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Short Communication

Conditions for the differentiation of melanocyte-precursor cells from human cord blood-derived mesenchymal stem cells

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The loss of skin pigmentation can induce compromised cutaneous immunity, which can result in conditions such as vitiligo. In this study, we evaluated various agents that are able to induce the differentiation of stem cells into melanocytes. We found that a mixture of forskolin (FK), stem cell factor (SCF) and endothelin-3 (EDN-3) induced melanocyte-like morphology in human cord blood-derived mesenchymal stem cells (CB-MSCs). In addition, significant expression of microphthalmia-associated transcription factor-M and tyrosinase-related protein-1 genes was observed. These results suggest that a mixture of FK, SCF and EDN-3 induces the differentiation of melanocyte-precursor cells (MPCs) from CB-MSCs.

Key words: mesenchymal stem cells, melanocyte-precursor cells, forskolin, microphthalmia-associated transcription factor-M, tyrosinase-related protein-1.

INTRODUCTION

Skin pigmentation makes a considerable contribution to the health and well-being of an individual. Pigment synthesized by cutaneous melanocytes protects individuals from various environmental assaults and potential cellular injury that can cause cancer and aging of the skin. The loss of skin pigmentation can also result in compromised cutaneous immunity, which can result in conditions such as vitiligo (Slominski et al., 2004). Vitiligo is an acquired depigmentary disorder of the skin in which melanocytes of the skin are destroyed (Slominski et al., 2004) resulting in melanotic lesions of variable size. The precise cause of vitiligo is complex and not well understood. However, there are some evidences that suggest that it is caused by a combination of auto-immune, genetic and environmental factors. Although psychological and social problems related to self-esteem and personal interaction can be induced by vitiligo (Porter et al., 1990), until now, there have been no effective treatments for this disorder.

Stem cells are undifferentiated cells with broad developmental potential. These cells are able to generate various specialized cell types and have the capacity to self-renew, which enables them to produce undifferentiated progeny that retain stem cell features (Minguell et al., 2001; Pittenger et al., 1999). Therefore, we evaluated various agents that are able to induce the differentiation of stem cells into melanocyte precursors to determine if they will be useful as an alternative treatment for hypopigmentation-related diseases and vitiligo.

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Abbreviations: FK, forskolin; **SCF,** stem cell factor; **EDN-3,** endothelin-3; **MITF,** microphthalmia-associated transcription factor-M; **TRP;** tyrosinase-related protein; **TYR,** tyrosinase; **CB-MSCs,** cord blood-derived mesenchymal stem cells; **MPCs,** melanocyte-precursor cells; **PCR,** polymerase chain reaction; **ACTH,** adrenocorticotropic hormone; □**-MSH,** □-melanocyte stimulating hormone; **CT,** cholera toxin; **IBMX,** isobutylmethylxanthine.

MATERIALS AND METHODS

Human cord blood-derived mesenchymal stem cells (CB-MSCs) were

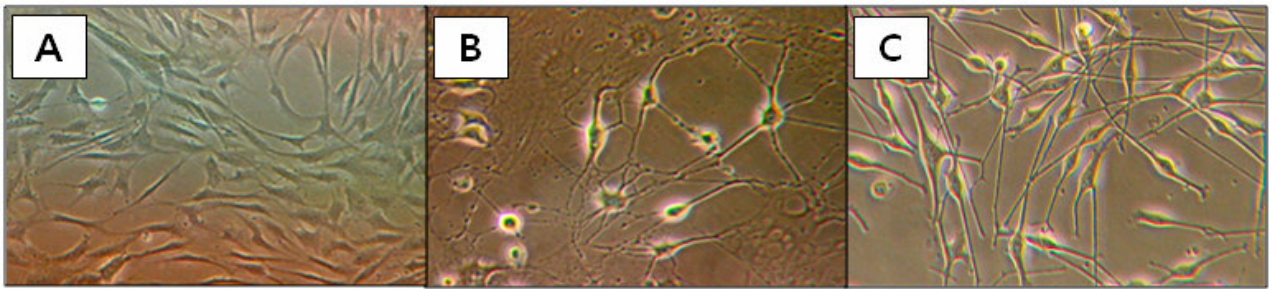

Figure 1. Differentiation of CB-MSCs into MPCs in melanocyte differentiation medium. (A) MSCs were grown on normal conditioned culture medium. (B) By day 30, differentiated populations homogeneously displayed melanocytic morphology. (C) Normal human melanocytes (positive control).

