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A 5α-reductase inhibitor, finasteride, increases differentiation and proliferation of embryonal carcinoma cell-derived-neural cells

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SUMMARY

Recent advances in stem cell biology have resulted in identifying new agents to differentiate stem cell-derived-neural cells. Different stem cell types have been shown to differentiate into neural cells. It has been shown that P19 line of embryonal carcinoma cells develops into neurons and astroglia after exposure to some hormones such as dehydroepiandrosterone (DHEA). Steroid 5α-reductase is a key enzyme in the conversion of several Δ4-3 keto steroids, such as testosterone into their respective 5α-reductase derivatives. Finasteride is a 5α-reductase inhibitor that inhibits conversion of testosterone to the more potent androgen dihydrotestosterone (DHT). Reduction in DHT and sustaining testosterone levels has an important impact on differentiation and proliferation of embryonal carcinoma cells to neural cells. We hypothesize that finasteride, a 5α-reductase inhibitor, will be differentiate embryonal carcinoma cell to the neural cell and increase their proliferation due to the elevation levels of testosterone, a neuroprotective neurosteroid.

Background

Culture of embryonal carcinoma (EC) cells revealed the potential application of these cells in developing assays of pharmacology, toxicology and cell therapy in a large spectrum of diseases. Protocols for controlled differentiation of EC cells ordinarily depend on the application of growth and/or differentiation factors in a defined temporal order.

The aggregation of EC cells to embryoid bodies is used to induce differentiation. The embryoid bodies represent multicellular structures which undergo spontaneous differentiation in derivatives of germ layers [1]. Initial differentiation of embryoid bodies occurs when the outer cells of the aggregate differentiate into endoderm-like cells that surround an undifferentiated core [1]. Embryonal carcinoma cells are derived from teratocarcinomas. Malignant teratomas also contain populations of undifferentiated stem cells that can grow as undifferentiated cell lines [2,3]. Examples of such cells that have been applicable are the human Tera-2 EC cells [4], mouse F9 EC cells [5] and P19 EC cells [6].

Early works showed that P19 EC cells are very suitable for isolating clonal sub-lines. In addition, hormones like retinoic acid (RA) and neurosteroids induce differentiation of EC cells, in monolayer culture, without an aggregation phase [7,8]. It was shown that relatively high concentrations of RA induce the formation of neuronal and glial cells [9], while 0.5–1% DMSO leads to the formation of a wide variety of endodermal and mesodermal tissues [10].

Dehydroepiandrosterone (DHEA) is a neurosteroid that has been shown to be a potential signaling molecule in neuronal differentiation during development. DHEA can increase proliferation of human neural stem cells. DHEA has recently been shown to be neuroprotective [11]. The adrenal cortex is the primary source of circulating concentrations of DHEA [12], which is secreted in response to adrenal corticotropin-releasing hormone [13]. DHEA act as the precursor to the approximately 50% of androgens in adult men and positively regulate elevation of testosterone [14]. DHEA production and concentration declines significantly and steadily during aging [15]. Testosterone is an important determinant of body composition in masculine. Its administration is associated with hypertrophy of muscle fibers and significant increases in myonuclear, satellite and neural cell numbers [16]. Transdifferentiation into neural stem cells and differentiating neurons was observed in human stem cells after exposure to certain combinations of steroids especially testosterone [17]. Testosterone enhances neurogenesis via increased cell survival through an androgen dependent mechanism [18].

Recently, finasteride was described as a potential clinical candidate for the treatment of androgen-dependent disorders. Finasteride is an inhibitor of 5α-reductase. Steroid 5α-reductase

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is a NADPH-dependent enzyme which converts testosterone to the more potent androgen, 5α-dihydrotestosterone (DHT), progesterone to dihydroprogesterone and deoxycorticosterone to dihydrodeoxycorticosterone, steroids modulating the action of γ-aminobutyric acid on GABA receptors [19]. In an extensive investigation of the effects of androgens on the proliferation, differentiation, and function of stem cells, it was clear that DHT inhibits the differentiation of stem cells. The final effect is a reduced number of fully differentiated cells [20]. Like DHEA, finasteride increase testosterone levels and decrease other androgen levels [21].

