormone-Responsive Cancer?

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<http://dx.doi.org/10.5772/intechopen.76499>

Abstract

Melanoma, a potentially fatal form of skin cancer is on the rise. This review not only underlines the close connection between skin and endocrine system, but also lists evidences from multiple sources epidemiological, clinical, previous in vivo and in vitro studies regarding the involvement of sex steroids in melanoma. Incidentally, clinical studies underscored the involvement of sex steroids in the protective function in melanoma in menstruating females. But, none of these studies identified the sex steroids involved in the protective function in melanoma in menstruating females. The sex steroid involved in this innate protection in melanoma in menstruating females has not been investigated by scientists, though advances have been made in immunotherapy with accompanying side effects. In this context, our in vitro studies on mouse and human melanoma cell lines, along with literature survey, pointed to progesterone as the possible female sex steroid involved in the protective function in melanoma. Based on our findings and previous studies, it is concluded in this review that melanoma is not a hormone-dependent cancer. But, it may be a hormone-sensitive or responsive cancer, as hormones (sex steroids) inhibited melanoma cell proliferation in vitro. This new understanding will help in developing new therapy or target for melanoma treatment.

Keywords: melanoma, epidemiological studies, clinical studies, in vivo and in vitro studies, protective function, progesterone, hormone-responsive cancer

1. Introduction

The question that has been raised over the years, whether melanoma is a hormone-dependent cancer or not still lacks a clear-cut answer [1–4]. However, in this review, an attempt has been made to collect evidences from multiple sources to point out the nature of melanoma. Melanoma, the fatal form of skin cancer accounts for less than 2% of skin cancer, but it is

responsible for 75% of deaths due to skin cancer [5]. According to the American Cancer Society reports known as Cancer Statistics, melanoma is on the rise. In 2018, in the United States, 91,270 new cases will be diagnosed with an estimated 9320 deaths [6]. Melanoma occurs mostly on the skin [7]; however, some rare forms of melanoma can occur in other areas such as cornea, uvea, and gastrointestinal tract [7]. Epidemiological data indicated an increased mortality rates in males than in females [8], suggesting a sex difference. Clinical studies showed that menstruating females were better protected (delayed metastasis and increased survival) in melanoma than postmenopausal women and men of any age [9], clearly indicating the role of sex steroid hormones in the protection function. It is important to point out that skin itself functions as an endocrine organ [10], even though it is not acknowledged as one. Skin possesses many of the enzymes necessary for synthesis of steroid hormones [11]. In fact, most of the peripheral conversions of dehydroepiandrosterone (DHEA) and androstenedione (AD) to testosterone and estradiol take place in the skin [12]. This area of endocrinology is known as intracrinology [12]. In addition, skin is also a target organ for various hormones. Sex steroids such as androgens, estrogens, and progestins are essential for a healthy skin [13]. Melanocyte, which is transformed to melanoma cell is also under the influence of melanocyte-stimulating hormone (MSH) from pituitary [14]. Hence, it is natural to ask the question whether melanoma is a hormone-dependent cancer like breast, prostate, and endometrial cancers. Generally, melanoma is not labeled as a hormone-dependent cancer because of the belief that UV rays from the Sun is the major cause for melanoma [15]. UV rays cause DNA damages and other inflammatory changes in the skin, which result in skin cancer. About 90% of melanoma is caused by environmental factors such as UV rays, radiations, and only 10% is inherited in the family. So, melanoma is never considered as a hormone-dependent cancer. However, existing evidences point to a hormone relatedness or a hormone-responsive nature of melanoma cancer:

1. Evidences of relationship between skin and endocrine system: there is a close connection between skin and endocrine system, as shown by the following examples.
 - a. All the components of a functional hypothalamo-pituitary-adrenal axis analog are present in the skin.
 - b. Presence of enzymes involved in steroid hormone synthesis in skin cells: the level of local steroid production depends on the expression of androgen- and estrogen-synthesizing enzymes present in specific cell types. Five major enzymes are involved in the activation and deactivation of androgens in the skin [13].
 - c. Actions of sex steroid hormones on skin: skin is a target organ for sex steroid hormones. Androgens are essential for differentiation and growth of Sebocyte and hair growth. Estrogen is responsible for skin pigmentation and skin cancer. Progesterone functions, though not clear is essential for treating acne [13].
 - d. Endocrine disorders manifested on the skin:
 - i. Association of insulin resistance and metabolic syndrome with acne: post-adolescent male patients with acne more commonly have insulin resistance [16]. This resistance may be a stage of prediabetes and the patients may develop hyperinsulinemia or type 2 diabetes in the future.

