Analysis of the function and subcellular localization of FAT/CD36 in hepatocytes and transfected cell lines of hepatic and non-hepatic origin

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Figure: **Follows Page:**

DECLARATION

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Presentations and publications from this study

Conference Presentations:

Eyre, N.S., L. Cleland, G. Mayrhofer. Uptake and incorporation of long-chain fatty acids into cellular lipids in FAT/CD36 transfected cell lines. *7th Congress of the International Society for the Study of Fatty Acids & Lipids incorporating th 6th International Congress on Essential Fatty Acids and Eicosanoids & PUFA in Maternal and Infant Health Annual Scientific Meetings*. Cairns, Australia. 2006 (poster presentation)

Eyre, N.S., L. Cleland, G. Mayrhofer. The localization and activity of FAT/CD36 is influenced by its carboxy-terminal cytoplasmic tail. *Heart Foundation Conference and Scientific Meeting: Cardiovascular Disease in the 21st Century: Shaping the Future.* Sydney, Australia. 2006 (poster presentation)

Eyre, N.S., L. Cleland, G. Mayrhofer. Factors influencing CD36-mediated long-chain fatty acid uptake. *Australian Society for Medical Research: Annual Scientific Meeting.* Adelaide, Australia. 2005.

Eyre, N.S., L. Cleland, G. Mayrhofer. Cellular localization of CD36 and its role in lipid uptake. *Australian Society for Medical Research: Annual Scientific Meeting.* Adelaide, Australia. 2004.

Publications:

Eyre, N.S., Cleland L.G., Tandon, N.N., Mayrhofer G (2007). Importance of the carboxyl terminus of FAT/CD36 for plasma membrane localization and function in long-chain fatty acid uptake. *J Lipid Res.* 48(3): 528-42. (see Appendix 2)

Eyre, N.S., Cleland L.G., Mayrhofer G (2007). Inducible expression of FAT/CD36 does not enhance oleic acid uptake by CHO cells. *Manuscript in preparation*.

Abstract

The class B scavenger receptor CD36, or fatty acid translocase (FAT), is an 88 kDa plasma membrane glycoprotein that is the founding member of the class B scavenger receptor family. It has a number of natural ligands and has different functions at various locations in the body. It contributes to adhesion of platelets via its binding to thrombospondin-1. In monocytes and macrophages, it contributes to recognition and phagocytosis of apoptotic cells and it mediates the binding and uptake of oxidatively damaged low-density lipoproteins (oxLDL). In adipose and muscle tissues, FAT/CD36 mediates high-affinity binding and uptake of long-chain fatty acids (LCFAs) and is therefore a key regulator of lipid storage (particularly in adipocytes) and mitochondrial beta oxidation (particularly in muscle). Interestingly FAT/CD36 also binds native lipoproteins (including high-density lipoproteins [HDL]) with high affinity *in vitro*, although the physiological significance of this is unclear at present.

Expression of FAT/CD36 by hepatocytes has not been recognised until recently, mainly because it is gender-regulated in both humans, and rats. However, the primary function of FAT/CD36 in the liver is unknown. The work described in this thesis has used various transfected cell lines to examine the possibility that FAT/CD36 contributes to hepatic LCFA uptake and/or the uptake of cholesteryl esters (and other lipids) from HDL. The subcellular localization of FAT/CD36 has been explored in rat liver and in cell lines of hepatic and non-hepatic origin, especially with respect to its association with specialized plasma membrane lipid raft microdomains known as caveolae. Furthermore, the importance of the cytoplasmic carboxyl-terminus of FAT/CD36 in both subcellular localization of the molecule and its activity as a LCFA transporter has been examined using truncated mutants and chimeric variants of FAT/CD36.

The results indicate that FAT/CD36 contributes to LCFA uptake by hepatocyte-derived cell lines. In these cells it resides in both non-raft and lipid raft domains of the plasma membrane that may not always include caveolae. The studies also indicate that the cytoplasmic C-terminus of FAT/CD36 contributes to the attachment of FAT/CD36 to membranes, including raft-derived detergent-resistant membranes. This domain is necessary also for correct targeting of the receptor to the plasma membrane and for its activity as a LCFA transporter. Finally, DNA constructs have been prepared and tested,

with the objective of producing transgenic mice in which expression of FAT/CD36 can be induced and over-expressed specifically in the liver. This model could be used to confirm whether FAT/CD36 has a role as a LCFA transporter in the liver and to explore whether it has additional significance as a hepatic transporter of HDL-derived cholesteryl esters or as a scavenger of oxidised LDL.