약학석사 학위논문

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Aminoacyl tRNA synthetase complex interacting multifunctional protein 1 induces proliferation of CD34^{+} hair follicle stem cell

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ABSTRACT

Aminoacyl-tRNA synthetase interacting multifunctional protein 1 (AIMP1) is a factor associated with a multi-tRNA synthetase complex (MSC). Once it is dissociated from the MSC complex, AIMP1 is secreted by various ques and has diverse functions such as immune activation, angiogenesis, and fibroblast and stem cell proliferation. Recent reports published that AIMP1 is secreted from the dermal papilla (DP) cell and induces proliferation of fibroblast and stem cell. Here, we figured out that AIMP1 is secreted from dermal papilla cell in sonic hedgehog (Shh) signal-dependent manner and demonstrated that secreted AIMP1 increases proliferation of hair follicle stem cells (HFSC) and affects hair growth. Interestingly, only the partial part of AIMP1 was detected in media and it was proven to be an N-terminal fragment as demonstrated by several antibodies with different epitope binding site. We found that sFRP1, widely known as wnt antagonist, was identified as an AIMP1 binding protein through GST pull-down assay. We also validated that AIMP1 specifically inhibits the role of sFRP1 and promotes wnt signal pathway in $CD34^+$ hair follicle stem cells. N-terminal fragment of AIMP1 increased the gene expression level of wnt-downstream as well as proliferation of isolated $CD34^+$ hair follicle stem cells. In addition, we generated the shorten peptide version corresponding N-terminal fragment of AIMP1 to be developed as a therapeutic peptide against hair loss-related diseases. Applying peptide topically on depilated mouse showed significant increase of hair growth and improved its efficacy by formulating with carbomer. Taken together, we

identified a novel secreted mechanism of AIMP1 and activity of its N-terminal fragment on hair follicle stem cells and these results shed light on new therapeutic approach in alopecia.

Key words: Alopecia, AIMP1, HF, DP, Shh, sFRP1, hair growth

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CONTENTS

LIST OF FIGURES

ABBREVIATION LIST

AIMP1: Aminoacyl-tRNA synthetase interacting multifunctional protein 1

N-AIMP1: N-terminal domain of AIMP1

sN-AIMP1: small N-terminal domain of AIMP1 (6-46 a.a.)

HF: Hair follicle

DP: Dermal papilla

ORS: Outer root sheath cell

GM: Germinal matrix cell

Shh: Sonic hedgehog

sFRP1: Secreted frizzled-related protein 1

LGR5: Leucine rich repeat containing G protein-coupled receptor 5

TCF7: Transcription factor 7

AXIN2: Axis inhibition protein 2

ELISA: Enzyme-linked immunosorbent assay

qRT-PCR: Quantitative real-time polymerase chain reaction

INTRODUCTION

Alopecia areata (AA) is an unpredictable and nonscarring hair loss condition. Among the many factors under investigation in the pathogenesis of AA, the main etiological causes have been genetic constitution (1, 2), organ-specific autoimmune reactions(3) and non-specific immune(4). But, the pathogenesis of AA is still unknown. At present, most treatments are palliative, only regulating the problem; they can't cure the cause of the disease definitely. All local treatments may help the treated areas, but do not prevent further spread of the condition. Minoxidil, which is most common baldness treatment(5), has been used to slow balding and promote hair growth. It has a direct effect on the proliferation and differentiation of follicular keratinocytes in vitro, and regulates hair physiology independent of blood flow influences(6).

Originally, Aminoacyl-tRNA synthetase interacting multifunctional protein 1 (AIMP1) was reported as a consist of multi-ARS complex in mammalian(7). As well as, AIMP1 itself is secreted as a cytokine; it functions with various target cells including endothelial cells, monocyte/macrophage cells, and dendritic cells(8-10). Also, each function of AIMP1 domains was revealed by deletion mapping analysis(11).

N-terminal domain of AIMP1 (amino acid 6-46) was previously reported as an inducer of fibroblast and bone marrow-derived mesenchymal stem cells (BMMSCs) proliferation. Its proliferative activities were induced by activated

extracellular signal-regulated kinase (Erk) and β-catenin/Tcell factor (TCF) complex respectively(12, 13).

In recent studies, AIMP1 was found in medium of DP cells but its role in hair follicle environment was in veiled(14). Since many clues indicate that AIMP1 might have a role in hair follicle, we sought for its function in hair by testing the level of AIMP1 expression in skin. Interestingly, AIMP1 expression level is decreased by aging in hair follicle. So, in this paper, we investigated role of AIMP1 in hair follicle.

AIMP1 is secreted by the secreted signaling molecule sonic hedgehog (Shh), which is critical role in both embryonic hair follicle development and adult hair cycling(15, 16). In early anagen stage (periods of active hair growth), Shh expression is up-regulated for maturation of the dermal papilla(17). Interestingly, maturated dermal papilla cells by Shh only secrete partial part of AIMP1 including N-terminal domain. This part of AIMP1 binds to Secreted frizzled related protein 1(sFRP1), an antagonist of the wnt signal(18). And we demonstrated 6-46 sequences are the main functional domain for binding sFRP1 by GST pull-down assay. Consequentially, hair follicle is stimulated by secreted N-terminal domain of AIMP1, through the inhibition of sFRP1, thereby stimulating wnt pathway.

Since AIMP1 is incompatible for skin thereby with its penetration issue, we generated small peptide that is suitable for skin penetration with conserved hair growth activity.

At last, we carried out formulation study to increase the ability of peptide absorption and encourage the use of naturally generated peptide on patients with hair loss related diseases.

MATERIAL AND METHODS

Cell culture and materials

Human dermal papilla (DP), human germinal matrix (GM), human out root (ORS) cells were purchased from Sciencell and were cultured in Mesenchymal stem cell media 10% fetal bovine serum with 50ug/ml streptomycin and penicillin. at 37℃ in a 5% CO_2 incubator. And RAW 264.7 cells for TNFa ELISA were purchased from American Type Culture Collection bank. Human Shh was purchased from Milteny Biotec. Human recombinant sFRP1 was purchased from MyBioSource.

