New methods for studying the effects of redox biochemistry on protein function.

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Redox stress, the imbalance between oxidising species and the ability of the system to respond to these, and the cellular responses to oxidative stress, play important roles in many diseases [1]. Oxidative stress can cause direct damage to proteins, or oxidise other biomolecules such as lipids and sugars to generate reactive species that can modify proteins, which can alter their function [2]. An example of this is PTEN, which is inactivated during oxidative stress by the formation of a disulphide link in the active site of the enzyme, leading to activation of the Akt signalling pathway which can lead to cancer as it controls cell proliferation and survival. It has been suggested that PTEN is also involved in other cell processes important in diseases, such as DNA damage sensing and repair and cell cycle control. It has been postulated that changes in the proteins that interact with PTEN between the reduced and oxidised form are responsible for some of these effects.

Recently we developed an in vitro method for studying the interaction of PTEN with other proteins in its reduced and oxidised form [3]. This involved modifying PTEN in vitro, 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 does depend 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.

The novel proteomic and chemical biology methodology developed for studying PTEN demonstrates that the ability to modify a protein, fully characterise the modifications by proteomics methods, and then identify how the modified protein interacts with the components of the cell is a powerful technique that can lead to new understandings of disease processes. This has the potential to be useful in a range of other studies that look at the effects of protein modification on protein interactions, such as the effects of reaction between proteins and lipid and sugar oxidation products, the formation of nitrated and chlorinated amino acids (generated from molecules released during inflammation) or the sulfhydration by the signalling molecule hydrogen sulphide. This will give new insights into the effect of redox and oxidative stress in disease states, and identify targets for interventions to help to treat these diseases.

This project will further develop this methodology to study other redox sensitive proteins, chemical modification of proteins, or sulfhydration. The target proteins to be studied will be defined based on the interests of the student, but some examples are given below.

- The effect of proline and asparagine hydroxylation in redox sensing through the HIF pathway [4] (with Dr Alex Cheong)
- The modification of the appetite affecting protein ghrelin by reaction with lipid oxidation products and its potential role in diabetes [5] (with Dr James Brown)
- The sulfhydration of H-Ras, PTP-1B, Keap1 or NF-κB and its effect on signalling or function [6] (with Dr Colin Murdoch, Aston Medical School)

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