Anti-wrinkle effect of bone morphogenetic protein receptor 1a-extracellular domain (BMPR1a-ECD)

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Bone morphogenetic proteins (BMPs) have diverse and important roles in the proliferation and differentiation of adult stem cells in our tissues. Especially, BMPs are well known to be the main inducers of bone formation, by facilitating both proliferation and differentiation of bone stem cells. Interestingly, in skin stem cells, BMPs repress their proliferation but are indispensable for the proper differentiation into several lineages of skin cells. Here, we tested whether BMP antagonists have an effect on the prevention of wrinkle formation. For this study we used an in vivo wrinkle-induced mouse model. As a positive control, retinoic acid, one of the top anti-wrinkle effectors, showed a 44% improvement compared to the non-treated control. Surprisingly, bone morphogenetic protein receptor 1a extracellular domain (BMPR1a-ECD) exhibited an anti-wrinkle effect which was 6-fold greater than that of retinoic acid. Our results indicate that BMP antagonists will be good targets for skin or hair diseases. [BMB Reports 2013; 46(9): 465-470]

INTRODUCTION

In our body, when any tissue is lost or damaged, stem cells in each tissue start to replicate and then differentiate in a specific spatiotemporal manner so as to effect the repair of lost or damaged tissues (1, 2). In the skin stem cells keep dividing and differentiating in a series of steps, and undergoing apoptosis prior to shedding from the skin only to be replaced newly differentiated cells, which allows our skin to be rejuvenated (3, 4).

The occurrence of wrinkles is one of the spontaneous phenomena of the aging process (5). Many diverse factors such as the synthesis of collagen or elastin, UV exposure, and integrity of the skin epidermis layer are known to be involved in wrinkle formation. The synthesis and degradation of collagen or elastin in the skin dermis layer is one of main factors involved in wrinkle biology (6). UV irradiation exposure is also known to be an inducer of wrinkles (7). Nowadays new biological roles of known or unknown proteins are being identified by many research groups, using advanced techniques in proteomics. Diverse cell signal transduction pathways such as Wnt, FGF (fibroblast growth factor), Notch, TGF-β (transforming growth factor beta) are involved in skin stem cell proliferation and differentiation (8, 9). Recent research articles have supported that inappropriate skin stem cell biology is one of major inducers of wrinkle formation (3, 8).

Homeostasis in the skin epidermis layer is repeated throughout life, as stem cells continue to undergo a series of replication, stepwise differentiation, apoptosis, and shedding from the skin (4). The homeostasis interval time in our skin increases with age (10). For example, the homeostasis interval time for someone in their twenties is about 20 days whereas it is increased to almost 40 days for someone in their forties, which indicates that the turnover interval time increase with age also affects wrinkle formation, hair whitening, acne, hair growth, and atopic dermatitis.

Wnt ligands and BMPs (bone morphogenetic proteins) are the two main regulators of skin stem cell proliferation and differentiation (11, 12). In a dormant state, activated BMP2 and BMP4 signals repress skin stem cell proliferation. When BMP2 and BMP4 activity is repressed by endogenous antagonists or exogenous antagonist treatment, skin stem cell proliferation signal is activated and starts to produce multiple undifferentiated skin cells, an effect which is further augmented by an activated WNT signaling pathway, mainly induced by the wnt5a ligand. Interestingly, WNT and BMP signals are absolutely required for the proper differentiation into several lineages of finally differentiated skin cells (4).

Our aim is to screen proteins which prevent wrinkle formation or reduce the effect of pre-existing wrinkles. In this experiment, we used an in vivo hairless mouse model to test for anti-wrinkle effects. As a positive control, retinoic acid showed a 44% anti-wrinkle improvement activity. Surprisingly bone morphogenetic protein receptor 1a extracellular domain...
(BMPR1a-ECD) showed an extremely strong anti-wrinkle effect with an approximate 300% anti-wrinkle improvement, or about 6-fold greater than that of retinoic-acid.

RESULTS

Recombinant BMP2, noggin, and BMPR1a-ECD protein production

Recombinant BMP2 and noggin were purified by the published protocols (13, 14) (Fig. 1A, B). Recombinant BMPR1a-ECD is itself not well folded and is unstable when expressed in E. coli. To solve this problem, the fusion protein TRX-BMPR1a-ECD has been constructed by attaching the thioredoxin (TRX) gene as a fusion partner. TRX is commonly used as a fusion domain of an interest target protein to improve several shortcomings such as poor solubility, incorrect folding and a low expression level (15). BMPR1a-ECD was purified to a high degree through the established protocol (Fig. 1C, lane 2), where TRX is clearly removed.

Noggin or BMPR1a-ECD protein efficiently inhibits the SMAD1 signal triggered by BMP2 ligand in a concentration dependent manner

BMP ligands send distinct signals through sequential bindings of type II and type I receptors (16). Several kinds of type II and type I receptors have been identified, and distinct combinations of type II and type I receptors for each BMP ligand are necessary for the full signal transduction (17). BMP2 or BMP4, which are the main regulators of skin stem cell proliferation and differentiation, bind to bone morphogenetic protein receptor type 1a (BMPR1a) to send appropriate biological signals (18). Many endogenous BMP antagonists including Noggin, Chordin, Cerberus, glypican-3 and Follistatin have been identified, among which noggin is known to have a strong inhibition activity for the BMP signal pathways (19).

To ensure the biological activity of Noggin or BMPR1a-ECD, and to test the repression of this signal pathway by its treatment, we used the SMAD-1, 5, 8 luciferase assay, in which SMAD 1/5/8 signal is converged by BMP ligands (20). As expected, the SMAD 1/5/8 signal induced by BMP2 treatment was repressed by the addition of serially increased Noggin or BMPR1a-ECD, confirming their good BMP signal inhibition effects (Fig. 1D, E). When 20 nM Noggin was added to a 10 nM BMP2 treated C2C12 cell culture, the SMAD-1 signal index induced by BMP2 was about 50% decreased and similarly, was approximately 59% decreased by treatment of 20 nM BMPR1a-ECD.

Preparation of liposome-encapsulated protein samples

The skin follicle is a reservoir of stem cells and a location in which stem cell proliferation and differentiation actively occur (11). The bulge stem cells, located at the upper right side of skin follicles, are multi-potent progenitor cells and all the skin cells are derived from these bulge stem cells when aged through repeated turnover or when damaged. Also epidermis stem cells are continuously connected to the follicle stem cells to relay and share signals triggered by protein ligands in skin follicles. The orifice of a skin follicle is wide enough for protein delivery but is filled with fatty acid mixtures secreted by sebaceous gland, located in the upper side of the skin follicle (21). To allow for the successful movement of target proteins into skin follicles, hydrophilic proteins may be delivered by hydrophobic delivery vehicles. In this study, we used liposomes as a delivery carrier. Hydrogenated lecithin, one of several kinds of liposomes, was mixed with BMP2, Noggin or BMPR1a-ECD and was homogenized several times at 800bar to encapsulate each target protein into liposomes until liposome sizes ranged 100-200 nm (Fig. 2). It has been reported that 100-200 nm liposomes suitable for efficient delivery into skin follicles, based on accumulated data revealed by many groups, including our own (22). In Lipo/BMP2, Lipo/Noggin and Lipo/BMPR1a-ECD samples, the liposome sizes ranged 100-200 nm (Fig. 2).