In vivo efficacy test (Murine depilation model

Dorsal of wild-type mice (C57BL/6, postnatal 49 days) were depilated by clipper. Remained hair removed by hair removal cream. After 1 day later, 100nM sN-AIMP1 were mixed with PBS contained 20% glycerol and applied on dorsal skin by brush. As a vehicle control, PBS contained 20% glycerol is used. Specific-pathogen free (SPF) Mice were purchased from OreientBio (Seongnam, Korea). Whole experiments were conducted in Woojung BSC (Suwon, Korea)

Antibodies

Primary antibodies used AIMP1 [(N-terminal (goat, Novus), C-terminal (rabbit, Abcam) 6-46 (customized production, Younginfrontier)] AIMP2 (rabbit, Abcam) EPRS (rabbit, Abcam) Tubulin (mouse, NOVUS), sFRP1 (Rabbit, NOVUS)

Secondary antibodies used Goat anti-rabbit IgG Alexa 488 and 647 (Thermo fisher) used for immunofluorescence. Goat anti-mouse IgG HRP and Goat anti-rabbit IgG HRP (thermos fisher) used for immunoblot.

Quantitative real-time PCR

To compare gene expression, RNA was extracted by RNeasy mini kit (Qiagen) according to the manual. Maxima first strand cDNA synthesis kit was used for cDNA synthesis according to the manufacturers recommendations. qRT-PCR was performed using the 7500 Real-time PCR system (Thermo Fisher) and SYBR green Master Mix (Thermo Fisher) The following primers were used:

species	gene	primer	Sequence
mouse	**CD44**	Forward	5`-CGTCCAACACCTCCCACTAT-3`
		Reverse	5`-AGCCGCTGCTGACATCGT-3`
	LGR5	Forward	5`-TGCCCATCACACTGTCACTG-3`
		Reverse	5`-CACCCTGAGCAGCATCCTG-3`
	TCF7	Forward	5`-AGGCAGGGAACAGGACGATA-3`
		Reverse	5`-TTCTCCATGTACTCGGACGC-3`
	AXIN2	Forward	5`-GAGTAGCGCCGTGTTAGTGACT-3`
		Reverse	5`-CCAGGAAAGTCCGGAAGAGGTATG-3`
	GAPDH	Forward	5`-CGCTCTCTGCTCCTCCTGTTC-3`
		Reverse	5`-TTGACTCCGACCTTCACCTTCC-3`

Histology and Immunofluorescence.

To analyze histology, backskins were dissected, fixed overnight in 4% formaldehyde in PBS, and embedded in paraffin. Dorsal skins were cut using Reica microtome(8um). Sections from mouse dorsal skin were stained with

hematoxylin/eosin. Immunofluorescence analysis of mouse skin hair follicle were performed as described(19). Deparaffinized the slides in xylene, and followed by rehydration through a graded ethanol series Immerse slides in 10mM citric acid (pH 6.0) and incubate in microwave 15min. As blocking reagents mouse on mouse IgG blocker (Vector lab) and CAS blocker (Life technologies) were used. Primary Abs were incubated overnight at 4°C and secondary Abs conjugated to Alexa 488 and 647 were incubated for 1h at room temperature. Nucleus staining was done with DAPI in 1:1000. Images were acquired with Canon A1 confocal microscopy.

Isolation of single cells in mouse backskin

Mouse HFs and epithelial cell isolation method was based on previous report(20). To prepare single-cell suspensions from telogen backskin, subcutaneous fat was scraped off with a scalpel and backskin was placed (dermis side down) on trypsin (Gibco) at 37 °C for 1hour. To obtain single epithelial cell suspensions, HFs and epidermal cells were scraped off gently from all trypsinized backskins with a scalpel and filtered with strainers (70μm, followed by 40μm). After centrifugation at 500g for 20min, RNA was purified from cell using GeneJET RNA purification kit (Thermo) following as manufacturer recommendation

Preparation of recombinant human AIMP1 fragments

The constructs of whole AIMP1, AIMP1-(1-312 a.a.), and AIMP1 deletions [namely AIMP1-(1-192 a.a.), AIMP1-(193-312 a.a.)] were described previously(11). Each of the whole AIMP1 and AIMP1-deleted constructs was expressed as a GST-tag fusion protein in E. coli Rosetta and purified by glutathione

S bead as described previously(21). Lipopolysaccharide was removed by filtering through Mustang E membrane (0.2um, Pall life sciences).

GST Pull-down assay

To confirm interactions by the in vitro pull-down assay, sFRP1 protein(2ug/ml) were incubated with purified GST-AIMP1 fragment(4ug/ml) for 2 hours. Binding assay was conducted in 25 mmol/L Tris-HCl buffer (pH 7.4) containing 120 mmol/L NaCl, 10 mmol/L KCl, and 0.5% Triton X-100.

Dye conjugation and skin penetration validation

Peptides were incubated with Flamma 552 NHS ester(Bioacts) for 2 hours at RT. Non-conjugated dye was removed by PD-10 column (GE Healthcare) using size exclusion method. Mouse (C57Bl/6, male, postnatal 49 day) were dorsal back skin by clipper and hair removal cream. Next day, spread the dye conjugated peptide on dorsal area. Skin samples were embedded in OCT compound (Sakura, 4583) and cryo-sectioned (8uM) By cryostat (NA200F). Nucleus staining was done with DAPI in 1:1000.

