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Investigation of domain-specific functions and stability of VPS33A, responsible protein for mucopolysaccharidosis-plus syndrome

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Background and Objective: In 2017, we reported a novel disease named mucopolysaccharidosis-plus syndrome (MPSPS, OMIM #617303). MPSPS is an autosomal recessive disorder caused by the mutation p.R498W in the *VPS33A* gene, a member of HOPS complex which coordinate fusion between endosome/autophagosome and lysosome [1]. Its clinical phenotype is similar to mucopolysaccharidoses or mucolipidoses, and 20 patients have been found among native population of Yakutia in Russia so far [2]. Previously, we showed that pathogenic mutation did not affect endocytic and autophagic pathways. Since detailed pathophysiology of MPSPS is not elucidated, we investigated stability of VPS33A protein and domain-specific functions.
Methods: The wild-type and mutant pcDNA3.1(-)-VPS33A (D2-1, p.R200P; D2-2, p.R498W; D3a, p.D251E; D3b, p.Y438D) expression vectors were prepared, and transfected by lipofection. Knock-down of *VPS33A* was performed with commercially available siRNAs. Cells were incubated in the presence or absence of cycloheximide (CHX), proteasome (MG132) and lysosome (BafA1, NH_4Cl) inhibitors. We used the CRISPR-Cas9 genome editing technology for preparing cell lines containing different in-frame mutations of *VPS33A* gene in HeLa cells. Protein amount was analyzed by immunoblotting. Autophagic and endocytic functions were checked by LC3 expression and EGFR degradation assays respectively.
Results: We revealed that p.Y438D mutant, which disrupt HOPS/CORVET complexes formation, have no effect on VPS33A protein stability. Other mutations reduced the abundance of VPS33A protein. Autophagic flux and endocytosis were blocked in all clones with in-frame mutations in domains 2-2, 3a and 3b. Cells with mutations in domains 1 and 2-1 showed normal endocytic and autophagic function. Our study showed that ~20 % of normal protein is enough to maintain known functions. Results from knock-down of wild type *VPS33A* experiment suggested that reduction of VPS33A affects more on autophagic than endocytic function. Wild type or p.Y438D mutant VPS33A protein was maintained with MG132 treatment, suggesting that they are degraded in proteasome. However, other mutants were mostly maintained after treatment with lysosomal inhibitor, which suggests that they are degraded in lysosome.
Conclusion: Mutations in *VPS33A* gene may cause decrease in protein levels due to post-transcriptional modifications. We speculated that although p.R498W mutation is not disturb autophagic and endocytic functions, domain 2-2 of VPS33A might be involved in these intracellular pathways. In MPSPS patient's skin fibroblast, VPS33A protein with p.R498W is reduced into ~40 % of normal, and autophagic/endocytic function was not

affected. Our study showed that ~20 % of normal protein level does not affect autophagic/endocytic function so much, and supports our previous data from patient's skin fibroblast. Results of experiments with proteasomal and lysosomal inhibitors suggest that several mutants escape from ordinary proteasomal degradation pathway and go into lysosomal degradation, although detailed mechanisms including the involvement of autophagy are not clear. Investigation of the VPS33A protein will offer new insights and much more detailed molecular understanding of HOPS and CORVET complexes. Research is on-going.

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References

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