- ii. Association of cutaneous findings and systemic abnormalities in women suspected of having polycystic ovary syndrome (PCOS): Hirsutism and acne are the most reliable cutaneous markers of PCOS and require a comprehensive skin examination to diagnose [17].
- iii. Psoriasis severity may influence type 2 diabetes risk: people living with psoriasis are not only at higher risk of type 2 diabetes, but their risk also rises in line with the skin disease's severity [18].

It is evident from the above mentioned examples that skin is not only an endocrine organ which produces various hormones, but also has a close relationship with systemic endocrine system.

2. Evidences from epidemiological data

According to the epidemiological SEER data [8] known as Surveillance, Epidemiology and End Results program, a database maintained by NCI, there has been an increase in the incidence of melanoma. The incidence of melanoma ('03-'07) for men and women were 26.7 per 100,000 and 16.7 per 100,000 respectively. There has been an increase in death rate also. Even in the death rate, there was a difference between males and females. The mortality rate for males was 4.0 per 100,000, whereas for females it was 1.7 per 100,000. Males have increased mortality rate than females. Death rate was cut more than half in females. Similarly, malignant melanoma database (1971–2012) maintained by UK Cancer Research Council [19] also showed that the mortality rate was higher in males than in females over the years. The data [20] was almost similar from Australian continent, where the incidence of melanoma is the highest in the world. From 1982 to 2016, the number of melanoma diagnosed in Australia increased from 3526 to an estimated 13,280. The age-standardized incidence rate increased for both males and females, from 28 to 60 cases per 100,000 males, and from 26 to 39 cases per 100,000 females. Data showed males were more prone to melanoma than females [20]. Thus epidemiological data from three continents clearly showed that males were more affected by melanoma than females. These gender differences in melanoma demand an investigation of the effect of sex hormones on this malignancy.

3. Evidences from clinical studies

Clinical studies supported the epidemiological findings. Clinical studies showed that menstruating females were better protected (delayed metastasis and increased survival) in melanoma than post-menopausal women and men of any ages [9]. This very difference between menstruating females and postmenopausal women clearly indicated the involvement of steroid hormones in protecting menstruating females in melanoma. However, these data base were not correlated with the steroid status of females. Studies published between 1977 and 1966 showed women had better survival in all but 4 out of 22 epidemiologic studies [21]. Two female hormones could be involved in rendering protection, namely estrogen and progesterone. First, estrogen as the hormone protecting menstruating females in melanoma: estrogen receptor antagonist tamoxifen was evaluated as a single agent in 12 studies covering 213

patients with metastatic melanoma cancer [22]; the response rate was only 7%. Moreover, estrogen receptors were found in some cancers only by biochemical and histochemical tests but not by the immunohistochemical tests using monoclonal antibodies [23]. Second, progesterone as the possible female sex hormone involved in the protection: there were only limited in vitro studies [24, 25] and they were also not tied to the protective function in melanoma. According to the data published on pregnancy and melanoma, several studies reported statistically no significant differences in survival rates between controls (non-pregnant women with malignant melanoma) and women diagnosed with melanoma stage I or II during pregnancy [26–28]. Studies also found no association between melanoma and oral contraceptives [29, 30]. Data on the relationship between melanoma and hormone replacement therapy were meager and it seemed that exogenous hormones did not influence the risk for malignant melanoma [31, 32]. 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.

4. Evidences from animal studies

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

- a. Female survival benefit with metastatic melanoma was observed, when melanoma cells produced liver metastases preferentially in male compared to female mice [33].
- b. In another study, estrogen receptor-positive human melanomas cells grew more slowly in females than in males mice [34].
- c. Similarly, dihydrotestosterone was shown to stimulate proliferation. But, in a follow-up study, male mice transplanted with melanoma showed increased survival after treatment with anti-androgen receptor hydroxyflutamide [35].
- d. Male mice were significantly more susceptible to carcinogen-induced skin cancer than female mice [36].
- e. Similarly male mice were more susceptible to UV-B induced skin carcinogenesis than female mice [37].
- f. Research work presented in one study showed that metapristone (a metabolite of mifepristone (RU-486)) had a remarkable effect of preventing cancer metastasis of B16-F10 cells in vivo compared with mifepristone [38].

5. Evidences from previous cell-culture studies

Apart from epidemiological, clinical, and in vivo animal studies, various in vitro studies using a variety of melanoma cell lines showed the inhibitory effect of steroid hormones on melanoma cell growth, suggesting melanoma could be a hormone-sensitive or responsive cancer.

