acylglucosylceramides appear to be preferentially utilized for CLE-bound ceramide production rather than free (CLE-unbound) lipid production in the SC. Exact mechanisms for CLE formation have not been elucidated yet and it remains to be resolved whether preferential utilization of acylglucosylceramide for CLE formation occurs only in the present case or also in other SLS patients. Moreover, it is unknown how decrease in Cer 1, 6, 7 occur and whether barrier lipid abnormality in the patient was a primary event or a secondary phenomenon in the pathogenesis of SLS skin lesions. Cer 1 is essential lipid species to form epidermal permeability barrier formation. Thus, not only accumulation of free fatty acids, but also deficiency of specific ceramide species might contribute to formation of ichthyotic phenotype in SLS.

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References

[1] Rizzo WB, Carney G. Sjögren-Larsson syndrome: diversity of mutations and polymorphisms in the fatty aldehyde dehydrogenese gene (ALDH3A2). Hum Mutant 2005;26:1–10.
[2] Aoki N, Suzuki H, Ito K, Ito M. A novel point mutation of the *FALDH* gene in a Japanese family with Sjögren-Larsson syndrome. J Invest Dermatol 2000;114:1065–6.
[3] Shitake A, Akiyama M, Shimizu H. Novel ALDH3A2 heterozygous mutations are associated with defective lamellar granule formation in a Japanese family of Sjögren-Larsson syndrome. J Invest Dermatol 2004;123:1197–9.
[4] Sakai K, Akiyama M, Watanabe T, Sanayama K, Sugita K, Takahashi M, et al. Novel ALDH3A2 heterozygous mutations in a Japanese family with Sjögren-Larsson syndrome. J Invest Dermatol 2006;126:2545–7.
[5] Schreiner V, Gooris GS, Pfeiffer S, Lanzendörfer G, Wenck HW, Diembeck W, et al. Barrier characteristics of different human skin types investigated with X-ray diffraction, lipid analysis, and electron microscopy imaging. J Invest Dermatol 2000;114:654–60.
[6] Uhida Y, Cho Y, Moravian S, Kim J, Nakajima K, Crumbing D, et al. Neutral lipid storage leads to acylceramide deficiency, likely cobtributing to the pathogenesis of Dorfman-Chanarin syndrome. J Invest Dermatol 2010;130:2497–9.
[7] Rizzo WB, S'Aulis D, Jennings MA, Crumbing DA, Williams ML, Elias PM. Ichthyoisis in Sjögren-Larsson syndrome reflects defective barrier function due to abnormal lamellar body structure and secretion. Arch Dermatol Res 2010;302:443–51.
[8] Elias PM, Williams ML, Holleran WM, Jiang YJ, Schmuth M. Pathogenesis of permeability barrier abnormalities in the ichthyoses: inherited disorders of lipid metabolism. J Lipid Res 2008;49:694–714.
[9] Rizzo WB, Craft DA, Somer T, Carney G, Trafrova J, Simon M. Abnormal fatty alcohol metabolism in cultured keratinocytes from patients with Sjögren-Larsson syndrome. J Lipid Res 2008;49:410–9.
[10] Paige DG, Morse-Fisher N, Harper JI. Quantification of stratum corneum ceramides and lipid envelop ceramides in the hereditary ichthyoses. Br J Dermatol 1994;131:23–7.

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Letter to the Editor

BMP-4 down-regulates the expression of Ret in murine melanocyte precursors

Bone morphogenetic proteins (BMPs) have been implicated in a diverse array of biological processes including development and apoptosis [1]. Ret is involved in the physiological mechanisms of melanocyte activation and melanin production [2]. Ret expression in enteric neural precursors is initiated shortly after they emigrate from the neural plate.

