Identifying novel modulators of amyloid precursor protein (APP) processing

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The accumulation of amyloid β-peptide (Aβ) into insoluble plaques in the brain is a key event in the pathogenesis of Alzheimer’s Disease. The first step in Aβ formation is the cleavage of the amyloid precursor protein (APP) by the β-site APP cleaving enzyme (BACE1). The processing of APP by BACE1 can be regulated by a number of different proteins; in this study we sought to identify proteins that modulate BACE1 cleavage of APP using an siRNA screening approach. An siRNA screen was performed using a protease sub-library to knockdown the expression of a range of different proteases (430 targets) in human embryonic kidney 293 cells expressing an N-terminal alkaline phosphatase tagged APP695 construct (AP-APP) and overexpressing BACE1. APP and BACE1 siRNA knockdown were tested as positive controls and a WST-1 cell proliferation assay was used to check cell number. Following siRNA knockdown APP processing was monitored by analysis of conditioned media for alkaline phosphatase activity to measure soluble AP-APP fragments. 25 proteins were identified to significantly affect APP cleavage in the initial screen: 22 positive regulators (decreased APP cleavage) and 3 negative regulators (increased APP cleavage) of APP processing were identified. Of the 22 positive regulators, 5 were selected as potential hit candidates (Neurolysin, Bone Morphogenic Protein 1 (BMP-1), Lipoprotein A (Lpa), Kalikrein-related peptidase 6 (KLK6) and Matrix Metallopeptidase 7 (MMP7)) and were further tested for their effects on APP processing by analysis of sAPPβ levels by western blot. Neurolysin, BMP-1, Lpa and MMP7 were shown to not specifically affect BACE processing of APP as determined by analysing APPβ; KLK6, however, significantly decreased APPβ. The effect of siRNA knockdown of KLK6 on APP processing was further investigated by analyzing sAPPα, sAPPβ and Aβ using the Mesoscale Discovery System. KLK6 knockdown significantly decreased sAPPβ and also decreased Aβ38, Aβ40 and Aβ42, although not significantly. In addition KLK6 knockdown also decreased sAPPβ. These changes were not due to a change in total APP levels, as determined by western blot. These data indicated that KLK6 may affect APP processing and the effect of KLK6 on APP processing warrants further investigation. In addition, we have validated an siRNA screening approach for identifying novel regulators of APP processing.

Biography

Katherine Kellett received her PhD from King’s College London in 2005 and has since worked in the laboratory of Professor Nigel Hooper, researching into Alzheimer’s disease and mammalian proteases for the last 9 years. In this time she has worked on two grants funded by Alzheimer’s Research UK and has published 12 papers. Along with the rest of Professor Nigel Hooper’s research group, Kate recently moved her work to the University of Manchester.

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