RESULTS

AIMP1 N-terminal fragment is secreted by Shh in dermal papilla cell

Previously, AIMP1 was shown to be secreted from dermal papilla, and fibroblast cells(14). As dermal papilla cell regulates the phase of hair follicle growth upon different signals(22), we first tested with various signals that relates hair cycles. As a result, we found that cleaved form of AIMP1 was detected in medium upon Shh signal (Fig. 1A). As secreted from anagen phase, Shh is crucial for hair development and cycling. (23) And, to check the cell specificity, we tested secretion of AIMP1 in various hair follicle cells. But, AIMP1 fragment did not detected outer root sheath (ORS) cell and germinal matrix (GM) cell by Shh (Fig. 1B) This phenomenon was further validated by dose-dependent manner (Fig. 1C). Then we conduced immunoblotting with three AIMP1 antibodies with different binding epitopes. Surprisingly, only antibody with N-terminal epitope successfully captured cleaved AIMP1 (Fig. 1D). we hypothesized that cleaved N-terminal part of AIMP1 might play a critical role in the anagen phase of hair cycle.

Secreted AIMP1 N-terminal domain interacts with sFRP1

To investigate the role of cleaved AIMP1 in hair growth, we first searched for its known binding partners that has role in hair microenvironment. In fact, secreted frizzled-related protein 1(sFRP1), known as a wnt antagonist, was selected as a candidate and we tested whether cleaved form of AIMP1 binds to the sFRP1(21). After purifying several types of domain with GST tag, we carried out GST pull-down assay and found out that N-terminal domain of AIMP1 (N-AIMP1)

bound to sFRP1 stronger than any other fragments including full size of AIMP1 (F-AIMP1) (Fig. 2A). Considering development of applicable therapeutics for hair treatment, we focused on generating smaller peptides originated from 1-192 a.a. of AIMP1. Since central domain (101-192 a.a.) of AIMP1 is reported as inflammation inducer via interaction with CD23, we searched for the region that could avoid side effect generated from inflammation(21). In fact, selected 6-46 a.a. of AIMP1 was related with proliferation, we tested its effect on inflammation and hair growth. Using LPS as a control, we found that small N-terminal AIMP1 fragment (sN-AIMP1) does not induce TNF alpha secretion but 1-192 a.a. with significant increase of TNF alpha release (Fig. 2B). Next by competitive GST pull-down assay, we further validated that sN-AIMP1 (6-46 a.a.) decreased N-AIMP1 (1-192 a.a.) binding to sFRP1 in concentration dependent manner (Fig. 2C). we concluded that sN-AIMP1 is main domain of binding with sFRP1.

AIMP1 small N-terminal fragment (6-46 a.a.) promotes proliferation of $CD34^+$ hair follicle stem cells

Since sFRP1, antagonist of wnt signal, is secreted in telogen phase from hair follicle stem cells (HFSCs), secreted sFRP1 acts a role in maintaining the stemness of HFSCs in telogen phase(24). So, we thought that AIMP1 would block the function of sFRP1 and increase wnt signal to promote proliferation of hair follicle stem cells. The mechanism of AIMP1 ultimately acts an important function in transition from telogen phase to anagen. For validate this hypothesis, we isolated hair follicle stem cells by stem cell marker, CD34. We treated sN-AIMP1 to isolated $CD34^+$ hair follicle stem cells and analyzed wnt downstream genes by

qRT-PCR. As shown in result, sN-AIMP1 treated HFSCs wnt downstream genes are up-regulated compared to control. Furthermore, we checked the proliferation of hair follicle stem cells by sN-AIMP1. When sN-AIMP1 was treated, relative cell number of the hair follicle stem cell was sufficiently increased (Fig. 3B). So, we conclude that N-terminal domain of AIMP1 increases the proliferation of hair follicle stem cells through the inhibition of sFRP1.

AIMP1 is decreased with aging process in hair follicles

To analyze expression of AIMP1 in physiological condition, we examined aging-associated back skin of C57BL/6 mouse. Through H&E staining of 3 different aged group of mice, we found that mice were getting their hair lost and miniaturized HFs along their aging process. (Fig. 4A) Particularly, the number of HF was shown to decrease starting from 8 weeks old group (Fig. 4B) and miniaturized HF was observed only from 24 months old group (Fig. 4A, black arrow). As AIMP1 was previously reported as a proliferation inducer in certain cells such as fibroblast and stem cells (4, 5), we next checked the protein level of AIMP1 in each group of mice. Interestingly, AIMP1 expression was decreased dramatically along the aging process but other components in MSC complex such as AIMP2, and EPRS showed different staining patterns (Fig. 4C). The expression level of EPRS was not changed but AIMP2 was increased (Fig. 4D, E). AIMP2 was known as an apoptosis inducer under DNA damaged condition through interaction with p53(22). In fact, its commonly known that DNA damage is accumulated in aged animals skin and this natural process might explain the change

of AIMP2 level (23). With the decrease of AIMP1 expression in aging process, we concluded that AIMP1 has a role in hair follicle environment and suggested.

AIMP1 small N-terminal fragment (6-46 a.a.) has hair growth effect.

To observe the degree of hair growth promoting effect of sN-AIMP1, we shaved the dorsal back of seven-week-old wild type mice for synchronizing in telogen phase (resting phase). At the post depilation 13days, sN-AIMP1 treated mouse showed greater hair growth compared to its control (Fig. 5A). It is quantified by histology analysis. Most mice treated with sN-AIMP1 entered the anagen phase. As evidence of entry into the anagen phage, thickness of dermis also increased(25). (Fig. 5C) To analyze which signal is changed in hair follicles, we isolated epithelial and hair follicle cells from sN-AIMP1 applied mouse and control mouse. 8 hours later, G protein-coupled receptor 5 (LGR5) containing leucine-rich repeat known as a stem cell marker, and wnt signal target gene were increased(26). Under the same conditions, expression of wnt target genes including transcription factor 7 (TCF7), and axis inhibition protein 2 (AXIN2) was up-regulated (Fig. 5D)(27). Through in vivo data and qRT-PCR result, sN-AIMP1 promoted hair growth and starting anagen phase by stimulating wnt signal.

AIMP1 small N-terminal fragment (6-46 a.a.) penetrates from epithermal skin to hair follicle.