We established three distinct cell populations of mouse neural crest (NC) cells, NCCmelb4, NCCmelb4M5 and NCCmelan5. NCCmelb4 cells have the potential to differentiate into mature melanocytes, but since they express melanocyte markers such as tyrosinase-related protein 1, DOPAchrome tautomerase and Kit, we consider them to be immature melanocytes, not multipotent precursors that can differentiate into neurons, as well as glia [3]. NCCmelb4M5 cells belong to the melanocyte lineage, but are less differentiated than NCCmelb4 cells [4]. NCCmelb4M5 cells do not express Kit and grow independently of the Kit ligand; these cells have the potential to differentiate into NCCmelb4 cells, which are Kit-positive melanocyte precursors. NCCmelan5 cells demonstrate the characteristics of differentiated melanocytes. We have also established an oncogene Ret-transgenic mouse line, line 304/B6, in which skin melanosis, benign melanocytic tumors and malignant melanomas develop in a stepwise fashion [2]. A malignant melanoma cell line, Mel-Ret, was established from the Ret-transgenic mouse. We found that all four cell lines express BMP receptors using Western blotting analysis (data not shown).

Western blotting revealed expression of the Ret protein in NCCmelb4M5 and in Mel-Ret cells, but in contrast, there was no expression of the Ret protein in NCCmelb4 or NCCmelan5 cells (Fig. 1A). Immunostaining also revealed that NCCmelb4M5 (Fig. 1B) and Mel-Ret cells are positive for Ret, but NCCmelb4 and NCCmelan5 cells are negative for Ret. Thus, Ret protein is expressed in most immature melanoblasts, while melanocytes are negative for Ret. We then analyzed Ret protein expression in BMP-4-treated NCCmelb4M5 cells by Western blotting (Fig. 1C–F). BMP-4 was added to the medium and incubated for 3 days at varying concentrations. After incubation with 10 ng/ml BMP-4 for 3 days, Ret protein expression was decreased, and disappeared completely

