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Neuropathology

Reduction of Amyloid- β by Lentiviral Expression of Short hairpin RNAi for BACE1 in cultured cells

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We previously have shown that BACE1, an aspartyl protease necessary for the generation of amyloid- β peptides, is implicated in the pathogenesis of Alzheimer's disease (AD). Importantly, our demonstration that the deletion of BACE1 prevented β deposition and restored cognitive deficits occurring in a mouse model of β amyloidosis support the view that BACE1 is an excellent therapeutic target for amelioration of β amyloidosis in AD. However, it is not known whether β induced abnormalities are reversible. To address this issue, we plan to determine the window of opportunity for therapeutic intervention to β deposition by knocking down the expression of BACE1 by RNA interference (RNAi) in the brain of β amyloidosis AD mouse model.

As a first step in this approach, we assessed two short hairpins RNAi (shRNAi) for BACE1 for their ability to reduce BACE1 activity in a neuronal cell line (N2A). While both shRNAi reduced significantly the levels of BACE1 mRNA and protein in N2A cells, more importantly the secretion of β was decreased by ~75% in these cells, establishing that these shRNAi are effective. To assess the effectiveness of these shRNAi in primary neurons, we generated lentiviral vectors expressing shRNAi for BACE1. These lentiviral shRNAi-BACE1 vectors (which co-express a green fluorescence protein (GFP)) efficiently infected primary neuronal culture as judged by strong expression of GFP in both somata and neurites. We are now testing the impact of these lentiviral vectors on β secretion in primary neuronal cultures as well as in brains of wildtype and transgenic models of β amyloidosis.