Chapter

A Review of Progesterone Effects on Human Melanoma Cell Growth In-Vitro

Pandurangan Ramaraj

Abstract

Progesterone, a female sex hormone not only has a role in reproduction, but also in protecting females in melanoma. A survey of steroid hormones actions steroid hormones actions survey on melanoma cells and literature survey showed that progesterone inhibited mouse and human melanoma cell growth significantly in-vitro. Progesterone not only inhibited cell growth, but also affected adhesion and migration functions (essential for metastasis) in-vitro. This observation correlated with the clinical studies where they had shown showed an increased survival and delayed metastasis in menstruating females in melanoma. Further, progesterone level in menstruating females (1000–1500 ng/dL) compared to post-menopausal females (20–100 ng/dL) also correlated with previous clinical studies. Progesterone action on melanoma cells, as reported by other researchers also supported the findings from this lab. Hence, progesterone could be the steroid hormone protecting menstruating females in melanoma. Moreover, our recent studies showed that progesterone suppressed pro-inflammatory cytokine IL-8 secretion by the melanoma cells, which decreased melanoma cell growth in-vitro. Hence, progesterone apart from reproductive function may also be involved in protecting menstruating females in melanoma.

Keywords: progesterone, menstruating females, protection in melanoma, IL-8 secretion

1. Introduction

Skin is not only a largest organ in the body, but also an endocrine organ and a target site for other hormones [1–3]. Skin has all the elements of a functional hypothalamo-pituitary-adrenal axis [4]. So, it has CRH (corticotropic releasing hormone), POMC (pre-opiomelano cortin) and associated peptides ACTH (adreno corticotropic hormone), α-MSH (α-melanocyte stimulating hormone), β-endorphin [5]. Expression of these peptides is environmentally regulated and their dysfunction can lead to skin and systemic diseases [6]. Skin neuroendocrine system acts by preserving and maintaining the skin structural and functional integrity [7]. This network allow skin to maintain local and global homeostasis that is vital for survival [6, 7]. Skin and hair follicles not only have functional melatonin receptor, but to a larger extent serve as an extra-pineal organ to synthesize melatonin [8]. Skin has the ability to synthesize glucocorticoid from cholesterol or from

steroid intermediates of systemic origin [6]. By interacting with glucocorticoid receptor, immune functions and physiological functions of epidermal, dermal and subcutaneous structures are regulated [9]. Since, synthesis and site of actions of hormones are nearby, it suggests auto, para and intracrine mode of actions. Levels of local production changes in response to environmental stress. This local glucosteroidogenesis is essential for skin homeostasis and prevent skin pathology. Sex steroids such as androgens, estrogens and progestins are essential for a healthy skin [1, 2]. Melanocyte, which is transformed to melanoma is also under the influence of melanocyte stimulating hormone (MSH) from pituitary. Generally, melanoma is not labeled as a hormone dependent cancer because of the fact that ultraviolet (UV) rays from the Sun is the major cause for melanoma [10]. UV rays cause deoxy ribonucleic acid (DNA) damages and other inflammatory changes in the skin, which result in skin cancer [11]. 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 to survival or a hormone responsive nature of melanoma [12, 13].

2. In-vitro studies from our lab

In-vitro studies from our lab showed the involvement of progesterone in the regulation of mouse and human melanoma cell growth.

2.1 Effect of Steroids on mouse melanoma (B16F10) cell growth

Initially four sex steroids viz., dehydroepiandsterone (DHEA), androstenedione (AD), testosterone (T) and progesterone (P4) were used to find out their effect on mouse melanoma (B16F10) cell growth. Though all four steroids showed a dose-dependent decrease in cell growth, yet progesterone alone showed a significant inhibition of the mouse melanoma cell growth [14].

2.2 Dose-response curves with mouse (B16F10) and human (BLM) melanoma cells

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 steroids on melanoma cell growth inhibition. Mouse (B16F10) and human melanoma (BLM) cells showed a dose-dependent cell growth inhibition, suggesting the inhibition was a biological action and not a toxic inhibition of cell growth at high concentrations [14, 15].

2.3 Effect of related steroids on mouse melanoma cell growth

A weak androgen DHEA also inhibited mouse melanoma cell growth, but it was not as significant as the inhibition of progesterone on mouse melanoma cells. But, RU-486 a progesterone receptor antagonist and also a glucocorticoid receptor antagonist showed significant inhibition of mouse melanoma cell growth [14, 15].

2.4 Mechanism of progesterone and RU-486 actions

Since progesterone and its receptor antagonist (RU-486) showed significant inhibition on melanoma cell growth, it raised the question whether progesterone

receptor was involved in this action. However, a co-incubation experiment of progesterone and RU-486 showed an additive effect on melanoma cell growth inhibition, suggesting that the action was not mediated through progesterone receptor and that each hormone acted through different mechanisms resulting in an additive effect on the inhibition of melanoma cell growth. Similarly human melanoma cell growth showed an additive effect on cell growth inhibition, when progesterone at fixed concentration (10 μM) was co-incubated with varying concentrations of RU-486 (10, 50 and 100 μM).