- a. In one study, 2-methoxyestradiol (2-ME), an estrogenic metabolite inhibited the growth of all melanoma cells tested, without inhibiting the growth of non-tumorigenic cells [39].
- b. Data from another study suggested that 17- β -estradiol, progesterone, and dihydrotestosterone suppressed the growth of melanoma cells by inhibiting interleukin-8 production in a receptor-dependent manner [25].
- c. However, Feucht et al. investigated three human melanoma cell lines and found no effect either by estradiol or tamoxifen on melanoma cell growth in vitro [34].
- d. Another in vitro study indicated a direct inhibitory effect of testosterone on growth of an amelanotic strain which in vivo grew faster in female hamsters [40].
- e. The findings in another study indicated that glucocorticoids exerted some influence on the growth of human melanoma cells and this effect was mediated through glucocorticoid receptor [41].
- f. Only study which showed a stimulatory effect was with melanocyte, where α -MSH stimulated melanocyte proliferation in a dose-dependent manner, but its stimulatory effect required bFGF and/or the activation of protein kinase C [42].
- g. Another in vitro study showed that melatonin at physiological concentrations (1 nM to 10 pM) inhibited metastatic mouse melanoma (B16BL6) cell growth [43].

6. Evidences from our studies on mouse melanoma (B16F10) cell line

- a. Progesterone effect on mouse melanoma (B16F10) cell growth: based on previous research work and literature survey, initially four sex steroids were checked for their effect on mouse melanoma (B16F10) cell growth [44]. Of the four steroids checked [dehydroepiandrosterone (DHEA), androstenedione (AD), testosterone (T) and progesterone (P)], progesterone showed significant inhibition (87%) of mouse melanoma cell growth. Though other steroid hormones also showed inhibition of cell growth, but it was not as significant as that of progesterone inhibition (**Figure 1**).
- b. Progesterone dose-response study with mouse melanoma cells: as the initial experiment was carried out at high concentrations (100, 150, 200 μ M) of hormones, a follow-up dose-response study was carried out with progesterone alone. Dose-curve study with progesterone showed a dose-dependent decrease in mouse melanoma cell growth (**Figure 2**).
- c. Further studies with mouse melanoma cell line: further studies (data not shown) showed that the effect of progesterone on mouse melanoma cells was not a toxic, not a spurious or not a non-specific effect [44]. The only other steroid which showed a significant inhibition of mouse melanoma cell growth was progesterone-receptor antagonist RU-486, a synthetic steroid (**Figure 3**).
- d. Mechanism of progesterone action on mouse melanoma cell line: since RU-486 also showed a dose-dependent inhibition of mouse melanoma cell growth, it was decided to find out whether the actions of progesterone and RU-486 were mediated through progesterone

receptor. A co-incubation study was carried out with fixed concentration of progesterone (50 μM) and varying concentrations of RU-486 (10, 50, 100 μM). Co-incubation study showed an additive effect (data not shown) on mouse melanoma cell growth suggesting that the action was not mediated through progesterone receptor [44].

