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In vitro neural differentiation of *CD34*$^+$ stem cell populations in hair follicles by three different neural induction protocols

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Abstract Differentiation of hair follicle stem cells (HFSCs) into neurons and glial cells represents a promising cell-based therapy for neurodegenerative diseases. The hair follicle bulge area is reported as a putative source of new stem cell population for many years. In vitro studies have implicated neural differentiation of HFSCs. Here, we report the identification and purification of *CD34*$^+$ cells from hair follicle by magnetic activated cell sorting (MACS). We next determined the cytotoxic effects of *all-trans* retinoic acid (RA) by using cell viability assays. Moreover, the neural differentiation potential of *CD34*$^+$ cells was evaluated in the presence of RA, serum-free condition, and neural differentiation medium (NDM) treatments by using immunocytochemistry and reverse transcription polymerase chain reaction (RT-PCR). Our results showed that the isolated *CD34*$^+$ stem cells were 12% of the total cells in the bulge area, and the neural cells derived from the stem cells expressed *nestin*, microtubule-associated protein 2 (*MAP2*), and glial fibrillary acidic protein (*GFAP*). Interestingly, all the neural induction media supported neuronal differentiation most effectively, but treatment with serum-free medium significantly increased the number of *GFAP*-positive glial cells. Moreover, increasing RA concentration (≥10 μM) leads to increased cell death in the cells, but a lower concentration of RA (1 μM) treatment results in a decrease in *CD34*-expressing stem cells. These findings show an instructive neuronal effect of three neural induction media in HFSCs, indicating the important role of this induction media in the specification of the stem cells toward a neural phenotype.

Keyword *CD34* · Isolation · Hair follicle stem cells · *All-trans* retinoic acid · Neuronal cell · Glial cell

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Introduction

First hair follicle stem cell cultures were established from mouse, rat, and human several years ago (Kamimura *et al.* 1997; Kobayashi *et al.* 1993; Yu *et al.* 2006). The putative hair follicle stem cells (HFSCs) are located in the bulge area of hair follicles (Tumbar *et al.* 2004; Myung and Ito 2012). They are slow-cycling stem cells expressing *nestin* (Li *et al.* 2003; Amoh *et al.* 2005b), *CD200* (Ohyama *et al.* 2006), and *CD34* (Cotsarelis 2006; Poblet *et al.* 2006); they have a long lifespan and play critical roles in the morphogenesis of hair follicles (Blanpain *et al.* 2004; Oshima *et al.* 2001), epidermis (Lenoir *et al.* 1988), and sebaceous glands (Morris *et al.* 2004).

These easily accessible adult stem cells are well-known for their differentiation potential into ectodermal, mesodermal, and endodermal lineages such as neural (Amoh *et al.* 2008, 2005b), endothelial, and muscle cells (Amoh *et al.* 2004; Hoffman 2006). It has also been demonstrated that HFSCs express neural crest genes as well as neural stem cell markers, indicating that neural differentiation potential of HFSCs is fascinating (Sieber-Blum *et al.* 2006). Neurons generated