Poster Presentation

MS009.P36

Notum – an extracellular protein deacylase that suppresses Wnt signalling.

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Secreted Wnt proteins trigger cellular signalling pathways that are essential for embryogenesis and adult tissue homeostasis. These ligands are distinctive in requiring a lipid/palmitoleoylate modification for receptor binding and activity. Wnt signalling is finely balanced to ensure normal development and homeostasis while avoiding diseases such as cancer. This balance is achieved in part by Notum, a feedback antagonist which is secreted by cells in response to Wnt activation to switch off further signalling. Notum is highly conserved in sequence from fly to humans. It has been thought to act as a phospholipase, shedding glypicans and associated Wnt proteins from the cell surface. However, we have demonstrated that Notum does not function by cleaving the glycophosphatidylinositol anchors of glypicans. Instead we have discovered that Notum is a carboxylesterase that removes an essential palmitoleate moiety from Wnt proteins and thus constitutes the first known extracellular protein deacylase. Our x-ray crystallographic analyses showed glycosaminoglycan binding sites on Notum located distant from the active site, consistent with Notum binding to glypican glycosaminoglycan chains likely helping co-localization with Wnt proteins. Notably, our crystal structures of human and Drosophila Notum identify a large hydrophobic pocket that accommodates palmitoleate at the active site. We are characterising the binding properties of this cavity, most recently by carrying out an XChem fragment screen at Diamond beamline I04-1 (in collaboration with Frank von Delft and the XChem team). We were able to collect high resolution (1.6-1.8 A) data sets of Notum crystal soaks for 700 fragments using the highly streamlined process for soaking, harvesting, automatic data collection and data analysis implemented at Diamond.

[1] Kakugawa et al. (2015) Nature 519, 187-192.

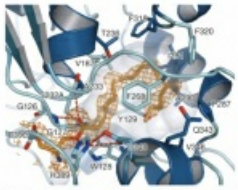

Fig 5. Crystal structure of Notum with palmitoleate (Kakugawa et al 2015).

Keywords: Wnt signalling, extracellular protein deacylase, fragment screen