We conducted mouse skin penetration experiments to confirm whether sN-AIMP1 only stays on the skin or it absorbs down to the hair follicle. Since peptide of sN-AIMP1 was not suitable to penetrate directly through the skin, we

checked its absorption ability along the hair follicle. To check skin penetration, we prepared sN-AIMP1 conjugated with Flamma® 552 HHS ester as a dye. After incubating sN-AIMP1 with dye, fractions were eluted by PD-10 column using size exclusion method. Fractions were checked with fluorescence plate reader to check the intensity of sN-AIMP1 conjugated with dye (Fig. 6A). Fluorescence microscopy was used to analyze the penetration of sN-AIMP1 in time dependent manner. Free dye was observed at 12 hours having intercellular penetration while sN-AIMP showed follicle penetration at 24 hours (Fig. 6B). We observed that sN-AIMP1 stimulates hair growth by follicle penetration.

Hair growth is enhanced by formulation

In order to enhance the hair growth effect of sN-AIMP1, we have tried formulation. Carbomer, a large polymeric chemical composed of acrylic acid monomers, was used as the formulation reagent because it is widely used in cosmetics especially in skin care products(28) to control viscosity and moisturizing. So, mixture of sN-AIMP1 and carbomer was applied on the dorsal skin of mice to check of enhanced effect. Noticeable increase in hair growth was observed (Fig. 7A). So, we provisionally concluded to increase its effectiveness in carbomer-based. We hypothesized that the increase in the hair growth effect of sN-AIMP1 using carbomers is due to the continuous signal transfer in stable compounds. We tracked using sN-AIMP1 conjugated with the florescent dye in same method as we did for penetration analysis. Carbomer maintain a much thicker and longer lasting florescence signal than PBS (Fig 7B). So, we conclude sN-AIMP1 can be developed as a therapeutic peptide for hair loss.

Figure 1. AIMP1 N-terminal fragment is secreted by Shh in dermal papilla cell

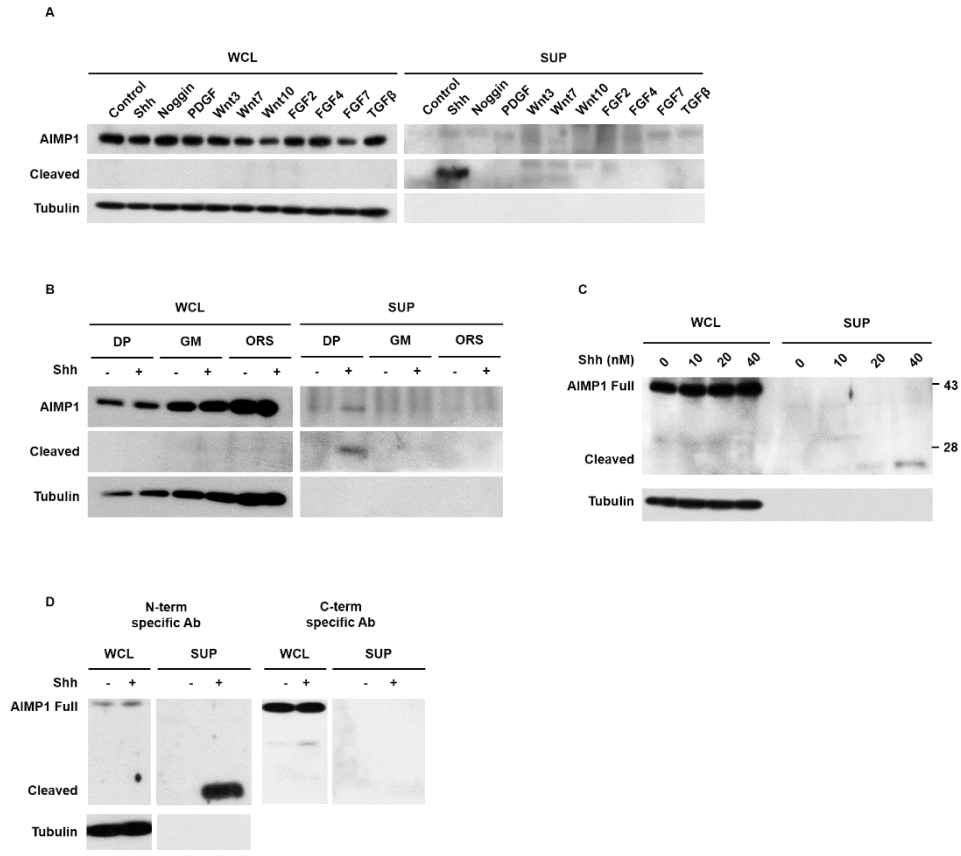

Figure 1. N-AIMP1 is secreted by SHH in dermal papilla cell

A) Screening for which signal promotes secretion of AIMP1 N-terminal fragment from dermal papilla cells among the secreted signals in hair follicle. AIMP1 detected by immunoblot analysis from cell lysate or TCA-precipitated media. Blots were probed with AIMP1 N-terminal antibody.

B) AIMP1 secretion shows Shh concentration dependent.

C) Among the various cells forming the hair follicle, only DP cell secrete AIMP1 fragment by Shh signal D) Detection of secreted AIMP1 fragment by 3 different epitope antibodies.

