The characterisation of the ectodomain shedding of a Low Density Lipoprotein receptor (LDLR)

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The low density lipoprotein receptor (LDLR) is a 160-kDa membrane protein that forms part of the LDLR family. The LDLR provides the cell with cholesterol, ensuring the maintenance of low cholesterol levels in plasma. A 140-kDa form of the receptor was detected in the medium of cultured cells, and was shown to be a product of shedding (1). An example of a sheddase is tumour necrosis factor-α converting enzyme (TACE) and it is thought that it is responsible for the shedding of the receptor, as the secretase increased the production of soluble LDLR in the presence of Phorbol Myristate Acetate (PMA) and activity was inhibited by TNF- Protease Inhibitor (TAPI) (1). To determine the role of TACE in LDLR shedding and to identify the cleavage site, the LDLR was tagged with a fluorescent protein. Green fluorescent protein (GFP) was used to enable quick and easy screening of clones in addition to assessing the production of soluble LDLR by a fluorimetric assay. GFP and a GFP-LDLr were constructed by PCR and sub cloned into pcDNA3.1(H)-.GFP constructs will be visualised by fluorescent microscopy. Untagged LDLR was stably transfected into TACE+/+ and TACE-/- mouse fibroblasts to investigate shedding in response to PMA and TAPI. Possible shedding of LDLR via TACE may play a role in the regulation of the LDLr and hence influence cholesterol maintenance.

[1] Begg, M. J., Sturrock, E. D., v.d.Westhuyzen, D. R. (2004) Soluble LDL-R are formed by cell surface cleavage in response to phorbol esters, Eur J Biochem. 271 (3):524-33.