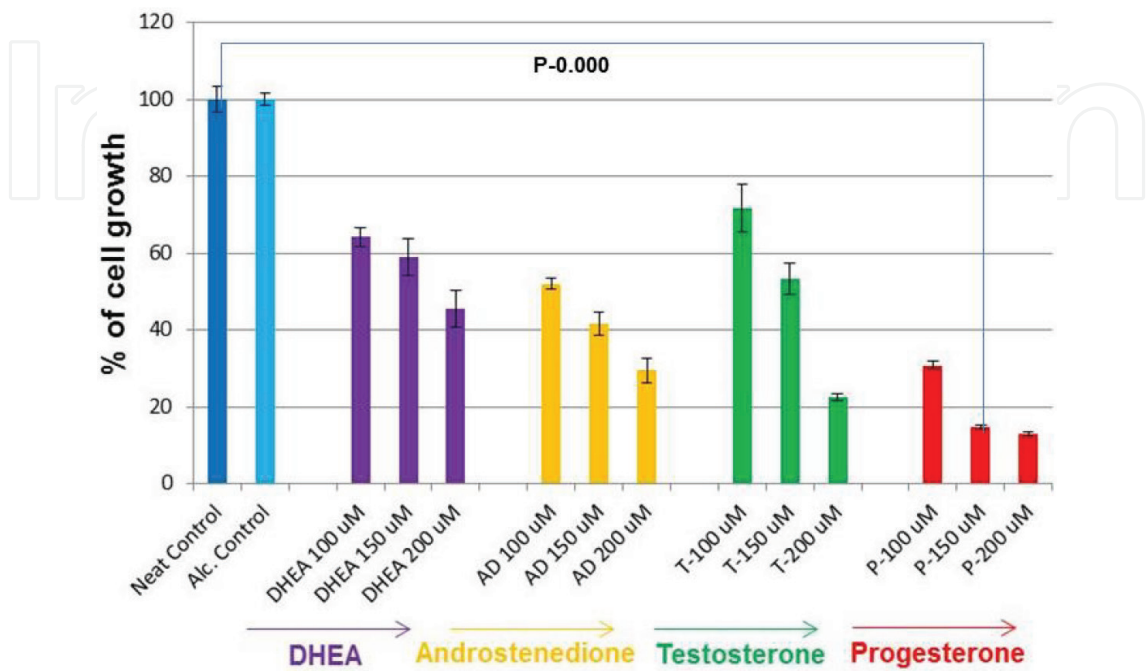


Figure 1. Various steroid hormones effect on mouse melanoma (B16F10) cell growth: three androgens (DHEA, AD, T) and one female sex steroid hormone (P) were checked for their effect on mouse melanoma cell growth at 100, 150, and 200 μM concentrations. Cells were incubated with the hormones separately for 48 h. After 48 h, cell growth was assessed by MTT assay. All the steroids checked showed dose-dependent decrease in cell growth. But, progesterone showed a significant inhibition of cell growth (87%) at 200 μM concentration.

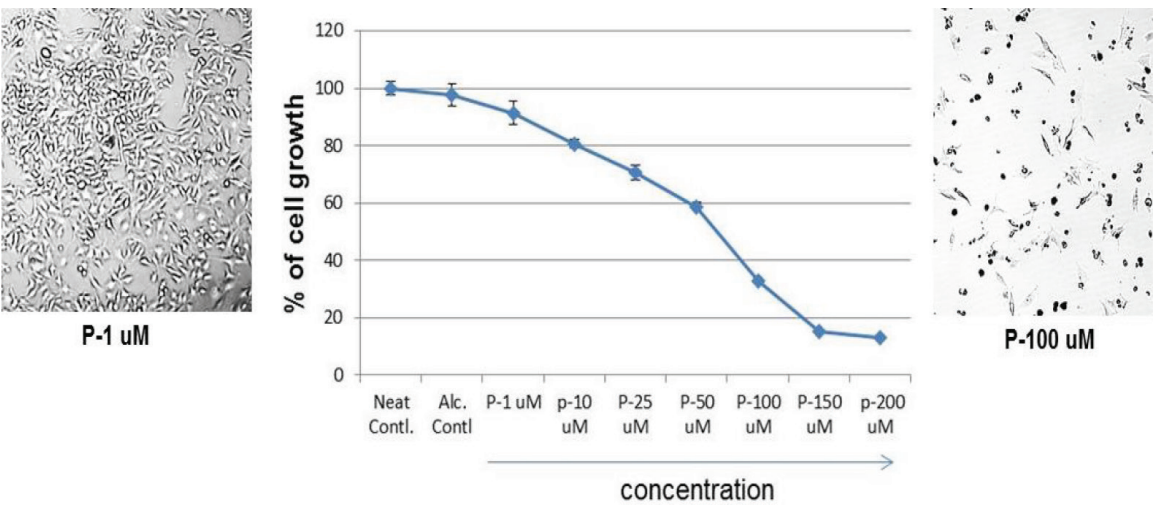


Figure 2. Dose-response study with mouse melanoma cell line: since the initial study was carried out at high concentrations, a dose-response study was carried out with progesterone starting from 1 to 200 μM . Progesterone showed a dose-dependent decrease in mouse melanoma cell growth with significant inhibition at 200 μM concentration. Cell growth was monitored by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

