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Determination of Presence of ADAMTS7 in several Human Cell Lines

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INTRODUCTION

ADAMTS7, a member of A Disintegrin And Metalloprotease with Thrombospondin type 1 repeats (ADAMTS) family, was first identified and cloned in 1999 [1]. The family of proteases consist of 19 secreted enzymes with proteolytic activity against extracellular substrates, especially extracellular matrix proteins [2,3]. The importance of ADAMTS proteases is implicated in the establishment of tissue architecture during development and in tissue degradation during disease states such as cancer, Alzheimer's disease, and chronic inflammatory conditions [2,3]. Recently, two independent genome-wide association studies (GWAS) have identified the ADAMTS7 gene as a novel locus for the development of coronary atherosclerosis [4,5]. To understand the regulation of ADAMTS7, we have cultured the following laboratory available human cell lines to 3 or 4 passages: Hela, Human gingival fibroblast (HGF-1), Liver hepatocellular carcinoma (HepG2), and Human umbilical vein endothelial cells (HUVEC).

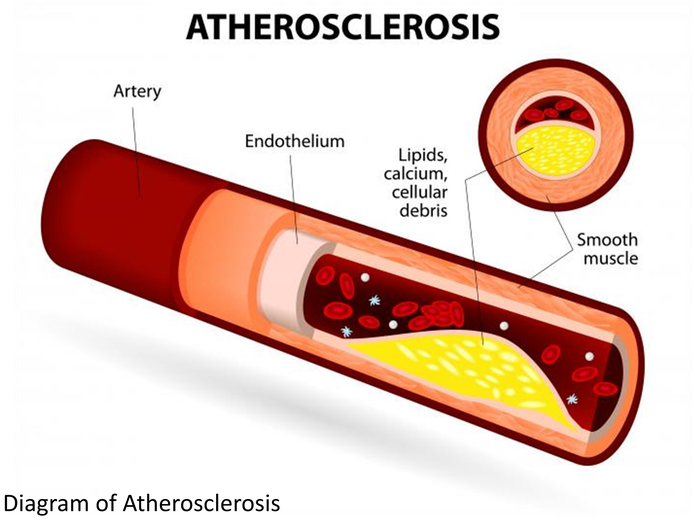

Fig 1: Diagram of Atherosclerosis

METHODS

We took the cell lines such as Hela, Human gingival fibroblast (HGF-1), Liver hepatocellular carcinoma (HepG2), Human umbilical vein endothelial cells (HUVEC) from cryogenic liquid and grew into T-25 flasks in DMEM or F-12K media with additives respectively. After subculturing 3 to 4 passages into the T-150 flask, we replaced the media with Freestyle media when the cells are 70 to 80% confluent. We harvested the media after 48 hour and took samples for Western blotting. Then 25 ml conditioned media was concentrated by using viva spin columns and a sample of the concentrated media was taken for Western blotting.

RESULTS

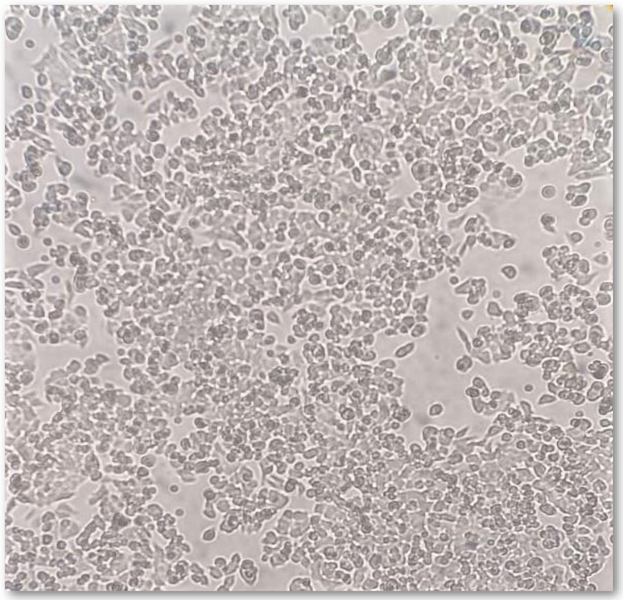

Fig 2: Hela cells;70-80% confluent;20X, low density

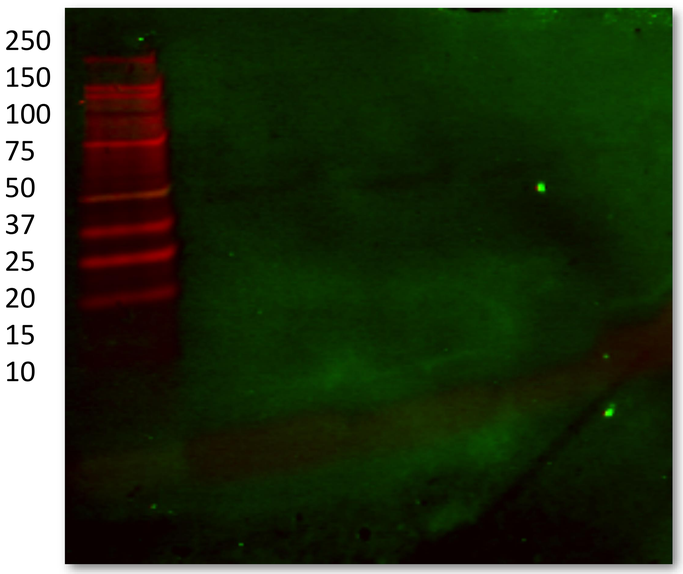

Fig 3: Western Blot Image with concentrated Hela cells

CONCLUSION

Western blotting with the concentrated and unconcentrated media to determine the expression of ADAMTS7 in specific human cell lines. During the Western blot technique, primary antibody will bind with ADAMTS7 if this enzyme is present in the specific cell culture media.

FUTURE ASPECTS

Additionally, we will study that which cell lines are appropriate for ADAMTS7. We will further study the localization of ADAMTS7 by using imaging and microscopy.

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