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Protein Size Differentiation in ADAMTS7 TurboID Experiment

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INTRODUCTION

Turbo ID is an enzyme that can catalyze the biotinylation of endogenous protein, which fuses to the protein of interest to cause an interaction with the proteins or a protein complex. Biotinylation is the process of attaching biotin to protein. This technique involves enzyme catalyzed Proximity Labeling (PL). PL assay is performed by labeling the protein of interest with an enzyme which biotinylates biomolecules in a proximity dependent manner

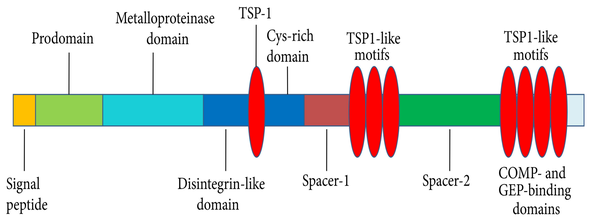
OBJECTIVES

In research for ADAMTS7's substrate, a question arose about the size of Turbo ID constructs. TurboID's technology serves as a convenient protein - protein interaction detection method. Differences in sizes of the protein should be observed based on variations of ADAMTS7 being added to the biotinylation protein.

METHODS

After confirming the plasmid sequences were correct by DNA sequencing, the plasmids Del Pigeon, E151, E151Q ,E389 and E389Q were transfected into HEK 293 cells. The transfected cells were placed in Freestyle media for 48 hours for expression. The conditioned media was then harvested and concentrated. The conditioned, concentrated media was run on a Western blot and imaged for ADAMTS7 protein bands.

Domain Structure and organization of ADAMTS7



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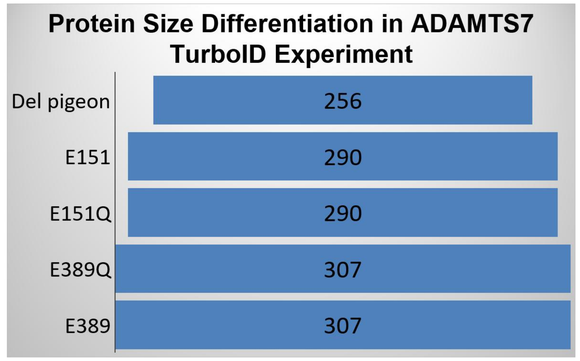
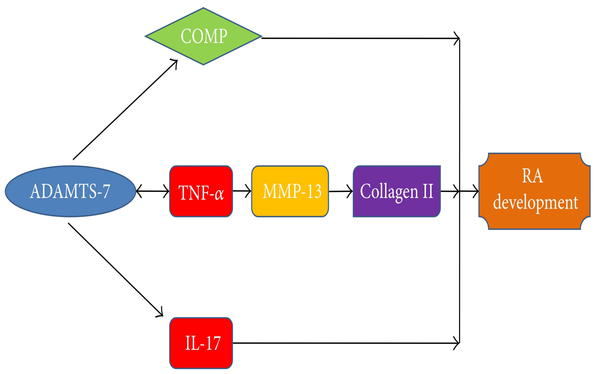
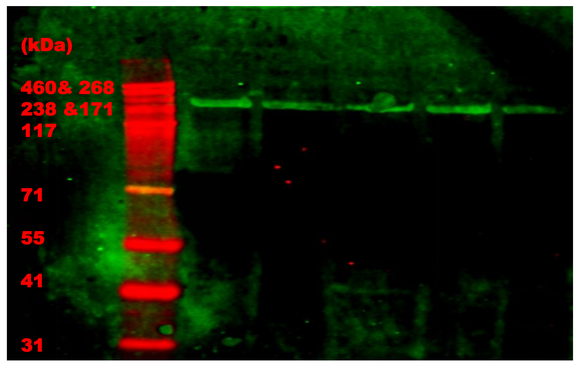


The function and roles of Adamts7 in inflammatory Diseases



Western Blot of TurboID Proteins



Western Blot using Anti-ADAMTS7 as primary antibody and Goat-anti Rabbit secondary antibody
Lanes: HiMark Ladder, E389Q Clone 20, E389 Clone 18, E151Q Clone 16, E151 Clone 18, and Del Pigeon Clone 2.

CONCLUSION

We anticipated the Del pigeon with 256kDa would migrate faster towards the anode while E151, E151Q , E389Q and E389 would migrate more slowly because of their bigger sizes. The size difference is very subtle on the Western blot shown here. In future, the gel could be run longer to get more size differentiation. A new transfection of Del Pigeon will be performed, and the conditioned, concentrated media tested via Western blot to confirm the size.

REFERENCES

[1]E. C. Arner, "Aggrecanase-mediated cartilage degradation," Curr. Opin. Pharmacol. 2002, vol. 2, pp. 322-29
[2]X. H. Bai, D. W. Wang, L. Kong, Y. Zhang, Y. Luan, T. Kobayashi, H. M. Kronenberg, X. P. Yu, and C. J. Liu, "ADAMTS-7, a direct target of PTHrP, adversely regulates endochondral bone growth by associating with and inactivating GEP growth factor," Mol. Cell Biol. 2009, vol. 29, pp. 4201-19
[3]A. Bateman and H. P. Bennett, "Granulins: the structure and function of an emerging family of growth factors," J. Endocrinol. 1998, vol. 158, pp. 145-51
[4]Coronary Artery Disease (C4D) Genetics Consortium., "A genome-wide association study in Europeans and South Asians identifies five new loci for coronary artery disease," Nat. Genet. 2011, vol. 43, pp. 339-44
[5]A. Didangelos, U. Mayr, C. Monaco, and M. Mayr, "Novel role of ADAMTS-5 protein in proteoglycan turnover and lipoprotein retention in atherosclerosis," J. Biol. Chem. 2012, vol. 287, pp. 19341-45