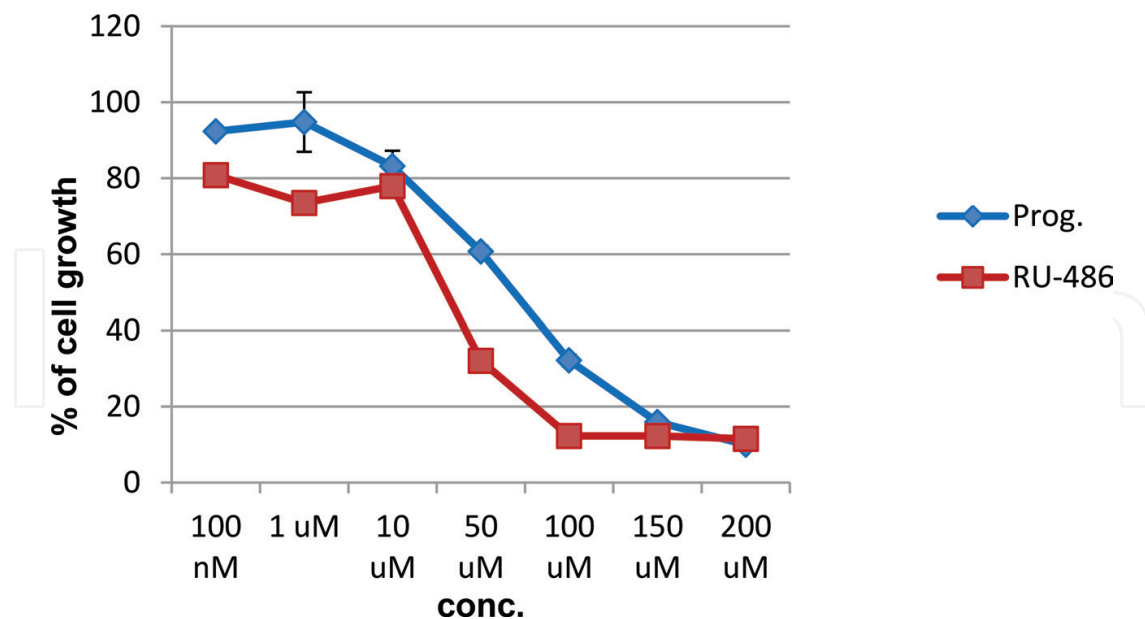


Figure 3. Comparison of dose-response curves of progesterone and RU-486: both progesterone and RU-486 were treated separately for 48 h. After 48 h, cell growth was assessed by MTT assay. Dose-response curve of RU-486 was compared with that of progesterone. The dose-response curves were very similar to each other.

7. Evidences from our studies on human melanoma (BLM) cell line

- a. Dose-response study with progesterone and RU-486 on human melanoma cell line: the sex steroids (progesterone and RU-486), which showed inhibition on mouse cell line were checked on human melanoma (BLM) cell line for their effect [45, 46]. Progesterone and RU-486 also showed a dose-dependent inhibition of human melanoma cell growth (**Figure 4**).

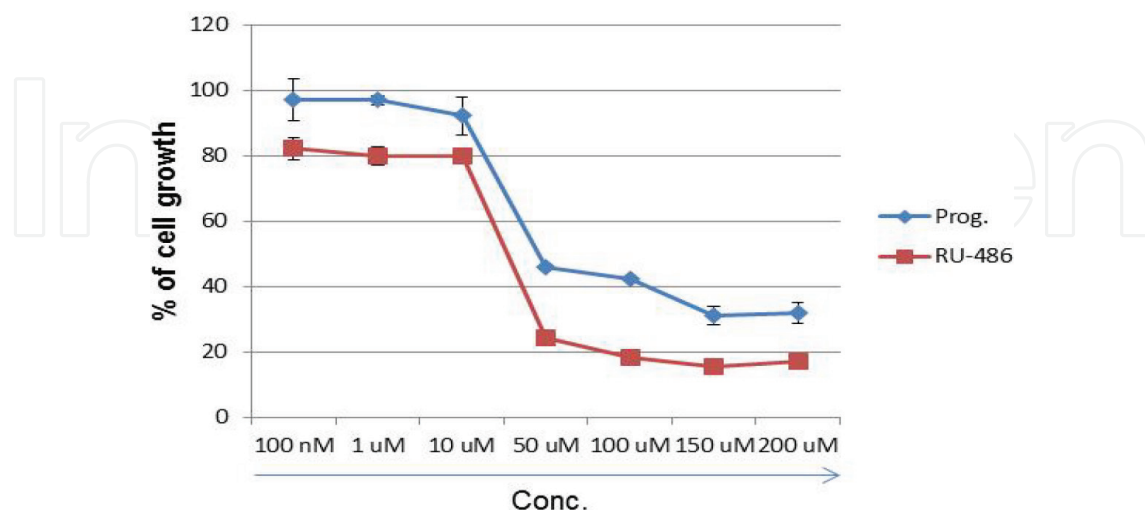


Figure 4. Dose-response study of progesterone and RU-486 on human melanoma cell line: progesterone and RU-486 were incubated separately with human melanoma cells for 48 h. After 48 h of incubation, cell growth was measured by MTT assay. Dose-response study of both progesterone and RU-486 showed a dose-dependent decrease of human melanoma cell growth.

- b. Mechanism of action of progesterone on human melanoma cells: since progesterone and RU-486 separately showed a dose-dependent inhibition of human melanoma cell growth, it was decided to find out whether these actions were mediated through progesterone receptor on human melanoma cell line. Therefore, co-incubation study, just like the one on mouse melanoma cell line was carried out. Co-incubation study showed that the effect was not mediated through progesterone receptor (**Figure 5**). In fact, co-incubation of the two steroids (progesterone and RU-486) showed an additive effect on cell growth inhibition, suggesting the actions were mediated through two different mechanisms.
- c. Mechanism of inhibition of human melanoma cell growth by progesterone: since the co-incubation study suggested that the mechanism of action of progesterone and RU-486 could be different, it was decided to find out the mechanism of inhibition of human melanoma cell growth by progesterone. After having ruled out necrosis and apoptosis as the mechanism of inhibition of cell growth, it was found out that autophagy was the cause for cell growth inhibition by co-incubating progesterone and 3-methyl adenine (3-MA) on melanoma cells. 3-methyl adenine (3-MA) had been used in various studies to check or

