

# SUMO wrestling with Pim1 for cancer treatment

## What is the problem?

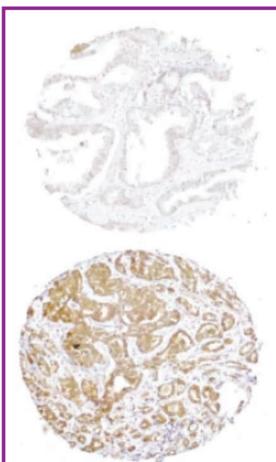


Figure 1 shows the amount and distribution of Pim1 protein (brown) in normal prostate tissue (top) and prostate cancer tissue (bottom). From Dhanasekaran et al., 2001.

Cancer is fast emerging as a leading cause of deaths worldwide. It is estimated that one in three individuals will develop some form of cancer during their lifetime. It is not only important to find cures for this disease, but also to understand why it happens in the first place.

A normal cell becomes cancerous when it starts to grow and multiply in an uncontrolled manner. At present, it is challenging to design a 'wonder drug' that will work against all types of cancer. In order to come up with effective treatments, various cancer-initiating or promoting proteins in a cell have been identified. If we could find a way of switching the activity of these proteins ON/OFF, we could potentially stop the cancer from growing.

One such protein is Pim1, which is maintained at very low levels in normal cells but is found to be elevated in tumours (see Figure 1). Pim1 is increasingly being considered as a therapeutic target, and various small molecular inhibitors, designed to switch off its activity, are currently undergoing clinical trials. Unfortunately, these have proven to be either ineffective or require large doses of drug, which can result in adverse side effects. Hence, there are no clinically approved Pim1-targeted drugs available on the market.

## What are we interested in?

While considerable research has been conducted on studying the effect of inhibition of Pim1 in cancer cells, there is very little information available on how Pim1 protein levels are regulated in normal or cancer cells. Since no major post-translational modifications of the protein have been reported to date, we were interested in investigating this aspect of the protein.

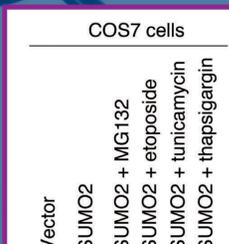
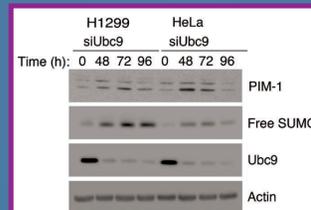
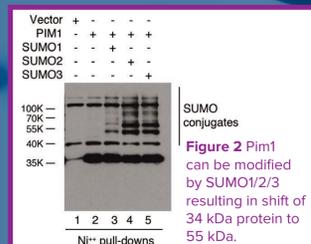
## What did we do?

We employed a variety of techniques to look at the interaction of Pim1 with other proteins and how this affected its structure. We used Western blotting to look at changes in the level of Pim1 by extracting protein from cells, separating them on the basis of their size and detecting the proteins using antibodies that specifically recognised the protein(s) of interest. We also used transfection to deliver artificial DNA or RNA into a cell to enable us to make our desired proteins inside the cell or reduce the levels of proteins already present inside the cell.

1. We analysed the Pim1 protein in various cancer cells by Western blotting, and found variants of the protein of higher molecular weight in the cancer cells. By looking at the amino acid sequence of Pim1, we identified a sequence (called a SUMO-motif) that indicated the protein might undergo post-translational modification. SUMO-motifs can be bound to by another protein called SUMO, which is known to modify proteins, and we wanted to identify what, if anything, SUMO might do to Pim1. We transfected cells with DNA for Pim1 alone or with Pim1 and SUMO (SUMO 1, 2 and 3) proteins.
2. We reduced the levels of a protein (Ubc9) that attaches SUMO to proteins in a cell by adding a type of RNA called siUbc9.
3. We blocked protein degradation in cells by treating cells with a drug called MG132.

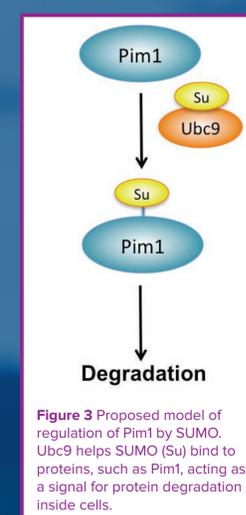
## What did we find?

1. We found that co-expression of Pim1 and SUMO led to the appearance of a bigger Pim1 protein in cells. This shows that SUMO (20 kDa) attaches itself to Pim1 (35 kDa) resulting in a higher molecular weight 55 kDa form of Pim1 (see Figure 2).
2. By reducing levels of Ubc9 by adding siUbc9, as expected, we observed an increase in levels of free SUMO in cells. Interestingly, we observed an increase in levels of Pim1 protein suggesting that SUMO affects Pim1 levels in a cell (see Figure 3).
3. Treatment of cells expressing Pim1 and SUMO with MG132 led to a strong accumulation of SUMOylated Pim1 (SUMO bound to Pim1). This suggests that SUMOylated Pim1 would normally be degraded inside the cells (see Figure 4).



## What does it mean?

It was believed that Pim1 does not undergo any post-translational modifications, but I discovered and showed that it could be modified by SUMO. Attachment of SUMO to Pim1 can inhibit the activity of Pim1 and can also cause its degradation, which could be exploited as a therapeutic strategy (see Figure 5). This gives rise to a possibility that use of a SUMO-pathway activator or inhibitor could be used to increase efficacy of drugs targeting Pim1. Efforts should also be made to identify new chemical compounds that would induce SUMOylation of Pim1 and, hence, its degradation. One such approach is already being used in the clinic, for example, the use of arsenic trioxide to treat patients with acute promyelocytic leukemia.



## Who am I?

I am a PhD student, currently in my final year, at the University of Dundee. Prior to this, I obtained my undergraduate degree in Biotechnology from Amity University (India), and a Masters by Research degree in Cancer Biology from the University of Dundee. During the course of my research, I have tried to fill in the crucial gaps in our understanding of the Pim1 protein. I wish to pursue academic research in the future to identify novel drug candidates, and study mechanisms of drug resistance in cancer.

I am grateful to Medical Research Scotland, the University of Dundee and CXR Biosciences for funding my studies. I am also thankful to my supervisor, Dr David Meek, for his constant support and positive suggestions.