Investigating the effect of familial Alzheimer's disease mutations on Amyloid-ß deposits in 3D cell culture

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Introduction

Alzheimer's disease (AD) is the most common cause of dementia but currently there is no critical cure for this progressive disease. Therefore, research into AD is needed to improve fundamental understanding of the disease and to find potential therapies. In the amyloid cascade hypothesis, Aß, formed from the proteolytic cleavage of the amyloid precursor protein (APP) by ß-secretase and then γ-secretase, is seen as a key molecule in initiating AD. More than 25 mutations in APP have been identified to cause familial AD or hereditary cerebral angiopathy (CAA). In this project, I examined ① the effect of Swedish, Italian and Iowa mutations on APP proteolytic processing and ② differences in Aß deposits between cells containing APP wild-type and APP with mutations using a 3D cell culture model.

Materials and Methods

Site-directed mutations were introduced in human APP695 within pIREShyg vector (Accession, #U89672; Clontech, #6061-1) to obtain APP695 Swedish, APP695 Italian, and APP695 Iowa. Human neuroblastoma cells (SH-SY5Y cells) were transfected with these vectors by electroporation. Next, cell lysates and culture supernatants obtained from each transfectants were subjected to Western blot to determine the effect of mutations on APP proteolytic processing. In order to determine any difference in Aß production in SH-SY5Y cells expressing mutated APP695, Aß in conditioned cell medium was quantified using an ELISA based system developed by Meso Scale Discovery (MSD, #K15200E). In addition, SH-SH5Y cells were cultured in Matrigel (Corning, #354277), which contains the extracellular matrix (ECM) to identify differences in Aß deposits in cells containing APP wild-type and APP with mutations in 3D cell culture.

Results and Discussion

It was found that proteolytic processing pattern of APP was not identical among mutant proteins (**Fig. 1**). This difference may contribute to the different pathologies associated with the APP695 Italian and APP695 Iowa mutations. In addition, it was suggested that the double point mutation of APP695 Swedish caused an increase in Aß production (**Fig. 2**). Moreover, I tried to compare extracellular Aß deposits stained with 4G8 antibody by using Matrigel and was able to detect an immunostaining signal (not shown). However, more data from repeated experiments are required for concluding that Aß deposits in 3D cell culture are affected by APP695 Swedish, Italian and Iowa mutations.

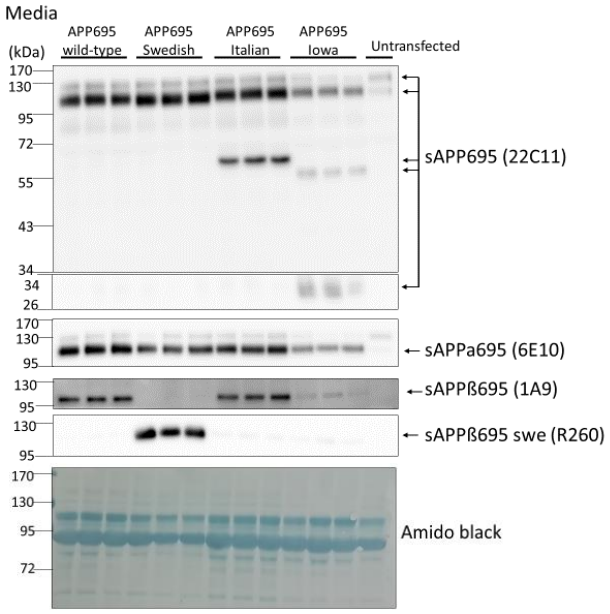

Fig. 1. Proteolysis of APP695 constructs

The concentrated conditioned cell medium was subjected to immunoblot analysis. 22C11, 6E10, 1A9, and R260 antibodies were used to detect APP695, sAPPα695, sAPPß695, and sAPPß695 Swedish, respectively. Protein-transferred membrane was stained with Amido black to confirm equal amount of proteins loaded in each lane.

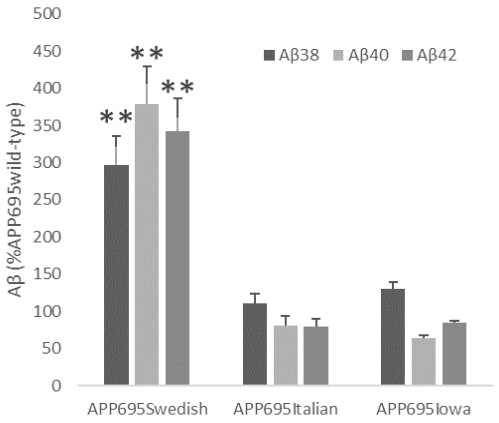

Fig. 2. Quantification of Aß38, Aß40 and Aß42 in conditioned cell medium from SH-SY5Y cells expressing APP695 mutant proteins

ELISA of Aß38, Aß40 and Aß42 in the conditioned cell medium from SH-SY5Y cells expressing APP695 Swedish, APP695 Italian, or APP695 Iowa, as compared to the amount observed in SH-SY5Y cells expressing APP695 wild-type, error bars are ± S.E.M. ** $p<0.01$, $n=3$.