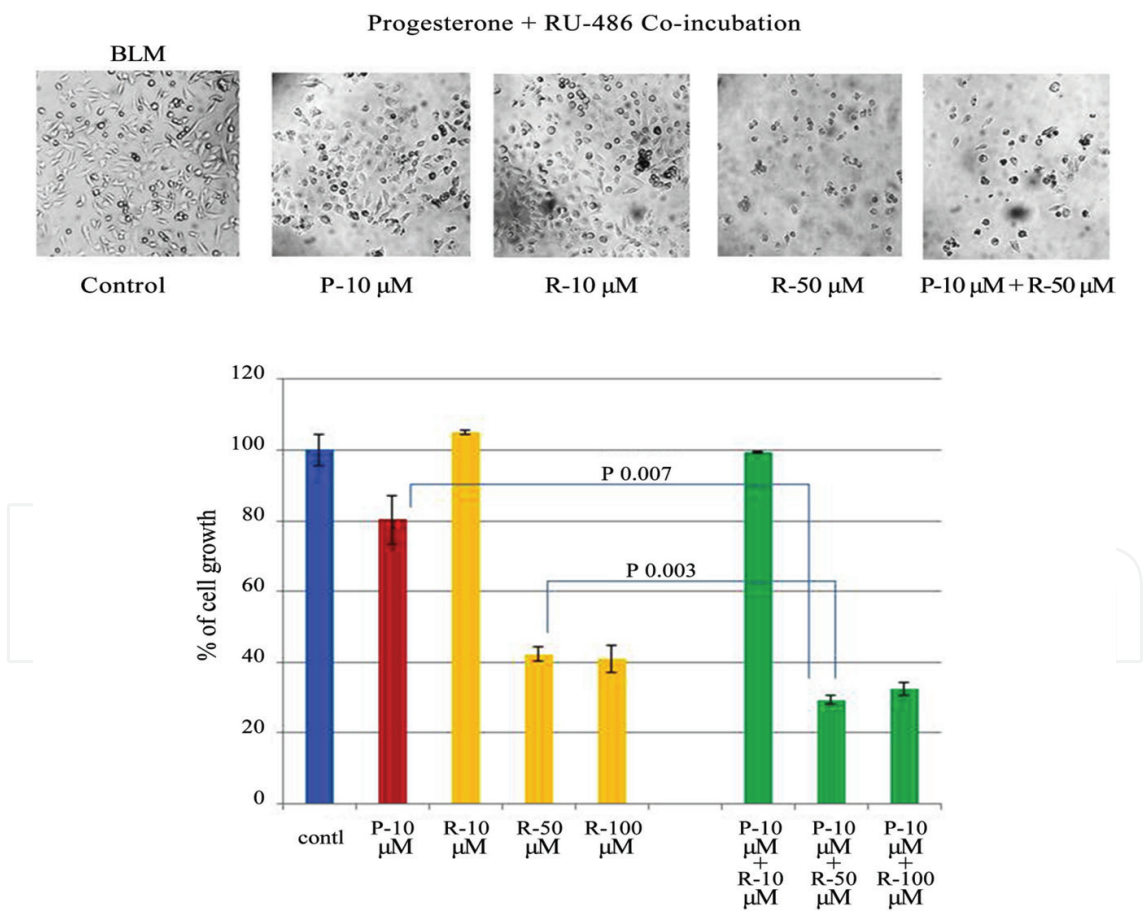


Figure 5. Co-incubation of progesterone and RU-486: a fixed concentration of progesterone (10 μ M) was co-incubated with varying concentrations of RU-486 (10, 50, 100 μ M). Co-incubated cells showed an additive effect on cell growth inhibition, suggesting the action was mediated through different mechanisms and not through progesterone receptor.

inhibit autophagy [47–49]. Therefore, the mechanism of inhibition of human melanoma cell growth by progesterone was due to autophagy (**Figure 6**).

- d. Suppression of adhesion and migration functions of human melanoma cells by progesterone: metastasis of cancer involves adhesion, migration, and invasion functions. Progesterone ability to suppress metastasis was checked by in vitro adhesion and migration assays after treatment with progesterone for 48 h [50]. Progesterone at 100- μ M concentration decreased adhesion function to 71% compared to untreated control cells at 100%. Similarly progesterone at 50 μ M significantly decreased migration to 20% compared to untreated control cells at 100%. Adhesion and migration assays suggested that progesterone could be playing a role in delayed metastasis, as reported in clinical studies [9] (**Figure 7**).