Figure 2. Secreted AIMP1 N-terminal fragment interact with sFRP1

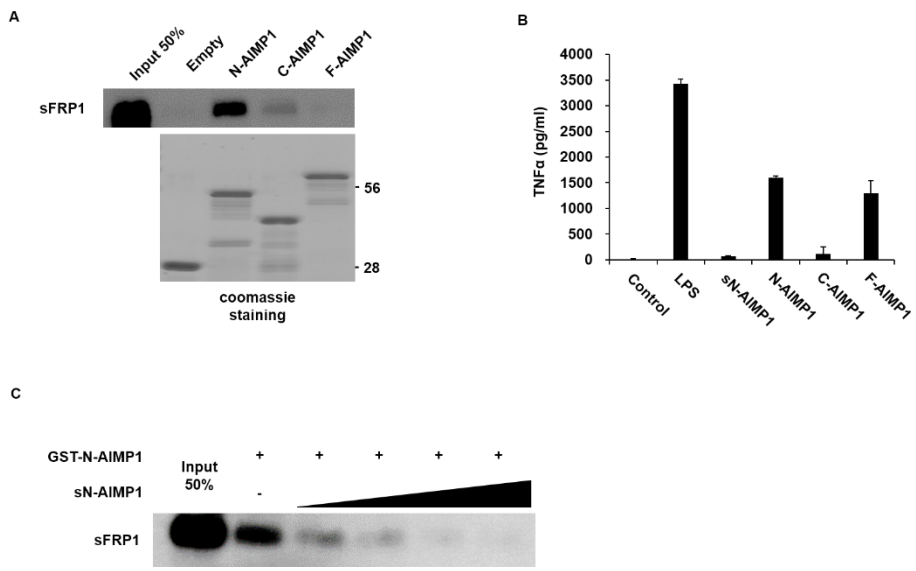

Figure 2. Secreted AIMP1 N-terminal fragment interact with sFRP1

A) GST pull-down experiment revealed that AIMP1 N-terminal domain interact with sFRP1.

B) The effects of AIMP1 and its fragmentation on TNFα induction. RAW cells were treated with AIMP1 and its deletion constructs, and TNFα secretion was monitored. Empty vector (EV) was used as the control.

C) Competition GST-pull-down assays. AIMP1 6-46 was pre-incubated with sFRP1 and mixed in the presence of GST AIMP 1-192 fragment bound beads.

Figure 3. Secreted AIMP1 N-terminal fragment promotes proliferation of CD34^{+} hair follicle stem cell

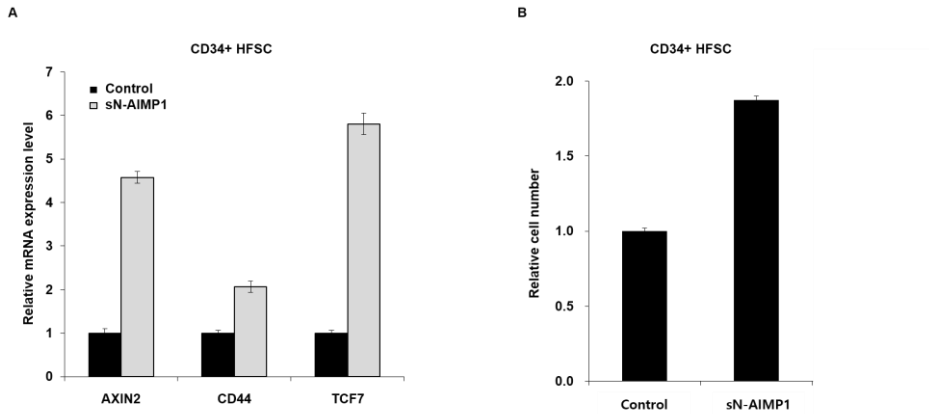

Figure 3. Secreted AIMP1 N-terminal fragment promotes proliferation of CD34+ hair follicle stem cells

A) Quantitative RT-PCR analysis of AXIN2, CD44, and TCF7 mRNA expression in isolated CD34+ hair follicle stem cell in sN-AIMP1 treated cells and control cells.

B) Proliferation of HFSC in sN-AIMP1 and control

Figure 4. AIMP1 is decreased with ageing process in hair follicle

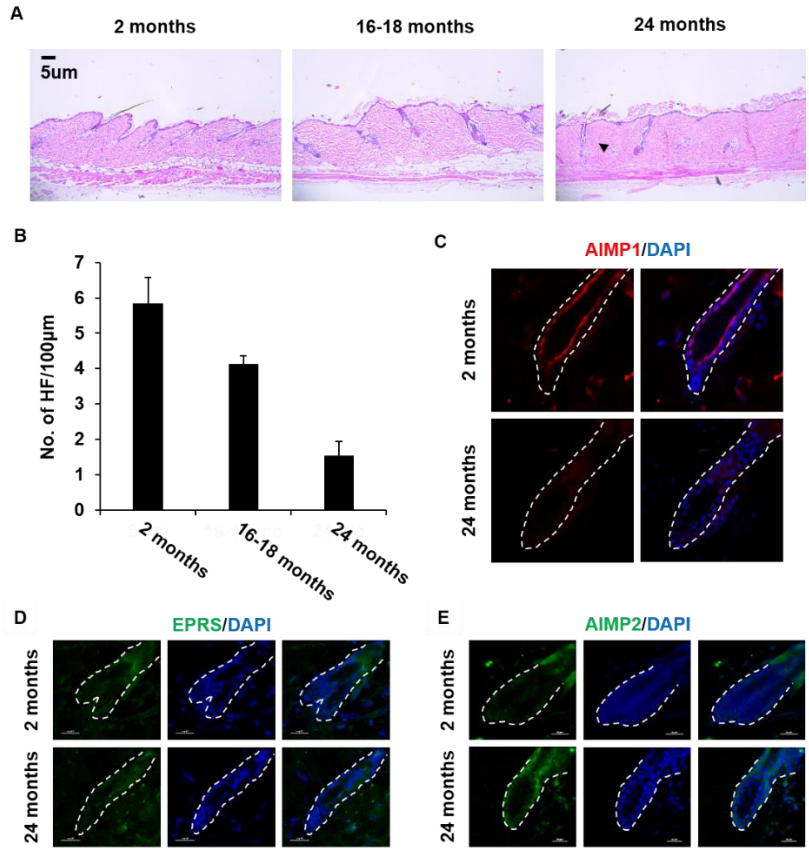

Figure 4. AIMP1 is decreased with age in hair follicle.

A) Histology of the dorsal skin from young (8 weeks), middle-aged (12-18 months) and aged (24 months) wild-type C57BL/6 by hematoxylin and eosin (H&E) stain. The arrow is miniaturizing HF.

B) Quantification of HF number per 100um.

C) IF images of HFs at 8 weeks and 24 months for AIMP1.