2.5 Dose-curve with cholesterol to check non-specific action of steroids

Since inhibition was seen with DHEA, progesterone and RU-486, it raised the question whether it was a specific effect on melanoma cell lines or common effect on all cancer cell lines? So, cells were incubated with cholesterol the parent compound of all steroids. Though, cholesterol showed initial decrease in cell growth, it failed to show a dose-dependent inhibition of cell growth. It was almost flat line from 10 μM to 200 μM, suggesting that the inhibition by progesterone and RU-486 was specific to melanoma cells.

2.6 Effect of progesterone and RU-486 on human gastric cancer cell (NUGC3) line

The effect of progesterone and RU-486 seen on mouse and human melanoma cells raised the question whether it was a non-specific effect on melanoma cell lines. So, progesterone and RU-486 were incubated separately with human gastric cancer (NUGC3) cell line. Progesterone and RU-486 did not show a significant inhibition as seen that of on melanoma cells, suggesting the inhibition was specific to melanoma cells.

2.7 Effect of progesterone and RU-486 on normal rat vascular smooth muscle cells

So far experiments were carried out on transformed cells and hence the effect on normal cells was not known. So, normal rat vascular smooth muscle cells were used. Progesterone and RU-486 were incubated with smooth muscle cells, which did not show a significant inhibition, suggesting progesterone and RU-486 inhibition were specific to melanoma cells.

2.8 Mechanism of inhibition of human melanoma cell growth

As progesterone showed a dose-dependent inhibition of human melanoma (BLM) cell growth, the mechanism of inhibition was determined. After ruling out necrosis and apoptosis, autophagy [12] as the mechanism was detected by co-incubation with 3-MA (methyl adenine) and progesterone. Results showed a partial increase in cell growth (rescue of cell growth) with 3-MA and progesterone co-incubation compared to cells incubated with progesterone alone, suggesting the mechanism of inhibition of cell growth was due to autophagy.

2.9 Other in-vitro functions inhibited by progesterone

Progesterone not only inhibited cell growth, but also other in-vitro functions such as adhesion and migration. Both adhesion and migration functions were essential for metastasis. Clinical study showed that menstruating females were

better protected in melanoma in terms of increased survival and delayed metastasis than post-menopausal women and men of any age. Literature survey also revealed that progesterone level in menstruating females was 100–150 ng/ml in the follicular phase and 1000–1500 ng/dL in the luteal phase [16]. Whereas, post-menopausal females' progesterone level was 20–100 ng/dL and males' levels were between 27 and 90 ng/dL. The last two groups were not protected in melanoma, as per the clinical studies. In fact, progesterone in-vitro action also suggested the same. Study of progesterone effect on melanoma cells by other researchers also showed the effect of progesterone on other human melanoma cell lines. Fang et al. from China showed inhibition of A375 and A875 cell line growth by progesterone and RU-486 and that their actions were not mediated through progesterone receptor [17]. Moroni et al. [18] repeated the study with the same lines using progesterone concentrations up to 1000 μM. Kanda and Watanbe [19] already showed the inhibition of human melanoma cell growth by progesterone along with dihydrotestosterone (DHT) and estradiol (E2). However, these studies did not correlate progesterone with the protective function as reported by the clinical studies.

3. Summary

Progesterone, a female sex steroid significantly inhibited mouse and human melanoma cell growth significantly in-vitro. RU-486, a progesterone receptor antagonist also significantly inhibited melanoma cell growth significantly. But the action was not mediated through progesterone receptor. In addition, effect of progesterone and RU-486 were found to be not a spurious or a toxic action. In-vitro studies also showed that progesterone inhibited human melanoma cells and the mechanism of inhibition was due to autophagy. Progesterone also inhibited adhesion and migration functions (essential for metastasis) of human melanoma cells in-vitro. This observation correlated well with the previous clinical studies which reported that menstruating females were better protected (increased survival and delayed metastasis) in melanoma than post-menopausal women and men of any age. Research works around the globe also showed inhibition of human melanoma cells by progesterone. Progesterone action was mediated by the suppression of IL-8 secretion by melanoma cells.

4. Conclusion

As shown by the in-vitro studies, progesterone could be protecting menstruating females in melanoma. In fact progesterone could be the appropriate steroid because progesterone is anti-inflammatory in nature. Further studies from our lab showed that progesterone suppressed pro-inflammatory cytokine IL-8 [20]. In fact, progesterone could be the appropriate steroid because progesterone is anti-inflammatory in nature. So, progesterone action could be mediated by the suppression of pro-inflammatory cytokine IL-8, which decreased melanoma cell growth in-vitro. Hence, survival of menstruating females in melanoma may be dependent on progesterone. So, progesterone apart from its effect on reproduction has also a role in protecting females from melanoma.