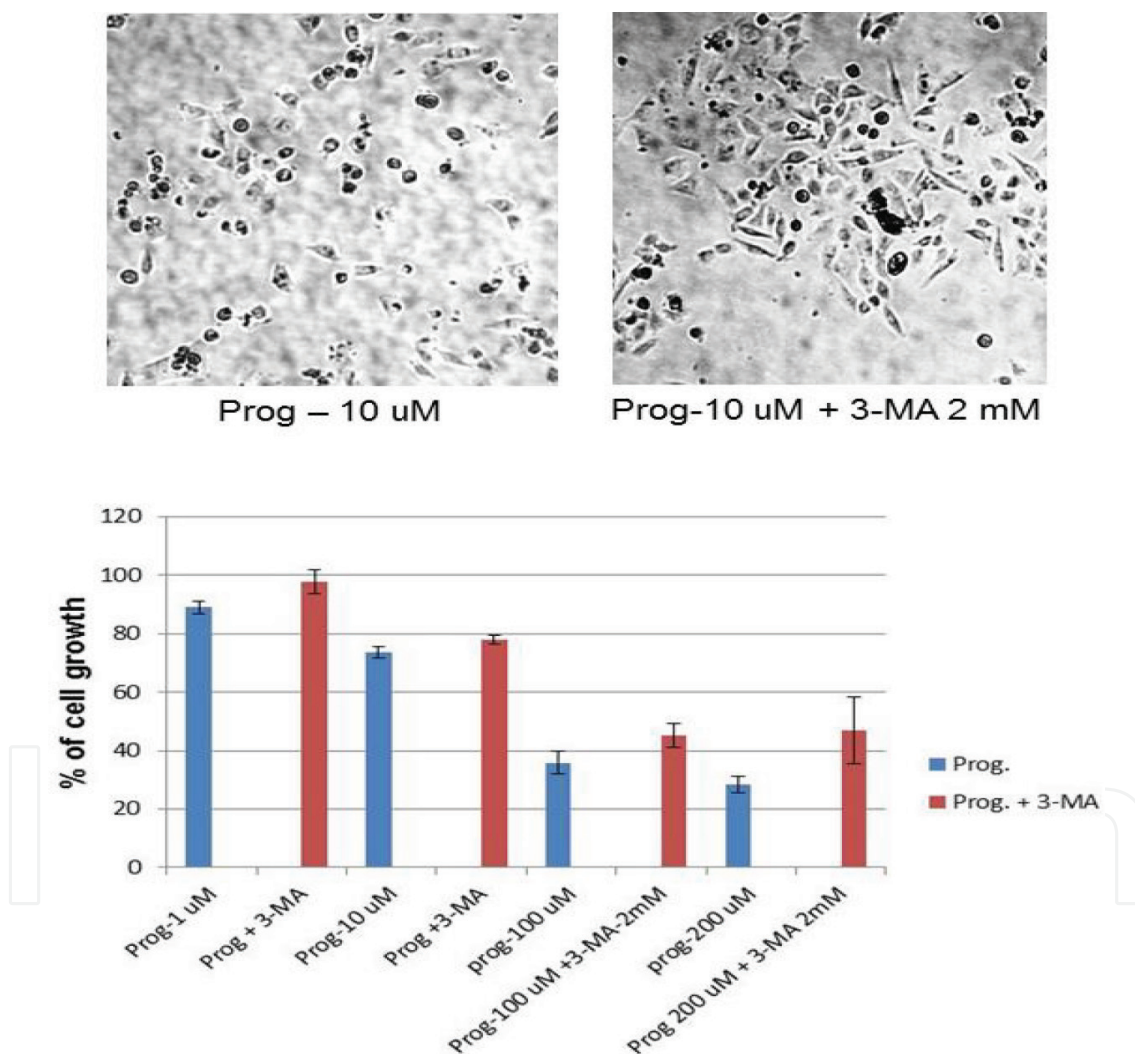


Figure 6. Mechanism of human melanoma (BLM) cell growth inhibition by progesterone: after having ruled out necrosis and apoptosis as mechanism of inhibition, it was decided to find out whether autophagy was the mechanism of inhibition of cell growth. So, cells were co-incubated with progesterone and 3-MA (2 mM) for 48 h. After 48 h, cell growth was monitored by MTT assay. 3-methyl adenine (3-MA) partially rescued melanoma cell growth, showing a slight increase in co-incubated cell growth compared to progesterone alone treated cell growth. 3-methyl adenine (3-MA) had been shown to disrupt the formation of autophagosome/lysosomal degradation in various studies [47–49].