obtained from Cha University (Seongnam, Gyunggi, Korea). The cells were cultured in Dulbecco's modified eagle medium containing low glucose levels and supplemented with 15% fetal bovine serum, 1% penicillin and streptomycin at 37℃. To induce melanocytic differentiation, cells (3 x 10^5) were seeded on 60 mm culture dishes (Nunc, Roskilde, Denmark) in differentiation medium M254 (Cascade Biologics, Portland, OR, USA) containing 50 ng/ml stem cell factor (SCF) (Cell Signaling Technology, Beverly, MA, USA), 100 nM endothelin-3 (EDN-3) (Calbiochem, San Diego, CA, USA) and forskolin (FK) (Calbiochem). Differentiation medium was replaced twice per week.

Real-time quantitative polymerase chain reaction (PCR) was conducted in triplicate in 384-well plates. A 384-well high-throughput analysis was then performed using the ABI Prism 7900 Sequence Detection System (PE Applied Biosystems, www.appliedbiosciences.com). In addition, white 384-well plates (ABgene, Hamburg, Germany) were used to intensify the fluorescent signals by a factor of three. The system operates using a thermal cycler and a laser that is directed via fiber optics to each of the 384 sample wells. The fluorescence emission from each sample is collected using a charge-coupled device-camera and the quantitative data were analyzed using the Sequence Detection System software (SDS version 2.0, PE Applied Biosystems). Reaction mixtures contained 10 pmol/µl of each primer and 2X SYBR Green PCR Master Mix (PE Applied Biosystems, www.appliedbiosciences.com), which includes the HotStarTaqt DNA-Polymerase in an optimized buffer, the dNTP mix (with dUTP additive), the SYBR Green I fluorescent dye and ROX dye as a passive reference. Each of the 384-well real-time quantitative PCR plates included serial dilutions (1, 1/2, 1/4, 1/8 and 1/16) of cDNA, which were used to generate relative standard curves for each gene. All samples were amplified using the following conditions: 50℃ for 2 min, followed by 95℃ for 10 min and then 40 cycles of 95℃ for 30 s, 60℃ for 30 s and 72℃ for 30 s. To exclude the presence of unspecific products, melting curve analyses were conducted routinely after amplification using a high-resolution data collection system in which an incremental temperature increase from 60 to 95℃ with a ramp rate of 0.21℃/s was employed. The number of real-time PCR cycles required for a positive result was converted to the weight of the genes (ng) using the equation generated by the melting curve analysis. The real-time PCR analysis was performed using an Applied Biosystems Prism 7900 Sequence Detection System (PE Applied Biosystems) and A375 melanoma cells were used as a positive control.

RESULTS AND DISCUSSION

In order to examine the ability to induce the differentiation of stem cells into melanocyte precursors, we cultured human CB-MSCs in the presence of a mixture of FK, SCF and EDN-3 for 30 days. We found that after 2 - 3 weeks of culture in the differentiation medium, the CB-MSCs began to exhibit melanocytic morphology (data not shown). In addition, after 30 days of culture, the melanocytic morphology became more apparent, which suggests that the differentiation medium may induce the CB-MSCs to differentiate into MPCs (Figure 1).

To further evaluate the melanocytic characteristics of MPCs, real-time quantitative PCR was performed. Microphthalmia-associated transcription factor (MITF), tyrosinase-related protein-1 (TRP-1), tyrosinase-related protein-2 (TRP-2), tyrosinase (TYR), c-Kit and Sox-10 were included as specific markers of melanocyte. As shown in Figure 2, of all the 6 marker genes of melanocyte, only expression of MITF and TRP-1 genes increased significantly when compared to the untreated controls. In addition, although co-treatment with EDN-3 and SCF resulted in increased expression of the MITF and TRP-1, morphological change of the CB-MSCs into MPCs was not observed. These results suggest that the cyclic AMP (cAMP) elevating agent, FK, may play another role that is crucial to the differentiation of melanocytes from CB-MSCs.