IntechOpen

Author details

Pandurangan Ramaraj
Department of Biochemistry, Kirksville College of Osteopathic Medicine, A.T. Still University, Kirksville, MO, United States

*Address all correspondence to: pramaraj@atsu.edu

References

[1] Zouboulis CC, Chen WC, Thornton MJ, Qin K, Rosenfield R. Sexual hormones in human skin. Hormone and Metabolic Research. 2007;**39**(2):85-95

[2] Zouboulis CC. The human skin as a hormone target and an endocrine gland. Hormones. 2004;**3**(1):9-26

[3] Zouboulis CC. Human skin: An independent peripheral endocrine organ. Hormone Research. 2000; **54**:230-242

[4] Slominski A, Wortsman J. Neuroendocrinoloy of the skin. Endocrine Reviews. 2000;**21**(5):457-487

[5] Slominski A, Wortsman J. Self-regulated endocrine systems in the skin. Minerva Endocrinologica. 2003; **28**(2):135-143

[6] Slominski A, Zbytek B, Nikolakis G, Manna PR, Skobowiat C, Zmijewski M, et al. Steroidogenesis in the skin: Implications for local immune functions. The Journal of Steroid Biochemistry and Molecular Biology. 2013;**137**:107-123. DOI: 10.1016/j.jsbmb.2013.02.006. Epub 2013 Feb19

[7] Nikolakis G, Stratakis CA, Kanaki T, Slominski A, Zouboulis CC. Skin steroidogenesis in health and disease. Reviews in Endocrine & Metabolic Disorders. 2016;**17**(3):247-258

[8] Cos S, Garcia-Bolado A, Sanchez-Barcelo E. Direct antiproliferative effects of melatonin on two metastatic cell sub-lines of mouse melanoma (B16BL6 and PG19). Melanoma Research. 2001;**11**(2):197-201

[9] Labrie F. DHEA and its transformation into androgens and estrogens in peripheral target tissues: Intracrinology. Frontieres in Neuroendocrinology. 2001;**22**(3): 185-212

[10] Rass K, Reicharth J. UV damage and DNA repair in malignant melanoma and non-melanoma skin cancer. Advances in Experimental Medicine and Biology. 2008;**624**:162-178. DOI: 10.1007/978-0-387-77574-6_13

[11] Gray-Schopfer V, Wellbrock C, Marais R. Melanoma biology and new targeted therapy. Nature. 2007; **445**:851-857

[12] Ghosh R, Ott AM, Seetharam D, Slaga TJ, Kumar AP. Cell cycle block and apoptosis induction in a human melanoma cell line following treatment with 2-methoxyostradiol: Therapeutic implications? Melanoma Research. 2003;**13**(2):119-127

[13] Disorbo DM, McNulty B, Nathanson L. In vitro growth inhibition of human malignant melanoma cells by glucocorticoids. Cancer Research. 1983; **43**:2664-2667

[14] Ramaraj P, Cox JL. In-vitro effect of sex steroids on mouse melanoma (B16F10) cell growth. CellBio. 2014;**3**:60-71. DOI: 10.4236/cellbio.2014.32007

[15] Ramaraj P, Cox JL. In-vitro effect of progesterone on human melanoma (BLM) cell growth. International Journal of Clinical and Experimental Medicine. 2014;**7**(11):3941-3953

[16] Häggström M. Reference ranges for estradiol, progesterone, luteinizing hormone and follicle-stimulating hormone during the menstrual cycle. WikiJournal of Medicine. 2014;**1**(1):1. DOI: 10.15347/wjm/2014.001

[17] Fang X, Zhang X, Zhou M, Li J. Effects of progesterone on the growth regulation in classical progesterone

receptor-negative malignant melanoma cells. Journal of Huazhong University of Science and Technology Medical Sciences. 2010;**30**(2):231-234. DOI: 10.1007/s 11596-010-0220-3

[18] Moroni G, Gaziano R, Bue C, Agostini M, Perno CF, Sinibaldi-Vallebona P, et al. Progesterone and melanoma cells: An old story suspended between life and death. Journal of Steroids and Hormonal Science. 2015;**S13**:158. DOI: 10.4172/2157-7536.1000158

[19] Kanda N, Watanbe S. 17-β-estradiol, progesterone and dihydrotestosterone suppress the growth of human melanoma by inhibiting interleukin-8 production. The Journal of Investigative Dermatology. 2001;**117**:274-283

[20] Miller A, Fulcher A, Dock P, Ramaraj P. Biochemical basis of protection by progesterone in melanoma based on curcumin pre-treatment of human melanoma cell models. Endo2018Home. 2018. Available from: http://www.abstractsonline.com/pp8/#!/4482/presentation/7469