MECHANISM OF DISEASE

The lipid connection

Alzheimer's disease (AD), which is associated with the generation of amyloid-β peptide (Aβ), has been linked to lipid regulation. However, the cellular mechanisms that link Aβ to lipid metabolism have been poorly understood. Now, a report in *Nature Cell Biology* provides, for the first time, a functional basis for the relationship between lipids and AD.

The precursor of Aβ, amyloid precursor protein (APP), is first cleaved by β-secretase, and subsequently by γ-secretase, to produce Aβ40 and Aβ42. Depletion of cholesterol has been linked to a decrease in γ-secretase activity and a resulting decrease in Aβ. The authors first looked at presenilin-1 and presenilin-2 (PS1 and PS2), either of which can function as the active centre of the γ-secretase complex. Mouse embryonic fibroblasts (MEFs) that lack PS1 and PS2 showed a rise in the levels of cholesterol and sphingomyelin, whereas wild-type neurons in which γ-secretase was inhibited showed a rise in sphingomyelin levels that was accompanied by a drop in sphingomyelinase (SMase) activity.

To analyse the effect of sphingomyelin on Aβ levels, Grimm *et al.* inhibited SMase in neurons, and noted a rise in sphingomyelin and a decrease in the levels of intracellular and secreted Aβ. In MEFs that lacked APP and amyloid-β precursor-like protein-2 (APLP2), the levels of sphingomyelin, SMase and cholesterol were similar to those in cells lacking PS1/2 or γ-secretase activity. Inhibition of γ-secretase in these $App^{-/-}$ $Aplp2^{-/-}$ cells did not alter cholesterol levels, indicating that the cleavage products of APP and APLP2 are responsible for the change in lipid levels. To determine if Aβ, the most prominent cleavage product, mediates this effect, the authors looked at the specific metabolic pathways of cholesterol and sphingomyelin.

In $App^{-/-}$ $Aplp2^{-/-}$ MEFs, SMase activity was low, but could be restored to partial wild-type levels by the addition of Aβ-containing media. Indeed, homogenates of $App^{-/-}$ $Aplp2^{-/-}$ MEFs showed enhanced SMase activity on addition of synthetic Aβ. Further *in vitro* experiments showed that Aβ42 specifically affects SMase, whereas Aβ40 is specific for hydroxymethylglutaryl-CoA reductase (HMGR), which is involved in cholesterol metabolism.

The authors propose a model in which an enzyme cascade of γ-secretase, APP processing and Aβ can affect cholesterol and sphingomyelin metabolism, resulting in altered levels of these lipids, which, in turn, regulates Aβ levels. The authors concede that additional cleavage products of APP other than Aβ might be involved. The functional basis of the link between Aβ and lipid levels has direct relevance to AD and, as the authors suggest, "…may provide a rational basis for therapy."

Helen Ross

References and links

ORIGINAL RESEARCH PAPER Grimm, M. O. *et al.* Regulation of cholesterol and sphingomyelin metabolism by amyloid-β and presenilin. *Nature Cell Biol.* 9 Oct 2005 (doi:10.1038/ncb1313)

TECHNOLOGY WATCH

Non-destructive development

Ethical issues surround human embryonic stem (ES)-cell research, and one of the most fundamental concerns relates to the fact that ES-cell derivation involves embryo destruction. However, two papers in *Nature* now describe techniques that might circumvent this problem.

In the first paper, Meissner and Jaenisch created mouse fibroblasts that carried a short hairpin RNA construct targeted against *Cdx2*. *Cdx2* encodes the earliest known protein that is involved in the development of the trophectoderm — the cell layer that is essential for the formation of the fetal–maternal interface. They then used nuclear transfer to derive mouse blastocysts from the donor fibroblasts. The cloned mouse blastocysts were morphologically abnormal and were incapable of implanting into the uterus and developing further. However, these blastocysts generated pluripotent ES cells when they were explanted into culture and the effects of the *Cdx2* knockdown were reversed.

In the second paper, Lanza and colleagues carried out single-cell biopsies on mouse embryos and showed that the resulting pre-implantation blastomeres could be used to establish ES cell lines. A similar single-cell biopsy technique is currently used in the pre-implantation diagnosis of genetic defects, and a significant advantage of this approach for ES-cell derivation is that the single-blastomere-biopsied embryos developed to term with no reduction in their developmental potential.

REFERENCES Meissner, A. & Jaenisch, R. Generation of nuclear transfer-derived pluripotent ES cells from cloned *Cdx2*-deficient blastocysts. *Nature* 16 Oct 2005 (doi:10.1038/nature04257) | Chung, Y. *et al.* Embryonic and extraembryonic stem cell lines derived from single mouse blastomeres. *Nature* 16 Oct 2005 (doi:10.1038/nature04277)

Sweet success

The field of functional glycomics concerns the study of glycan structure, function and recognition by carbohydrate-binding proteins (CBPs). However, the current glycan-array technology is limited by the difficulty in producing derivatives of free, reducing glycans with primary amines for conjugation. Now, though, in *Nature Methods*, Cummings and co-workers present a new method that allows the efficient derivatization of glycans for glycomics analyses.

They describe a simple approach that involves the derivatization of glycans using the inexpensive reagent 2,6-diaminopyridine (DAP) to create fluorescently labelled glycans (GDAPs). They were able to convert a broad variety of glycans to GDAPs, as confirmed by high-performance liquid chromatography and mass spectrometry. Importantly, the DAP part of each GDAP contains a primary amine that can be used for further conjugation, and the authors were able to conjugate GDAPs to, for example, biotin, microspheres and glass slides. All of the different types of conjugated glycan were recognized by the relevant CBPs. These results therefore show that GDAPs are a versatile new tool that will allow the visualization, quantification and covalent capture of minute quantities of glycans. These glycans can be studied structurally and functionally, and can also be used to generate glycan arrays from naturally occurring glycans.

REFERENCE Xia, B. *et al*. Versatile fluorescent derivatization of glycans for glycomic analysis. *Nature Methods* **2**, 845–850 (2005)