D) IF images of HFs at 8 weeks and 24 months for EPRS. E) IF images of HFs at 8 weeks and 24 months for AIMP2. Hair follicles are outlined with dotted lines.

Figure 5. AIMP1 N-terminal fragment (6-46) have hair growth effect.

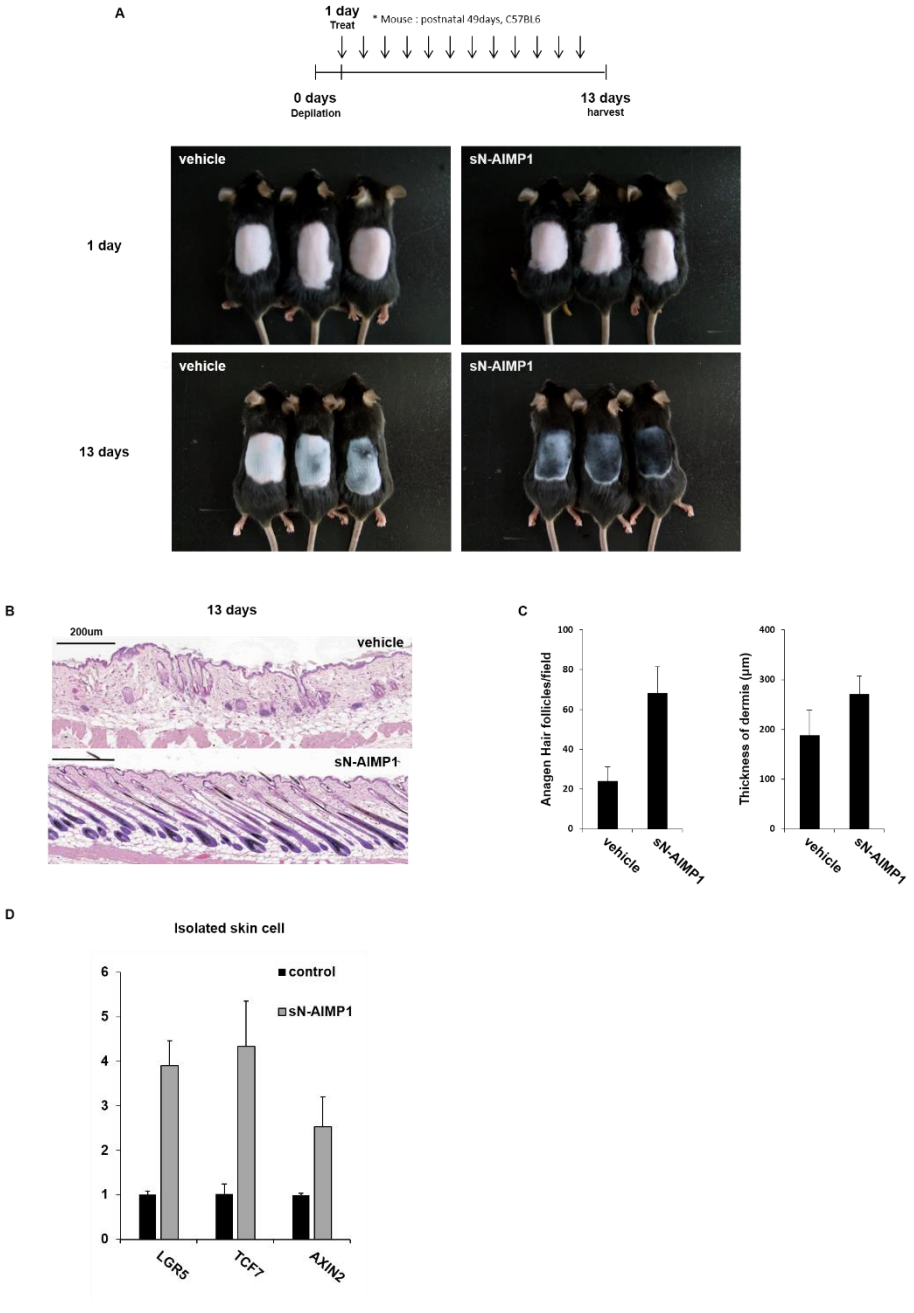

Figure 5. AIMP1 N-terminal fragment (6-46) have hair growth effect.

A) Representative images of hair coat after hair depilation treated PBS(vehicle), Neo-Pep at post depilation(PD) 13 days.

B) Histological images of control and Neo-Pep treated mouse skin.

C) Number of anagen hair follicle and thickness of dermis at PD 13 days.

D) Quantitative RT-PCR analysis of CD44, LGR5, TCF7, and AXIN2 mRNA expression in isolated skin cells in Neo-Pep treated mice (8hours later) and in control mice.

Figure 6. AIMP1 N-terminal fragment (6-46) penetrates from epithermal skin to hair follicle.

