PTEN Redox Signalling through protein interactions

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Redox signalling plays an important role in cellular homeostasis and responses to oxidative stress in disease [1]. Many of the basic redox sensing and signalling pathways have been elucidated, and depend on the reversible oxidation of key proteins. An example is PTEN, which is a phosphatase responsible for regulation of the Akt signalling pathway, where PTEN reverses the action of phosphatidylinositol-4,5-bisphosphate 3 kinase (PI3K) and switches off Akt signalling [2]. The Akt signalling pathway is one of the primary signalling pathways in the cell, controlling among other things cell proliferation and survival; uncontrolled activation of this pathway leads to cancer, hence PTEN is a known tumour suppressor as loss of PTEN activity results in hyperactivation of the pathway.

Protein oxidation plays an important role in cell signalling [3], for example PTEN is inactivated under oxidative stress by the formation of a disulphide link in the active site of the enzyme, resulting in inactivation of PTEN and activation of Akt signalling. However, PTEN is also implicated in a number of other processes relating to, for example, sensing of DNA damage and control of DNA replication, which also play roles in the development of diseases including cancer. It is not understood how PTEN is involved in this control, but it does not appear to depend on PTEN activity. Hence it has been postulated that the proteins that interact with PTEN change between the reduced and oxidised form due to changes in the shape of PTEN, and these changes are responsible for some of the effects of PTEN oxidation on control of cellular processes.

Recently we developed an in vitro method for studying the interaction of PTEN with other proteins in its reduced and oxidised form [4]. This involved modifying the PTEN by oxidation, confirming by detailed analysis how this modification had affected the PTEN, then immobilising PTEN on beads and fishing in a cell lysate for the proteins that interacted with the different states of PTEN. Through this we were able to demonstrate that the “interactome” of PTEN is redox sensitive; that is, how proteins interact with PTEN depends on its redox status. This may well explain some of the activity of PTEN and how it affects pathways depending on the status of the cell.

This project will continue these studies with PTEN, further developing the characterization of the redox interactome of PTEN and validating that these changes are relevant in whole cells. The main techniques to be used will be proteomics for the characterisation of proteins and quantitative measurement of protein interactions and chemical biology methods, along with genetic and chemical biology methods to identify the functional consequences of the changes in interactions.. The project will use molecular and chemical biology techniques

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