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LETTER TO THE EDITOR

Transcriptional activation of CCN1 and CCN2, targets of canonical Wnt signal, by ascorbic acid 2-phosphate in human dermal papilla cells

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Hair growth;
CCN1/Cyr61;
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β-Catenin;
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To the Editor

The mammalian hair follicle is highly organized, multi-layered, and dynamic organ. It contains dermal papilla and dermal sheath cells derived from the mesenchyme. It also contains epithelial cells of outer and inner root sheaths, matrix, and hair shaft, derived from the epithelium. The reciprocal interactions between the epithelium and mesenchyme are essential for post-natal hair growth as well as embryonic formation of hair follicle [1,2]. The post-natal hair follicle undergoes a cycle of anagen (the active growth phase), catagen (the apoptotic regression phase), and telogen (the resting phase) [1,2]. Dermal papilla (DP), a cluster of specialized fibroblasts, is believed to secret diffusible proteins which regulate the growth and activity of the various cells in the follicle, thereby plays a key role in the regulation of hair cycling and growth [1,2].

We have recently reported that L-ascorbic acid 2-phosphate magnesium salt (Asc 2-P), a derivative of L-ascorbic acid (Vitamin C), stimulates the growth of human dermal papilla cells, promotes the elongation of hair shafts in hair follicles in culture, and induces early conversion from a telogen phase to an anagen phase in mice [3]. These results suggest that Asc 2-P regulates expression of genes in dermal papilla cells and thereby affects hair growth and cycling. Consistent with this, we found that Asc 2-P induces expression of versican in human dermal papilla cells [4]. Versican is known as a target gene of canonical Wnt/β-catenin signaling pathway [5] and is suggested to play an important role in anagen induction and maintenance of the normal growing phase [6]. Versican promoter is known to be activated by nuclear accumulation of β-catenin which complexes with TCF transcription factors in vascular smooth muscle cells [7].

Recently, an expression profiling analysis of mesenchymal stem cells stimulated by Wnt3A revealed that CCN1/Cyr61 and CCN2/CTGF, members of the CCN family, were among the genes most significantly up-regulated by Wnt3A [8]. Si et al. [8] also demonstrated that CCN1/Cyr61 is a direct target gene of canonical Wnt/β-catenin signaling by chromatin immunoprecipitation analysis. These data, together with our previous finding of induction of versican by Asc 2-P [4] prompted us to examine whether Asc 2-P induces CCN1/Cyr61 and CCN2/CTGF transcription and nuclear accumulation of β-catenin in human dermal papilla cells.

Hair biopsy specimens were obtained from the non-balding occipital scalp region of patients with androgenic alopecia during hair transplantation at Kyungpook National University Hospital (Daegu, Republic of Korea) with patients' consents. Hair follicles were isolated and cultured by the method described previously [9]. Dermal papilla was isolated from the bulbs of dissected anagen hair follicles and cultured as described previously [10]. Primary cultured cells of the two to three passages were used in this study.

We observed that nuclear β-catenin is increased by Asc 2-P, as examined by the western blot analysis

