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Identification of ADAM proteinase substrates in neurodegeneration and neuroinflammation

Silvia Naus*, Simone Reipschläger, Dirk Wildeboer, Yuji MInai, Stefan F Lichtenthaler, Stefan Mitterreiter, Ziqiang Guan, Marcia L Moss and Jörg W Bartsch

Address: King's College, London, UK
* Corresponding author

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ADAM proteases are type I transmembrane proteins with extracellular metalloprotease domains. Like most ADAM family members, ADAM8 (CD156a, MS2) is involved in ectodomain shedding of membrane proteins and is linked to neuroinflammation and neurodegeneration. To identify potential substrates released under these pathologic conditions, we screened 10-mer peptides representing amino acid sequences from extracellular domains of various membrane proteins using the ProteaseSpot™ system. A soluble ADAM8 protease containing pro- and metalloprotease domain was expressed in *E. coli* and purified as active protease due to autocatalytic prodomain removal. From 34 peptides tested in the peptide cleavage assay, significant cleavage by soluble ADAM8 was observed for 14 peptides representing membrane proteins with functions in inflammation and neurodegeneration, among them the beta amyloid precursor protein (APP) and the Tumor Necrosis Factor alpha Receptor type I (TNFRI). The *in vivo* relevance of the ProteaseSpot™ method was confirmed by cleavage of full length APP with ADAM8 in human embryonic kidney 293 cells expressing tagged APP. ADAM8 cleaved APP with similar efficiency as ADAM10, whereas inactive ADAM8 mutant did not. Cleavage of TNFRI was assessed in primary cell lines deficient in ADAM8 and revealed a gene dosage-dependent effect. Exchanging amino acids at defined positions in the cleavage sequence of myelin basic protein (MBP) revealed sequence criteria for ADAM8 cleavage. Taken together, we identified novel substrates that could be cleaved by ADAM8 *in vivo* under pathologic conditions.