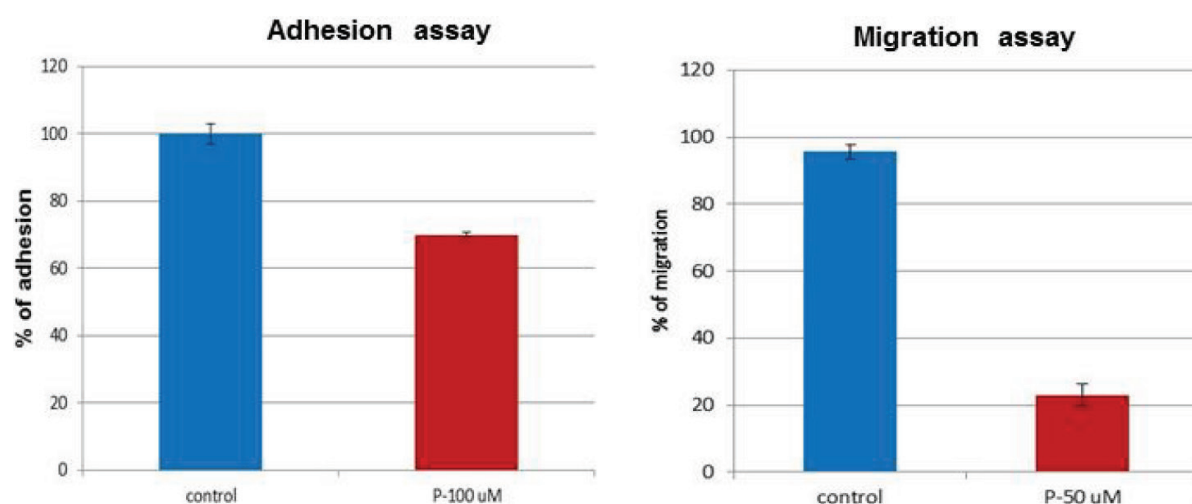


Figure 7. Suppression of adhesion and migration functions by progesterone: human melanoma cells were treated with progesterone for 48 h in petri dishes. After 48 h, cells were harvested from control and progesterone treated cells. Adhesion assay was carried out in a 96 well plate with 30,000 cells/wells. Cells were incubated for 60 min and washed. Cells attached to the plate were fixed with 2% paraformaldehyde and stained with 0.2% crystal violet dye. Purple color dye was eluted with isopropanol and assayed at 570 nm in a plate reader. For migration assay, treated cells were placed in a 24 well plate and allowed to become confluent. Cells were scratched in the middle of the plate with a tip, which was considered as 0 time point and cells were allowed to incubate for 24 h. After 24 h, percentage of cells migrated to the cleared space was calculated with a software.

8. Summary

There is a close connection between skin and endocrine system, as shown by the neuroendocrine properties of skin. Skin not only functions as an endocrine organ but also as a target organ for various hormones. Epidemiological studies highlighted the differences in mortality rate between males and females and hinted the involvement of hormones in melanoma. Clinical studies pinpointed the role of sex steroids, mainly female sex steroids, in melanoma. Animal studies also highlighted the involvement of sex steroids in melanoma. In vitro studies with steroid hormones showed inhibition of melanoma cell growth. Our studies showed the in vitro effect of progesterone on mouse and human melanoma cell growth. In our studies, progesterone showed significant inhibition of mouse and human melanoma cell growth. The mechanism of inhibition was due to autophagy and the effect was not mediated through progesterone receptor. In vitro study also showed suppression of adhesion and migration functions after progesterone treatment, suggesting progesterone could be involved in delayed metastasis of cancer. This in vitro finding supported the clinical studies which showed menstruating females (whose progesterone level vary between 1000 and 1500 ng/dL) were better protected in melanoma than post-menopausal women (whose progesterone level vary between 20 and 100 ng/dL) and men of any age. A similar study with different human melanoma (A375, A875) cell lines by Fang et al. [51] also showed that progesterone and RU-486 inhibited melanoma cell growth and this effect was also not mediated through progesterone receptor. Similar result was observed in another study using progesterone and the same melanoma cell lines by Moroni et al. [31]. Kanda and Watanabe [25] had already shown the inhibition of human melanoma cells by progesterone. Thus the inhibition of melanoma cell growth

by progesterone was observed by 3 other different groups. Thus, the preceding studies, our own studies, and previous studies by others lend support to the idea that melanoma is amenable to hormone action and that melanoma is sensitive or responsive to steroid hormones.

9. Conclusion

Evidences from multiple sources (epidemiological, clinical, in vivo, and in vitro) suggested the involvement of hormones in melanoma and that melanoma was amenable to hormone action. But, unlike breast, ovary, and prostate cancers, addition of hormones did not stimulate proliferation of melanoma cells, suggesting melanoma was not a hormone-dependent cancer. However, addition of hormones suppressed melanoma cell proliferation, suggesting melanoma might be a hormone-sensitive or responsive cancer. Therefore, acquisition of melanoma may not be hormone dependent, but survival (suppression of cancer cell proliferation) in melanoma may be hormone dependent. Hence, based on epidemiological findings, clinical studies, literature reports of previous in vivo and in vitro experiments, and our own experiments, melanoma may be considered as a hormone-sensitive or responsive cancer. This understanding will help in generating new therapy or therapeutic target for melanoma treatment.

Declaration of interest

Author has nothing to declare.

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