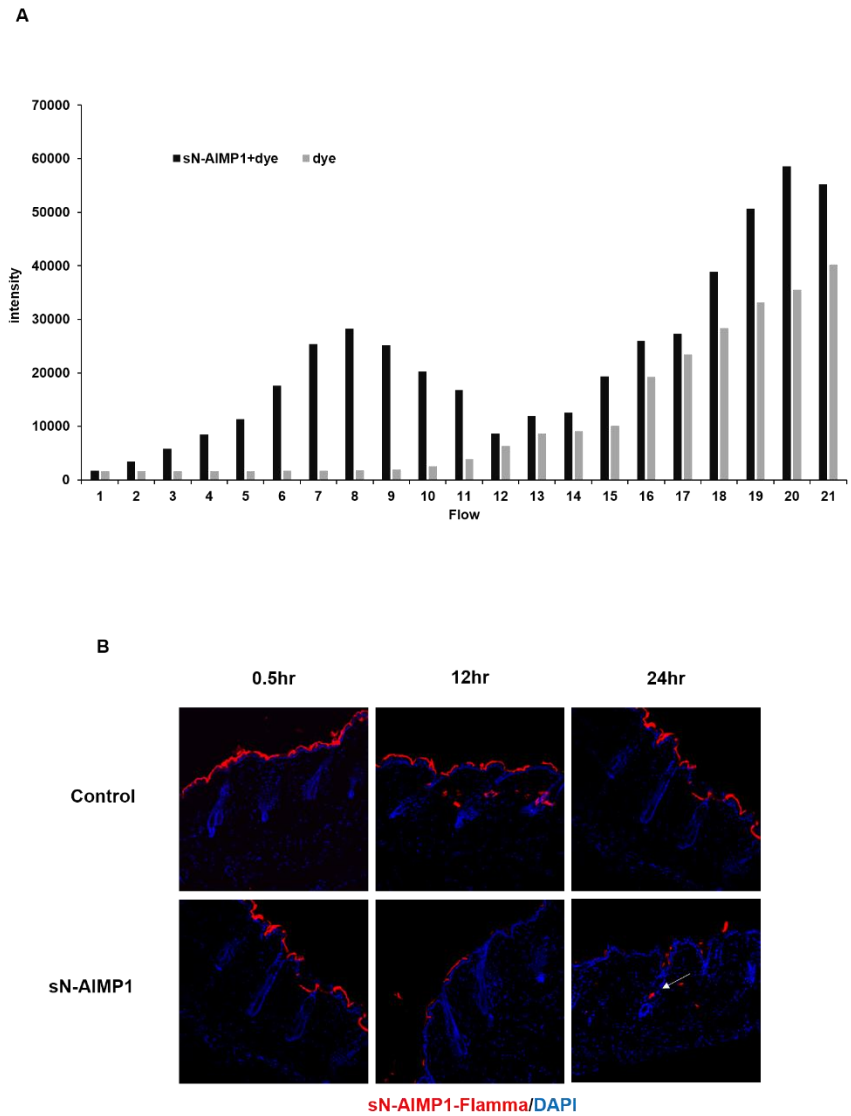

Figure 6. AIMP1 N-terminal fragment (6-46) penetrates from epithermal skin to hair follicle.

A) Seperation of Non-conjugated Flamma® 552 dye and dye conjugated peptide by size wxclusion PD-10 column. Fraction isolated drop by drop in 96well plate. And measured by florescent microplate reader.

B) Fluorescence microscopy of mouse skin sections 0.5, 12, and 24hr after application of Flamma 552-labeled peptides. Control is non-peptide conjugated dye.

Figure 7. Hair growth is enhanced by formulation

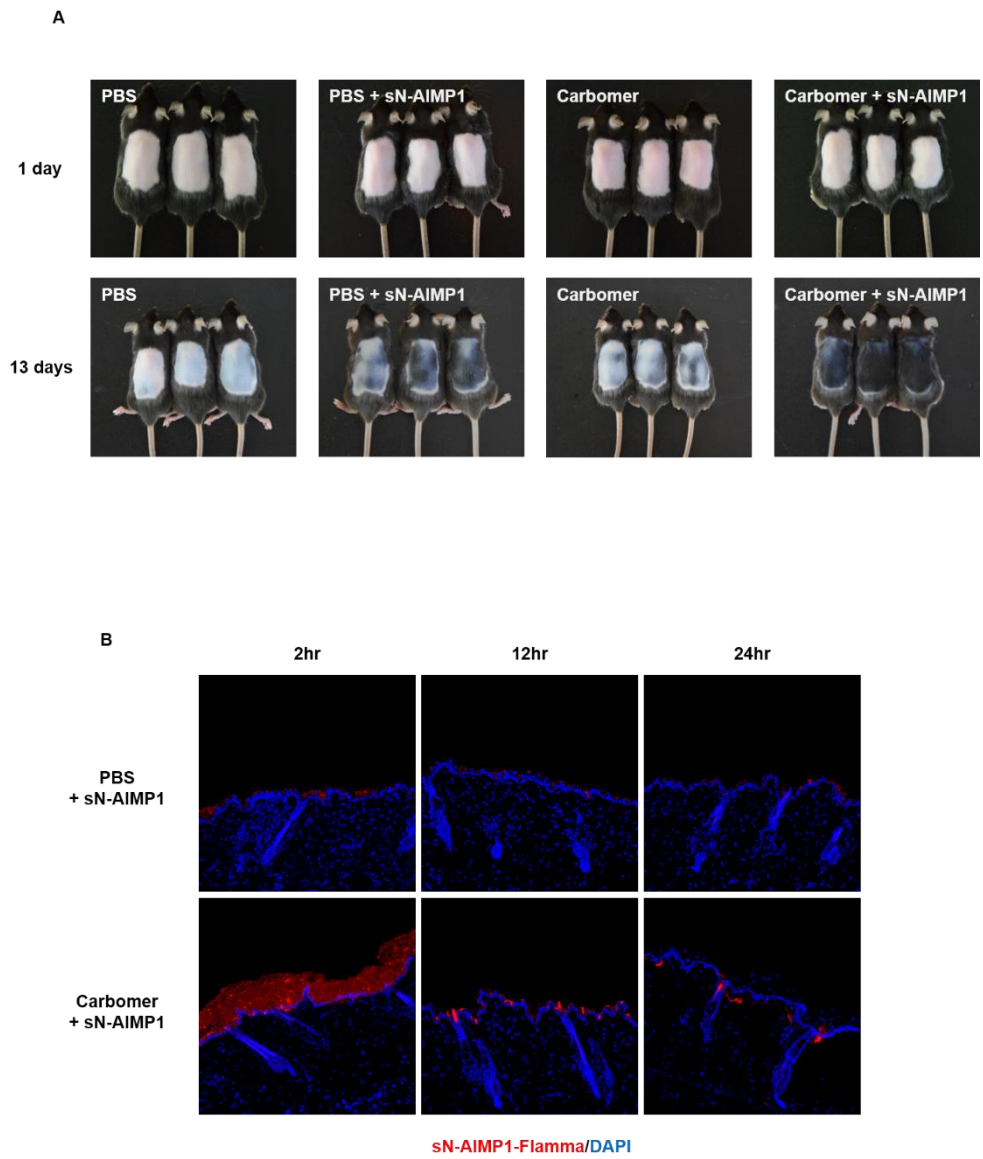

Figure 7. Hair growth is enhanced by formulation

A) In vivo formulation effect in hair depletion mouse model.

