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RNF213-Associated Ubiquitin Signaling with *UBC13*

Jiyeong Kim, Toshiyuki Habu*

Department of Food Sciences and Nutrition, Mukogawa Women's University, Hyogo, Japan

****Corresponding author:** Toshiyuki Habu, Department of Food Sciences and Nutrition, Mukogawa Women's University, Hyogo, Japan, Tel: +81 798-45-9754; E-mail: habu@mukogawa-u.ac.jp*

DESCRIPTION

Moyamoya disease (MMD) is a cerebrovascular disorder with unknown pathogenesis that features abnormal blood vessel formation with stenosis or an occlusion in the circle of Willis [1-4]. *RNF213* is a susceptible gene for Moyamoya disease (MMD), which encodes a huge protein with AAA+ATPase (ATPase associated with various cellular activities) and RING domains [5]. The molecular mechanisms of MMD are mainly unknown. Elucidation of the *RNF213* functions might lead to the understanding of MMD pathology. In this article, we discuss the current topics on *RNF213* ubiquitination activity and the regulation and point out some of the questions that remain unanswered.

RNF213 has been identified as a susceptibility gene for MMD by genome-wide linkage analysis in families with MMD. *RNF213* protein encodes AAA+ATPase and RING domains, exhibiting ATPase and ubiquitination activity [5-10]. The p.*R4810K* variant of *RNF213* protein, located far from these domains, has been identified as a founder mutation in East Asian populations. In extensive studies, the correlation between the variant and the angiogenesis has been reported. The HUVEC cells expressing the *RNF213 R4810K* mutant showed defects in tube formation and decreased wound healing activities, which influenced angiogenic activity in endothelial cells. Endothelial cells derived from MMD patients' iPS cells also showed the lowered angiogenic activities *in vitro*. These phenotypes indicated that the *R4810K* variant might down-regulate the angiogenic activity in endothelial cells in MMD.

The target proteins with mono or poly-ubiquitin are subsequently recognized by other enzymatic proteins to proceed with diverse intracellular signaling pathways [11-13]. According to the pattern of ubiquitination, ubiquitinated proteins go through different fate; Lys48 (K48)-linked polyubiquitination allows proteasome to target the substrate to degrade, whereas Lys63 (K63)-linked ubiquitin chains lead the protein to be involved in particular cellular processes, such as DNA repair and immune regulation [13-16]. Thus, it can be said that ubiquitylation embeds a key to various signaling processes. Three enzymes, E1, E2, and E3 are involved in an enzymatic cascade through which ubiquitin is covalently attached to proteins [17,18]. In these modification steps, E3 ligases attach the ubiquitin to target proteins with ubiquitin-conjugated E2 enzyme. E2 and E3 enzymes prominently determine specificity in the choice of ubiquitylation substrate and the type of ubiquitin chain, and individual E2 enzymes can determine the fate of modified protein through E2-E3 interaction. The RING domain of *RNF213* is a well-established E3 ubiquitin ligase domain. Hence, the identification of E2 enzymes which have interaction with *RNF213* expands the network of *RNF213* protein. Yeast two-hybrid screening is a powerful and physiological tool for identifying protein-protein

interaction. Many E2 partners for E3 protein have been identified [19,20].

Habu and Harada identified *UBC13* (UBE2N) as an E2 enzyme for *RNF213* with an *RNF213* RING domain as bait using E2 enzyme-specific libraries [21,22]. The interaction was dependent on Isoleucine residue, a hydrophobic and critical amino acid for E2-E3 interaction and E3 ligase activities. The RING domain-dependent interaction indicated the interaction-specificity and enzymatic activity. The interaction between these two proteins was shown *in vivo*. As expected, *RNF213* had the K63-linked auto-polyubiquitination activity with *UBC13* protein. The *RNF213-UBC13* enzyme complex might control the signaling pathway using K63-linked ubiquitination. Indeed, *RNF213* mutants within the RING domain, which showed a weak interaction with *UBC13*, showed lower ubiquitination activity and tube formation assay and wound healing assay using HUVEC cells.

CONCLUSION

RNF213 dependent K63-linked ubiquitination with *UBC13* might be involved in the angiogenic activity and consequently in MMD. Deubiquitinase of the K63-linked ubiquitin chain has been reported to play a critical role in angiogenesis. The regulation of K63-linked ubiquitination by deubiquitinase and *RNF213* may be essential for angiogenesis. These studies showed that ubiquitination activity influenced on cell motility. *RNF213* might regulate the cell motile activity in endothelial cells.

REFERENCES

[1] Suzuki J, Takaku A., *Arch Neurol*, **1969**, 20: 288.
[2] Suzuki J., *Arch Neurol*, **1969**, 20: 288.
[3] Takeuchi K., *Brain Nerve*, **1957**, 9: 37.
[4] Kudo T., *Neurology*, **1968**, 18: 485.
[5] Liu W, Morito D, Takashima S. *PLoS ONE*, **2011**, 6: e22542.
[6] Kamada F, Aoki Y, Narisawa A. *J Hum Genet*, **2011**, 56: 34.
[7] Hitomi T, Habu T, Kobayashi H. *Biochem Biophys Res Comm*, **2013**, 439: 419.
[8] Hitomi T, Habu T, Kobayashi H. *Biochem Biophys Res Comm*, **2013**, 438: 13.
[9] Kobayashi H, Matsuda Y, Hitomi T. *J Am Heart Assoc*, **2015**, 4: e002146.
[10] Kobayashi H, Hitomi T, Harada K. Environ Health Prev Med, **1998**: 17, 7151.
[11] Ciechanover A., *EMBO J*, **1998**, 17: 7151.
[12] Joazeiro C, Wing S, Huang H. *Science*, **1999**, 286: 309.
[13] Hochstrasser M., *Nat Cell Biol*, **2004**, 6: 571.
[14] Yin Q, Lin SCC, Lamothe B. *Nat Struct Mol Biol*, **2009**, 16: 658.
[15] Andersen PL, Zhou H, Pastushok L. *J Cell Biol*, **2005**, 170: 745.
[16] Jiang X, Chen ZJ. *Nat Rev Immunol*, **2012**, 12: 35.
[17] Pickart CM, Raasi S., *Methods Enzymol*, **2005**, 399: 21.
[18] Winkler G, Timmers H., *Methods Enzymol*, **2005,** 399: 355.
[19] Christensen DE, Klevit RE., *FEBS J*, **2009**, 276: 5381.
[20] Christensen DE, Brzovic PS, Klevit RE., *Nat Struct Mol Biol*, **2007**, 14: 941.
[21] Habu T, Harada KH., *FASEB BioAdvances*, **2021**, 3: 557.
[22] Miskinyte S, Butler MG, Hervé D, et al., *Am J Hum Genet*, **2011**, 88: 718.