

Molecular Signalling Laboratory

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The Molecular Signalling Laboratory examines sphingolipid-mediated cell signalling pathways, and how they contribute to cancer and other diseases. In particular, the primary focus of our work is the enzyme sphingosine kinase (SK), that controls the cellular levels of two important signalling molecules, sphingosine and sphingosine 1-phosphate (S1P).

Both sphingosine and S1P regulate a diverse range of cellular processes by acting as intracellular second messengers, while S1P also acts as a ligand for a family of S1P-specific cell surface receptors. Of greatest interest to our laboratory are findings that elevated cellular sphingosine kinase prevents programmed cell death (apoptosis), enhances cell proliferation, and leads to neoplastic cell transformation. This indicates an oncogenic role for sphingosine kinase, which is further supported by recent data showing elevated sphingosine kinase in a variety of human cancer cells and inhibition of tumor growth *in vivo* by genetic or chemical suppression of SK.

In addition to this role in tumorigenesis, SK and S1P appear central players in many other cellular processes, including; vascular endothelial cell activation, a hallmark of inflammatory diseases; enhancing blood vessel construction, and; enhancing constriction of airway smooth muscle cells. Thus, sphingosine kinase is also a potential target for therapeutic intervention in inflammation and atherosclerosis, hypertension and asthma.

Recent work in the Molecular Signalling Laboratory has concentrated on understanding the biochemistry of SK, identifying the mechanisms regulating the activity and localisation of this enzyme, and on the (patho-)physiological functions of signal transduction pathways it controls. Understanding these factors may allow for the development of novel anti-sphingosine kinase therapeutics.

Key recent discoveries:

1. The catalytic and functional activation of sphingosine kinase 1 by phosphorylation

We have identified that phosphorylation of SK at serine-225 by a member of the extracellular signal regulated protein kinase (ERK) family directly results in its activation. Strikingly, the oncogenic effects of SK are blocked by mutation of this phosphorylation site. This is despite this non-phosphorylatable mutant retaining full basal catalytic activity. More recently we have established that this single phosphorylation of SK not only directly increases its catalytic activity but also results in its translocation from the cytosol to the plasma membrane. Furthermore, we have shown that this phosphorylation-induced change in localisation of SK is critical in driving oncogenic signalling by this enzyme.

Pitson *et al.* (2003) Activation of sphingosine kinase 1 by ERK1/2-mediated phosphorylation. *EMBO J* **22**, 5491–5500.

Pitson *et al.* (2005) Phosphorylation-dependent translocation of sphingosine kinase to the plasma membrane drives its oncogenic signalling. *J Exp Med* **201**, 49–54.

2. Sphingosine kinase mediates oncogenic signalling by EF1A

We have recently identified that regulation of sphingosine kinase can be achieved through another protein called EF1A. Notably, EF1A has been implicated in inducing the formation of some solid tumours, but the mechanism was unknown. Our findings show that the oncogenic effects of EF1A are mediated by sphingosine kinase, and thus indicates that targeting this enzyme is a therapeutic option for these EF1A-induced cancers.

Leclercq TM, Moretti PAB & Pitson SM (2010) Guanine nucleotides regulate sphingosine kinase 1 activation by eEF1A and provide a mechanism for eEF1A-associated oncogenesis. *Oncogene* **30**, 372–378.

3. CIB1 controls oncogenic signalling by sphingosine kinase

We have revealed a crucial mechanism by which sphingosine kinase is regulated via its interaction with another protein called CIB1. We found that CIB1 binds to sphingosine kinase and transports this protein complex to the cell membrane, a localisation that we have previously shown to be a major

factor in the ability of sphingosine kinase to lead to tumour formation. These findings provide us with the unique opportunity to develop potential anti-cancer therapies to specifically target this pathway.

Jarman KE, Moretti PAB, Zebol JR & Pitson SM (2010) Translocation of sphingosine kinase 1 to the plasma membrane is mediated by calcium and integrin binding protein 1. *J Biol Chem* **285**, 483–492.

4. Pro-survival 14-3-3 proteins are regulated by sphingosine and FTY720

We have discovered that a family of 14-3-3 proteins that are critical for maintaining cell survival, bind to and are functionally altered by sphingosine. Sphingosine disrupts the pro-survival function of 14-3-3 proteins, leading ultimately to cell death. Additionally a new drug, FTY720 mimics the effect of sphingosine on 14-3-3 proteins suggesting that FTY720's anti-cancer effects are mediated by its effect on 14-3-3 proteins.

Woodcock JM, Ma Y, Coolen C, Pham D, Jones C, Lopez AF and Pitson SM (2010) Sphingosine and FTY720 directly bind pro-survival 14-3-3 proteins to regulate their function. *Cellular Signalling* **22**, 1291–1299.

Want to know more about sphingosine kinase?

Pitson SM (2011) Regulation of sphingosine kinase and sphingolipid signaling. *Trends Biochem Sci* **36**, 97–107.

Pitson SM, Powell JA and Bonder CS (2011) Regulation of sphingosine kinase in hematologic malignancies and other cancers. *Anti-Cancer Agents in Medicinal Chemistry*, in press.

Available Student Projects:

1. The molecular mechanisms of sphingosine kinase regulation

SK becomes rapidly and transiently activated in cells in response to growth factors and other regulatory agonists. This activation is critical in the signalling functions of this enzyme, and its dysregulation can lead to tumor formation. Thus, knowing how this activation occurs is important for understanding the function of this enzyme. As noted above, we have recently made a major advance in this area by establishing that phosphorylation of SK at serine-225 by a member of the ERK family directly results in its activation. Much is still not known, however, regarding how this phosphorylation is regulated, and whether other alternative regulatory mechanisms also control the activity and cellular location of this protein. Indeed, we have recently identified several proteins that interact with SK through the use of a yeast two-hybrid screen. We are currently examining some of these proteins to establish their possible roles in the regulation of SK activity and function. Several of these proteins represent exciting Honours projects.

2. Sphingosine kinase inhibitors as anti-cancer therapeutics

Using a structural model of the sphingosine kinase active site and in silico docking of small molecules, we have identified novel sphingosine kinase inhibitors that show efficacy in killing cancer cells in preliminary studies. Honours projects are available to examine these inhibitors as potential anti-cancer therapeutics on both solid tumor and leukemia cells, both in vitro and in mouse studies.

3. Sphingosine-regulation of the adapter protein 14-3-3 in apoptosis

As noted above, binding of sphingosine to 14-3-3 disrupts the anti-apoptotic function of this protein. To function 14-3-3 needs to be a dimer, but we have shown that binding of sphingosine results in phosphorylation of 14-3-3 at the dimer interface which blocks its dimerisation. Honours projects are available to examine the molecular basis for these effects of sphingosine, and also further develop our novel small molecules that mimic sphingosine as potential anti-cancer therapeutics

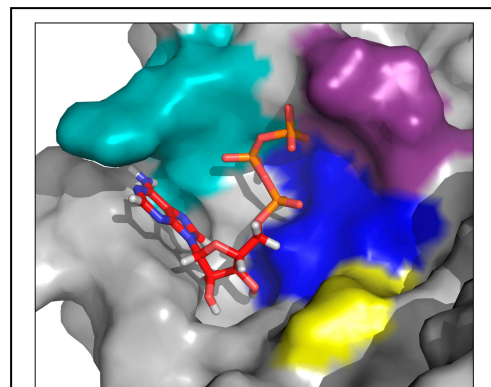


Fig 2. Molecular model of the sphingosine kinase active site