In melanocytes, cAMP is also known to play key roles in the regulation of skin and hair pigmentation in humans. For example, skin hyperpigmentation has been reported in patients suffering from Addison's disease or Cushing's syndrome (Lamerson and Nordlund, 1998) which are both characterized by an overproduction of adrenocorticotropic hormone (ACTH). In addition, skin hyperpigmentation as a result of α-melanocyte stimulating hormone (α-MSH) hypersecretion has been reported (Pears et al., 1992). Furthermore, administration of α-MSH analogs to humans induces skin pigmentation in the absence of ultra-violet (UV) exposure (Levine et al., 1991), and these effects can be mimicked by pharmacological cAMP elevating agents such as FK, cholera toxin (CT) or isobutylmethylxanthine (IBMX) (Englaro et al., 1995; Hearing and Tsukamoto, 1991). Taken together, our observations suggest that FK plays a pivotal role in the differentiation of melanocytes from stem cells.

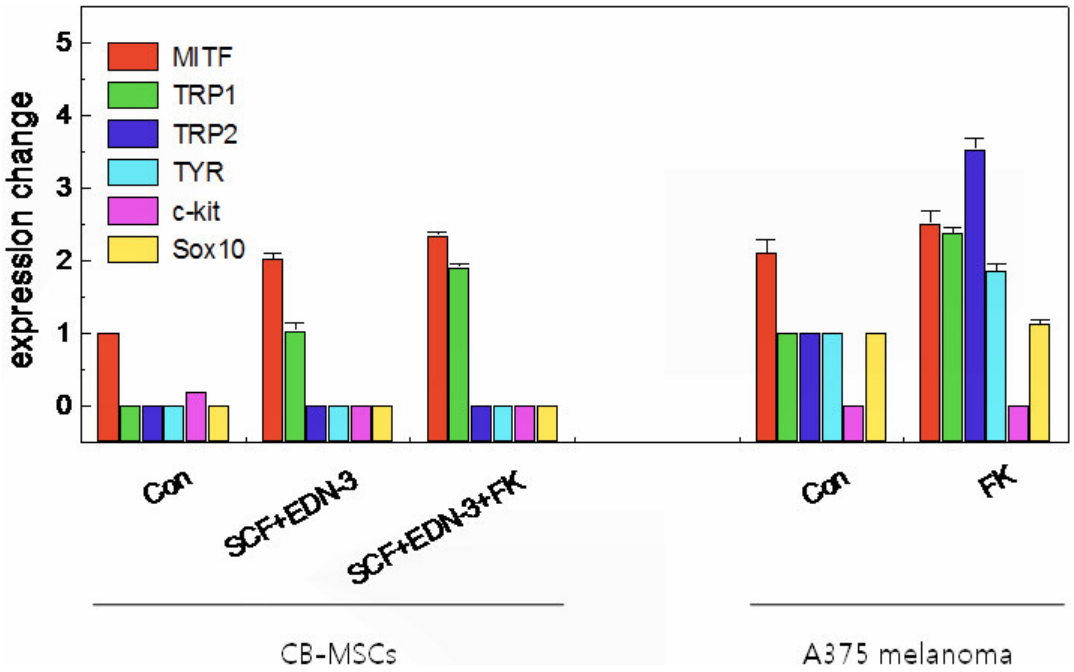

Figure 2. Expression of markers characteristic of the melanocytic lineage in MSCs-derived MPCs.

In summary, although there is still need for further study, we report the possibility that a mixture of forskolin, stem cell factor and endothelin-3 induces the differentiation of CB-MSCs into MPCs for the first time. These results suggest that CB-MSCs may be useful for the treatment of hypopigmentation-related diseases as well as vitiligo.

ACKNOWLEDGEMENT

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