B) Fluorescence microscopy of mouse skin sections 2, 12, 16hours after application of Flamma® 552-labeled peptides with PBS and Carbomer.

DISCUSSION

Hair follicle undergoes continuous self-renewal through the 3 major hair cycles. First, hair follicle has period of active growth (anagen phase). The cells in the hair follicle divide to produce new hair fibers, and the follicle elongate itself into the dermal layer of the skin during anagen phase(29). At the end of the anagen phase, hair follicle is regressed and shortened (catagen phase). In this phase, hair bulb from the blood vessel and moves upwards(30). After falling the hair shaft out, the follicle rests (telogen phase). This dormant phase prepares for another round of the anagen period(31). In the subsequently repeated hair cycles, complex morphogenetic process relies on a reciprocal exchange of signals between mesenchymal and epithelial cells(22).

Dermal papilla(DP), which is located at the base of hair follicles, serves as the central tower of hair growth and morphogenesis signal. In anagen phase(29), Shh is required for maturation of DP and accelerates the transition from telogen to anagen(15). In this paper, we demonstrated additional function of Shh induces secretion of AIMP1 N-terminal fragment in dermal papilla cell. We validated this mechanism by *in vitro*. However, we have not clarified whether Shh plays in the same mechanism *in vivo*.

sFRP1 as a secreted wnt antagonist, is expressed in outer bulge cell, including dickkopf (Dkk). When hair follicle enters the telogen phase, hair follicle stem cell (HFSC) activity is restricted by expression level change of various signal such as wnt, BMP, Shh and others.(32). As same manner, sFRP1 maintains quiescence of HFSC by inhibiting wnt signal. however, in telogen - anagen

transition phase, this function should be controlled for HFSC differentiation and maturation. So, we suggested that secreted AIMP1 N-terminal fragment inhibits sFRP1 and induces proliferation of HFSC to help hair follicle enter the anagen phase. We demonstrated this hypothesis by qRT-PCR and proliferation assay using isolated hair follicle stem cells. But, to validate this mechanism also apply to *in vivo*, it is necessary that tracing the signals secreted the protein and mRNA expression sites for each hair cycles, using immunofluorescence and in situ hybridization. Furthermore, generating AIMP1 knock-out mouse model would assure our hypothesis of AIMP1 mechanism in hair follicle region.

Next, we analysis expression level of AIMP1 in physiological condition. Almost HFSC related signals expression level is decreased as aging process(19). Likewise, AIMP1 is also shown that decreased with aging in hair follicle. So, we suggested that AIMP1 should be developed by alopecia treatment peptide.

Vast majority of protein generated for skin therapy have been obstructed because of skin penetration issue. To overcome this hurdle, we synthesized the shorten version of AIMP1 and observed its permeability into hair follicle. However, 41 amino acids are quite long for cost-effective therapeutics. Therefore, it is necessary to identify the active binding site of AIMP1 to sFRP1. Furthermore, we need to validate this effect in aging animal model.

To deliver peptide or protein into the skin, carbomer, as a synthetic polymer, was widely used for this purpose as it can stabilize substances by adjusting pH condition. Our AIMP1 in carbomer showed enhanced efficacy

compared to AIMP1 itself but other formulating reagents should also be tested to maximize the condition of AIMP1.

In summary, secreted AIMP1 N-terminal fragment bind to sFRP1, blocking its effect. And promotes hair growth effect by activated wnt signal. And small AIMP1 N-terminal fragment can present a novel therapeutic approach for hair loss treatment.

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요약 (국문초록)

AIMP1 의 CD34+ 모낭 줄기세포의 증식 유도효과 연구

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남문식

Aminoacyl-tRNA synthetase interacting multifunctional protein 1 (AIMP1)은 multi-tRNA synthetase complex (MSC)를 구성하는 요소 중 하나이다. AIMP1 이 MSC 에서 분리되면, AIMP1 은 세포밖으로 분비되고 면역활성화, 신혈관 생성, 섬유아세포 증식과 같은 다양한 역할을 수행한다. 또한 AIMP1 이 모유두세포에서 분비된다는 기존의 연구를 바탕으로 모발성장단계에서 중요한 Shh 신호에 의해 AIMP1 이 모유두세포에서 분비된다는 것을 밝혔다. 흥미롭게도 이렇게 분비된 AIMP1 은 N-말단 부분만을 가지고 있었다. 추가적으로, AIMP1 의 N-말단 부분은 wnt 의 경쟁적 저해제인 sFRP1 과 결합하여 wnt 신호 경로를 촉진한다는 메커니즘을 증명했다. 이러한 AIMP1 N-말단 부분의 작용기전은 특히 모낭 줄기세포에 작용하며 세포 증식을 유도한다는 것이 쥐에서 분리한 $CD34^+$ 세포를 통해 증명되었다. 추가적으로 AIMP1 의 발현양이 나이가 들수록 줄어든다는 것을 동물 모델을 통해 확인을 했고 이를 통해 AIMP1 의 N-말단 부분이 탈모 치료용

펩타이드로 개발이 가능할 것이라고 생각을 했다. 이후에, 탈모 치료용 펩타이드 개발을 위하여 N-말단 AIMP1 의 소형화를 시험해보았다. 인공적으로 털을 제거한 쥐의 등부분에 소형화한 N-말단 AIMP1 펩타이드를 도포했을 때, 대조군에 비해 털의 성장 속도가 굉장히 빨라졌음을 확인하였고 추가적으로 카보머를 이용한 제제연구를 통해 그 효과를 증가시켰다. 정리하자면 본 논문에서 우리는 AIMP1 의 N-말단 조각의 새로운 활성과 메커니즘을 밝히고 탈모 치료제로써의 가능성을 확인하였다.