Understanding the consequence actions of DHEA, DHT and finasteride inspired us to propose that finasteride, the 5α-reductase inhibitor, will be differentiate embryonal carcinoma cell to the neural cell and increase proliferation of neural cells due to elevation of testosterone, a neuroprotective neurosteroid.

Hypothesis

Testosterone levels have an important impact on differentiation and proliferation of embryonal stem cells to neural cells. The hypothesis we propose here is that finasteride, a 5α-reductase inhibitor, that could change the balance between testosterone and DHT will be differentiate embryonal carcinoma cell to neural cell and increase proliferation of neural cells. The hypothesis is due to elevation of testosterone and lessens in DHT levels. DHT, a non-aromatizable androgen, can decrease proliferation and differentiation of EC cells and its existence is contrarily to finasteride role. This idea would be important and has implication for future use.

Evaluation of hypothesis

Investigations of androgens effects on differentiation of stem cells revealed that DHT inhibits the differentiation of stem cells. Adipocytes differentiated from stem cells in the presence of DHT are smaller and accumulate fewer lipids [20]. Testosterone and DHT regulate the differentiation of pluripotent cells of mesenchymal origin; these androgens stimulate myogenic differentiation while inhibiting adipogenic differentiation [22]. Androgens regulate body composition by stimulating the commitment of pluripotent cells that are resident within the skeletal muscle and elsewhere into the myogenic lineage [22].

Finasteride blocks the actions of DHT that mainly has actions on the prostate and hair loss. Therefore, testosterone replacement can be safely prescribed alongside finasteride in patients with low testosterone [23]. The presence of 100 nM/L finasteride during the ischemic insult results in substantial protection. In some cases surviving rate of neurons is quantified by counting distinct functionally active neurons before and after insult [19].

Discussion

Androgens inhibit bodies fat mass; thus, their deficiency is associated with higher fat mass [24], and testosterone supplementation lessens whole body and inter-muscular fat mass [25,26]. DePergola has reported that testosterone inhibits differentiation of precursor cells, suppresses lipid uptake and lipoprotein lipase activity, and up-regulates the number of beta-adrenergic receptors [27].

Pregnenolone, a steroid precursor of testosterone, had no significant effect on the differentiation of stem cells, indicating that these effects are specific to androgenic steroids. Steroids affect neuronal operation through binding to cognate intracellular receptors which may act as transcription factors in regulation of gene expression [28].

Neuroactive steroids modulate ligand-gated ion channels via non-genomic mechanisms. Classical steroid hormones such as testosterone and progesterone are neuroactive steroids because they may act as functional antagonists at the 5-hydroxytryptamine receptor, a ligand-gated ion channel or distinct glutamate receptors. Although a binding's site for steroids at $GABA_A$ receptors is still a subject for further discussions, there is an evidence that steroids interact allosterically with ligand-gated ion channels at the receptor membrane interface [28].

Animal studies showed that testosterone is converted rapidly into GABAergic neuroactive steroids *in vivo*. It reduces locomotor activity in a dose-dependent fashion in Wistar rats. Both the genomic and non-genomic effects of steroids in the brain may contribute to pathophysiological disorders. Neuroactive steroids affect embryonal carcinoma cells and may represent a new treatment strategy for neurological disorders [29].

This discussion suggests a promising strategy for promoting neurogenesis by finasteride in the aged brain and potentially for restoration of neuronal populations in brains recovering from neurodegenerative disease or injury.

Conclusion

In summary according to the theory mentioned above, we would use the finasteride to induce development of neurons in cultures of P19 cells. Finasteride could provide a suitable inducer for differentiation of stem cell-derived-neural cells, including neurospheres and neurons.

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Conflicts of interest statement

None declared.

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