Targeting BACE1 to restore functional angiogenesis in type 2 diabetes

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Research Objectives

β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1) is a transmembrane protease infamous for its involvement in Alzheimer's disease (AD). Here, it cleaves APP to amyloid peptides which consequently aggregates and forms neurotoxic plaques. Importantly, molecular scientists have shown that BACE1 is not restricted to APP proteolysis and various bioinformatic and proteomic data sets suggest that novel BACE1 substrates are yet to be discovered. Moreover, BACE1 has been shown to cleave various angiogenic signalling factors including VEGF receptor 1 (VEGFR1), NOTCH ligands, the insulin receptor and occludin, proposing an important role for BACE1 in vascular homeostasis.

Like AD, BACE1 expression and activity are elevated in models of type 2 diabetes (T2D), indicating a possible function in aberrant vessel growth which is typical of diabetes-related ischemia. Therefore, identifying novel roles for BACE1 in angiogenic dysregulation will aid the progression of biomedical interventions to prevent debilitating diabetes-associated complications such as lower limb amputations.

Methods

The fibrin gel angiogenesis assay and immunofluorescence retinal staining were used to identify a role for BACE1 in vessel growth *in vitro* and *in vivo*, respectively. Human umbilical vein endothelial cells (HUVECs) treated with or without a highly specific BACE1 inhibitor or transfected to over-express BACE1 were used in the fibrin gel angiogenesis assay to measure sprout formation. Furthermore, endothelium of the developing retinal vasculature in $BACE1^{-/-}$ and wild type control (WT) mice was stained and imaged using confocal microscopy. Lastly, Western blots were performed using HUVECs and primary isolated pulmonary endothelial cells (PECs) isolated from $BACE1^{-/-}$ and wild type mice.

Results

HUVECs treated with a BACE1 inhibitor had increased sprouting (18.70%± 5.92, P=<0.05) as well as increased phosphorylation of eNOS (83% ± 22, P=<0.05) and Akt (85.5% ± 9.24, P=NS) compared to untreated cells. Moreover, HUVECs transfected to over-express BACE1 had decreased sprouting (35.22% ± 7.34, P=<0.01), decreased phosphorylation of eNOS (15% ± 4.44, P=NS) and Akt (25% ± 3.73, P= NS), as well as increased NICD1 (26% ± 3.05, P=0.01) and Jagged1 C-terminal fragment (38.7% ± 26, P=NS). Lastly, $BACE1^{-/-}$ retinas had increased branch points, vasculature area and filopodia compared to WT mice. Also, $BACE1^{-/-}$ PECs had reduced NICD1 (26.73% ± 14.15, P=0.05) and Jagged1 C-terminal fragment (28.48% ± 14.61, P=<0.05).

Conclusion

Our findings indicate a role of BACE1 in negatively regulating vascular sprouting, possibly via Jagged1/NOTCH1 or Akt/eNOS/NO signalling. This provides a potential therapeutic repurpose for BACE1 inhibitors, previously trialled to treat AD, in normalising BACE1 levels in individuals with type 2 diabetes and preventing associated microvascular complications.