32nd IFSCC Congress
London 19-22 September 2022

SUNSTAR

Poster ID : 040

Involvement of transglutaminase 3 (TG3) in hair stiffness changing with age by capture of zinc.

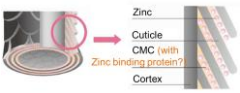
Ueda Seiko [1]; Inaba Megumi [1]; Hitomi Kiyotaka [2]

[1] Health & Beauty Innovation R&D, Sunstar Inc., Kobe, Japan
[2] Graduate School of Pharmaceutical Sciences, Nagoya University, Nagoya, Japan

Introduction:

We previously reported that zinc in the cuticle of hair contributes to hair stiffness, and the age-dependent decrease in zinc is one of the reasons for the decrease in hair stiffness. Although it is not clear why zinc level in hair decreases with age, we speculated that there are possible zinc-binding proteins which might also decrease with age in the cuticle cell membrane complex (CMC).

In this study, we tried to identify responsible proteins for binding zinc in the cuticle CMC. Then we examined zinc-binding activity, its distribution in hair root and age-dependent change of its expression level.



Methods & Results :

1.【Mass spectrometry of proteins extracted from hair with formic acid】

We washed identical Chinese female hair, then dried in the coiled shape like Fig1 (a). The hair was enclosed in a tube like Fig1 (b) as both cut sides of the hair were placed outside. The hair was immersed in formic acid at room temperature for 5 minutes. The formic acid-extracted protein solution was fractionated by SDS-PAGE (5 -20 %gradient gel) and analyzed by Q-Orbitrap mass spectrometry .

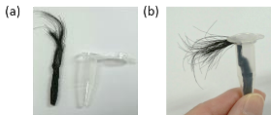


Fig. 1. Hair dried in a coiled shape (a) and the cut surface of the hair held outside the container by the tube lid (b).

A total of 136 proteins were detected in the formic acid extracted solution. Among them, Transglutaminase 3 (TG3) was found as a candidate protein that is capable of binding to divalent metal ions.

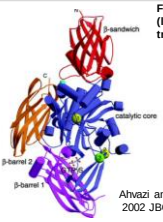


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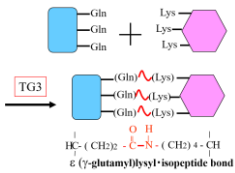
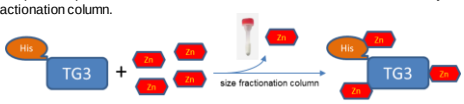
Fig. 2. Tertiary structure of human TG3 (left) and catalytic reaction of transglutaminases (right).



TG3 is a member of the enzyme family of transglutaminase, a protein cross-linking enzyme, that is activated by Ca^{2+} and forms an isopeptide bond between glutamine and lysine residues. Currently, it is not known whether TG3 can bind zinc *in vivo* and *in vitro*.

2.【Verification of recombinant TG3 binding to zinc】

To verify the binding of TG3 to zinc, recombinant hexahistidine-tagged human TG3 (His-TG3) was mixed with zinc sulfate, then removed unbound zinc by size fractionation column.



The amount of zinc eluted with His-TG3 was analyzed by inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7700).
Alkaline phosphatase (ALP) was used as a positive control.
His-tag was also capable of binding zinc. Therefore, EGFP (enhanced green fluorescence protein) and SPR3 (small proline-rich protein 3) were selected as proteins to which does not capture zinc. These His-tagged recombinant proteins (His-EGFP and His-SPR3) were prepared in *E. coli.*

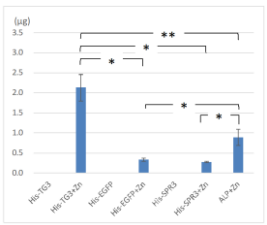


Fig. 3. Amounts of zinc (μg) bound to 1 nmol of protein.

The value of zinc bound to His-EGFP and His-SPR3 indicated that the amount of zinc bound to the His-tag portion was 0.3 μg per 1 nmol of protein.

=0.3 μg / 1nmol His-tag

Hence, 1.83 μg of zinc appeared to bind the TG3 portion.

=1.83 μg / 1nmol TG3

TG3 was able to bind about twice as much zinc as ALP.

3.【Immunostaining of TG3 in the cuticle of hair root】

TG3 was known to be expressed in hair and contribute to crosslink several structural proteins as in the case of skin epidermis. Hair was plucked from female Japanese volunteer, and the longitudinal section of the hair root was prepared and incubated with monoclonal antibody (C2D) against human TG3 followed by reaction with biotinylated secondary antibody [1] . Color development was performed using ImmPACT DAB peroxidase substrate kit. Mouse IgG was used for immunoreaction as a negative control.

TG3 appeared to be present in large amounts in the hair bulb area and also in the cuticle layer. Thus, TG3 is possible to contribute to retain zinc ion in the hair cuticle layer.

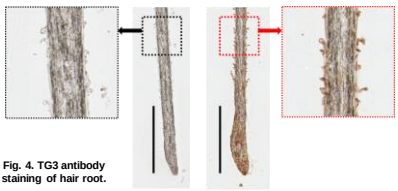


Fig. 4. TG3 antibody staining of hair root.

4.【qPCR analysis of TG3 expression in the hair root of various aged volunteers】

We collected 20 hairs from 49 Japanese volunteers aged 24-59 after obtaining informed consent. The total RNA in the hair root was extracted and expression quantification of each gene was performed by relative quantification method using ViiA™ 7 Real-Time PCR System (Applied Biosystems). Then the value of the expression levels of TG3 were corrected for 18S rRNA expression levels.

The expression of TG3 tended to decrease with age, particularly apparent after the age of 40s. Zinc in hair has also been reported to similarly decrease after the age of 40s [2].

These data indicate that possible amounts of zinc maintained in the hair will be also reduced corresponding with the decrease in TG3 depending on the age.

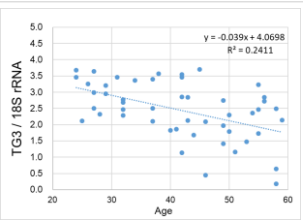


Fig.5. TG3 mRNA expression level in the hair at various aged group

Conclusions:

By a series of experiments, the decrease in zinc in the hair and also its firmness with age appeared to be partly due to the decrease in the expression of TG3 in the cuticle layer. TG3 has been known as an enzyme that provides hair stiffness by cross-linking structural proteins with strong isopeptide bonds [3] [4], but here we showed a novel role of this enzyme that also contributes to hair stiffness by retaining zinc in the cuticle layer of post-keratinization hair.
In the future, application of compounds that enhance TG3 expression to the scalp is strongly expected to keep hair firmness, by preventing age-dependent decrease in TG3 and its captured zinc in the hair.

Acknowledgements:

We would like to express our deepest gratitude to Professor Taniguchi and his laboratory at Tokushima University for conducting the protein mass spectrometry, and to Professor Joko at Sugiyama Jogakuen University for advising us on how to extract proteins from the hair cuticle layer.

References:

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