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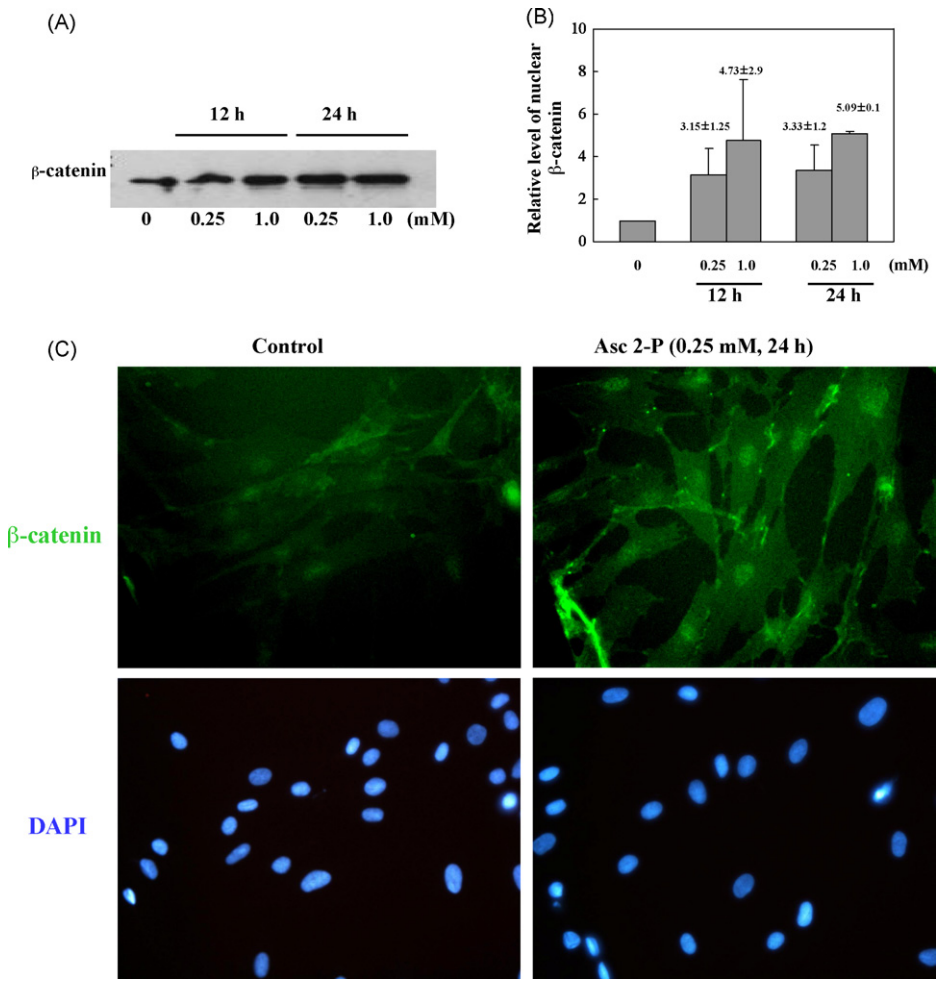

Fig. 1 (A) Western blot analysis of β-catenin. Dermal papilla cells were treated with Asc 2-P and total nuclear fractions (3 μg/lane) were probed with anti-β-catenin antibody. (B) The relative level of nuclear β-catenin as measured from the western blot analysis by densitometry. Values represent the mean ± S.D. from three independent experiments. (C) Immunostaining of β-catenin in dermal papilla cells. Cells were treated with 0.25 mM Asc 2-P for 24 h. DAPI nuclear staining is also shown in bottom panel.

(Fig. 1A and B). Immunocytochemical staining also showed nuclear accumulation of β-catenin by Asc 2-P treatment (Fig. 1C). Consistent with this, CCN1/Cyr61 and CCN2/CTGF expression, which is under Wnt/β-catenin control, were increased in dermal papilla cells after Asc 2-P treatment (Fig. 2A and B). We also found that dermal papilla *in vivo* indeed express β-catenin, CCN1/Cyr61 and CCN2/CTGF (Fig. 2C).

Our data together with the finding of Si et al. [8] demonstrate that Asc 2-P induces CCN1/Cyr61 and CCN2/CTGF via Wnt/β-catenin pathway in human dermal papilla cells. How the Asc 2-P regulates the Wnt/β-catenin pathway is not clear. However, we speculate that Asc 2-P causes nuclear β-catenin accumulation by inactivation of GSK-3β which is not only an upstream effector of β-catenin but also a downstream target of PKB. This idea is in line with the finding that Asc 2-P activates/phosphorylates PKB in human dermal papilla cells [4].

Since CCN family proteins are secreted extracellular matrix proteins regulating multiple cellular processes such as cell proliferation, migration, differentiation, angiogenesis [8], transcriptional activation of CCN1 (Cyr61) and CCN2 (CTGF) by Asc 2-P in human scalp dermal papilla cells may control human hair cycling/growth. For example, CTGF may play an important role in induction and maintenance of hair growth phase (anagen) by inhibition of molecules such as BMP and TGF-β family proteins which are important factors for regulating telogen-to-anagen and anagen-to-catagen transition in hair cycling [1]. In this regard, it is interesting to note that Asc 2-P not only promotes the elongation of human hair shafts in isolated hair follicles in culture [3], but also induces early