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Original Article

In vitro induction effect of $1,25(OH)_2D_3$ on differentiation of hair follicle stem cell into keratinocyte

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ABSTRACT

Background: Stem cells are characterized by self-renewal and differentiation capabilities. The bulge hair follicle stem cells (HFSCs) are able to convert to epithelial components. The active metabolite of vitamin D, $1,25(OH)_2D_3$, plays important roles in this differentiation process. In the present study has found that $1,25(OH)_2D_3$ induces the HFSCs differentiation into keratinocyte.

Methods: HFSCs are isolated from rat whiskers and cultivated in DMEM medium. To isolate bulge stem cell population, flow cytometry and immunocytochemistry using K15, CD34 and nestin biomarkers were performed. In order to accelerate the HFSCs differentiation into keratinocyte, HFSCs were treated with 10^{-12} M, $1,25(OH)_2D_3$ every 48 h for a week.

Results: Immunocytochemistry results showed that bulge stem cells are nestin and CD34 positive but K15 negative before differentiation. Subsequently flow cytometry results, showed that the expression of nestin, CD34 and K15 were 70.96%, 93.03% and 6.88% respectively. After differentiation, the immunocytochemical and flow cytometry results indicated that differentiated cells have positive reaction to K15 with 68.94% expression level.

Conclusion: It was concluded that 10^{-12} M, $1,25(OH)_2D_3$ could induce the HFSCs differentiation into keratinocytes.

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At a glance commentary

Scientific background on the subject

Stem cells, particularly adult stem cells, have shown promising results in both translational and clinical applications. Previous studies show that the bulge region of hair follicle contains pluripotent stem cells which have the ability to differentiate into epithelial components. Accordingly we believe that HFSCs may be used for therapeutic purposes in regenerative medicine. Since $1.25(OH)_2D_3$ regulates keratinocytes differentiation, in the present study we sought to investigate the effect of $1.25(OH)_2D_3$on HFSCs differentiation into keratinocytes.

What this study adds to the field

The novelty of our experiment is proving the efficient induction of $1,25(OH)_2D_3$ on HFSCs differentiation. In this study, for the first time we showed that, $1,25(OH)_2D_3$ (10^{-12} M) dissolved in ethanol could direct and accelerate the differentiation of rat HFSCs into keratinocytes cells.

Skin injuries resulting from diabetic ulcer or burns are major healthcare problem [1,2]. Hence, utilization of adult [3] or embryonic [4] stem cells in skin grafting and tissue engineering is very important in regenerative medicine. It is important to direct stem cell differentiation into the keratinocyte lineage in skin grafting and tissue engineering. To avoid immunological rejection, it is essential to utilize autologous skin grafts and to prevent additional surgery according to unaffected skin requirement, it would be preferable to utilize *ex vivo* cultured autologous keratinocytes [5].

Recently, adult stem cells are highly concerned in clinical applications because some of the problems associated with embryonic and fetal stem cells, such as immunological incompatibility are not found in adult stem cells [6,7]. Hoffman demonstrated the pluripotency of hair follicle stem cells use in regenerative medicine, because [1], they are readily available from essentially anyone [2], they are easily cultured and expanded [3], are highly pluripotent [4], they have been demonstrated to be able to support the regrowth of nerves [5], they do not carry the ethical issues that embryonic stem cells and fetal stem cells do [8].

Paus et al. declared that hair follicle adult stem cells are reserved in bulge area of the hair follicle [9]. Bulge region is located between the insertion of the arrector pili muscle and duct of the sebaceous gland [10]. Li et al. showed that the population of cells in the bulge region expresses Nestin (intermediate filament) as a marker of neural stem cells [6–8,11,12]. Trempus et al. first described the expression of CD34 (the surface protein) as a marker of mouse bulge cells of hair follicle [13]. Lyle et al. for the first time reported that K15 (intermediate filament) as a keratinocytes marker is expressed in human bulge cells of hair follicle [14]. Liu et al. also showed that K15 has been expressed in adult mouse bulge cells [15].

Vitamin D is responsible for maintenance of plasma phosphate [16], regulating bone formation and calcium homeostasis [17]. Vitamin D, like other steroid hormones, regulates gene expression in different cell types [18]. Peehl et al. reported that active metabolite of vitamin D, $1,25(OH)_2D_3$ regulates cell proliferation and differentiation of several cell types including keratinocytes [19]. Hosomi et al. found that $1,25(OH)_2D_3$ accelerates terminal differentiation of cultured mouse epidermal cells. Similarly Smith et al. demonstrated this marker is able to enhance human keratinocytes differentiation. Thus, $1,25(OH)_2D_3$ is assumed to have an important role in keratinocytes differentiation [20].

The present study investigates the effect of $1,25(OH)_2D_3$ on HFSCs differentiation into keratinocytes cells for the first time.

Materials and methods

Animals and housing conditions

All animal experiments were carried out according to the guidelines of the Iranian Council for Use and Care of Animals and approved by the Animal Research Ethical Committee of Iran University of Medical Sciences. Male Wistar rats (n = 40, 150–200 g of body weight) were purchased from the Animal Center of Iran University of Medical Science. All rats were permitted free access to food and water at all times and were maintained under light–dark cycles.

Hair follicle isolation and cultivation

The rats were deeply anesthetized with chloroform, and the whisker follicles were dissected as described by Amoh et al. and Gilanchi et al. with modification [6,7,21]. After rinsing the animal's head, with betadine and 70% ethanol for 3 min, the upper lip was completely shaved and trimmed into small pieces. The samples were incubated at 37 °C and 5% CO2 in Collagenase I/Dispase II solution (Sigma–Aldrich) in incubator for 7 min. Then, most of the connective tissue and dermis around the follicles were removed, and whisker follicles were extracted with fine forceps. The follicles were transferred into another sterile 35-mm dish. The bulge region located in the middle third of the hair follicle, were dissected from the upper follicle by making two transversal cuts at the site of the enlargement spots of outer root sheath (ORS) with a fine needle, and the collagen capsule was incised longitudinally. 20–30 isolated bulges submerged in amphotericin B for 3 min. Afterward they were incubated in Trypsin–EDTA 0.25% for 10 min and cut into small pieces, and plated into 25-cm^2 tissue culture flasks (TCFs) pre-coated with collagen type I (Sigma–Aldrich). Before the cultivation, the flasks were pre-incubated with medium and after 2 h the medium was eliminated. Bulges submerged in a 3:1 supplemented mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium (DMEM/F12) containing 10% fetal bovine serum, antibiotics (100 U/ml penicillin and 100 μg/ml