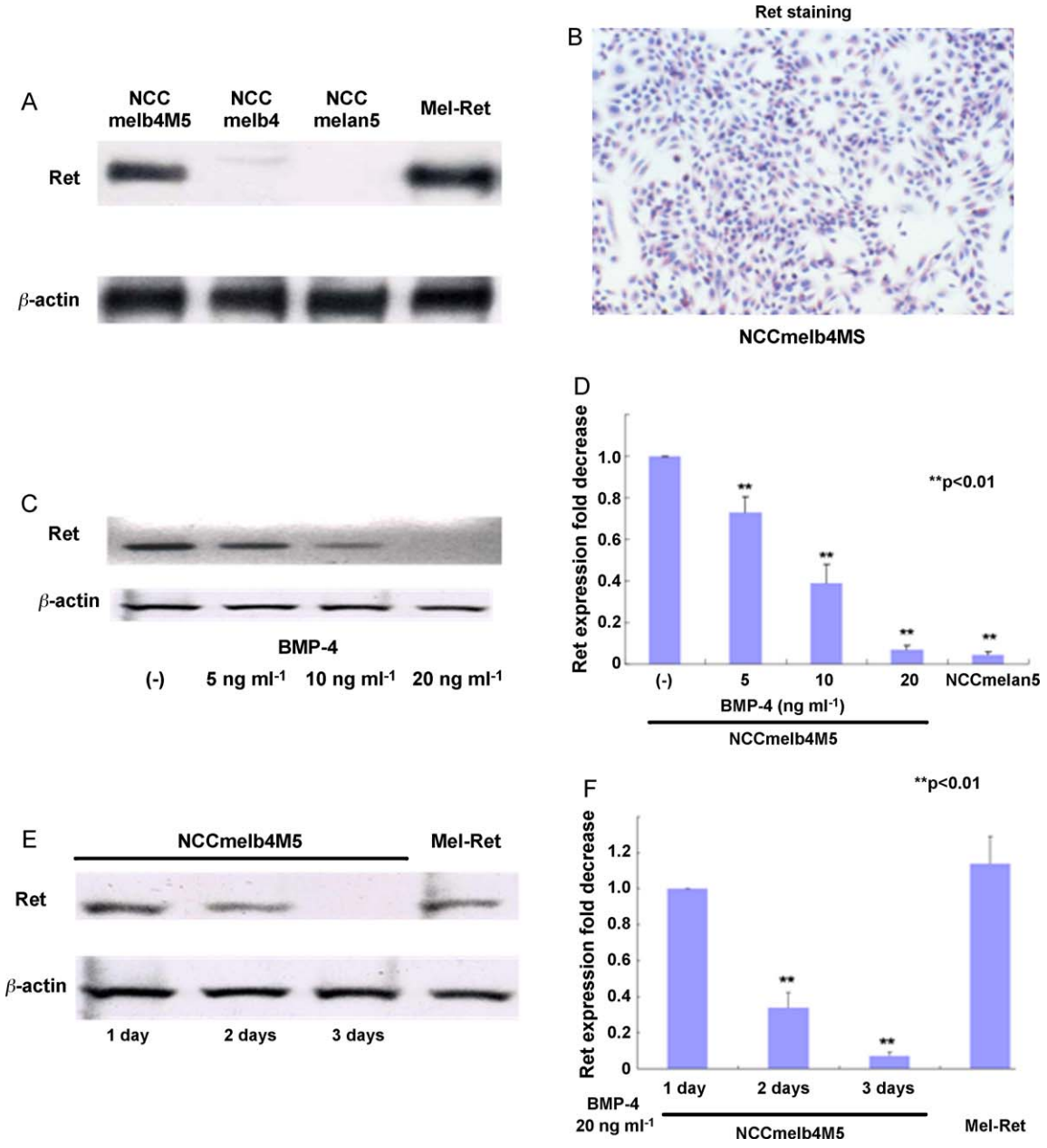

Fig. 1. (A) NCCmelb4M5, NCCmelb4, NCCmelan5 and Mel-Ret cells were cultured and were assessed by Western blotting, which showed that Ret protein is detectable in NCCmelb4M5 and in Mel-Ret cells but not in NCCmelb4 or NCCmelan5 cells. (B) NCC melb4M5 cells were immunochemically stained using an anti-Ret antibody (IBL, Fujioka, Japan). The red cells in the photomicrograph represent strongly Ret-positive NCC melb4M5 cells. (C) Ret protein expression in NCCmelb4M5 cells in the presence of BMP-4 was assessed by Western blotting. Various concentrations of BMP-4 as noted were added to the medium for 3 days. Ret expression almost disappeared at a concentration of 20 ng/ml BMP-4. (D) Ret expression levels were normalized to the amount of β-actin and are shown relative to Ret expression in untreated cells. Ret expression in NCCmelb4M5 cells was lowest in the presence of 20 ng/ml BMP-4. The statistical significance of differences was evaluated by the Mann–Whitney U, test and $p < 0.05$ is considered significant. Values reported are means ± SD of triplicate determinations, and all experiments were repeated more than four times. (E) Analysis of the time-response of treatment with BMP-4 at 20 ng/ml. BMP-4 was added to the basic medium for varying incubation times as noted. Ret protein expression decreased in a time-dependent manner during the first 72 h. (F) Ret expression levels were normalized to the amount of β-actin and are shown relative to expression at day 1. Ret protein expression in NCCmelb4M5 cells was lowest after 3 days in the presence of 20 ng/ml BMP-4. The statistical significance of differences was evaluated by the Mann–Whitney U test, and a $p < 0.05$ is considered significant. Values reported are means ± SD of triplicate determinations, and all experiments were repeated more than four times.

when BMP-4 was added at 20 ng/ml. Ret protein expression in NCCmelb4M5 cells showed a dose-dependent decrease in expression elicited by BMP-4 (Fig. 1D). We further examined the time-dependent relationship between BMP-4 treatment and Ret expression in NCCmelb4M5 cells (Fig. 1E). Ret expression decreased in a time-dependent manner following 1, 2 or 3 days of incubation with 20 ng/ml BMP-4 (Fig. 1F). In contrast, there was no significant difference in expression of Ret protein by NCCmelb4 cells, NCCmelan5 cells or Mel-Ret cells in the presence of BMP-4. Based on these findings, the combined effects of Ret and BMP-4 might provide important clues towards understanding the roles and working mechanisms in melanocyte development.

To understand the precise mechanisms of BMP-4 signaling, we tested Smad proteins which have been implicated as downstream effectors of BMP signaling. Smad activation of BMP signaling was detected using an antibody against phosphorylated Smad 1/5/8 (p-Smad 1/5/8) (Fig. 2A). Levels of p-Smad 1/5/8 increased in NCCmelb4M5 cells treated with BMP-4