

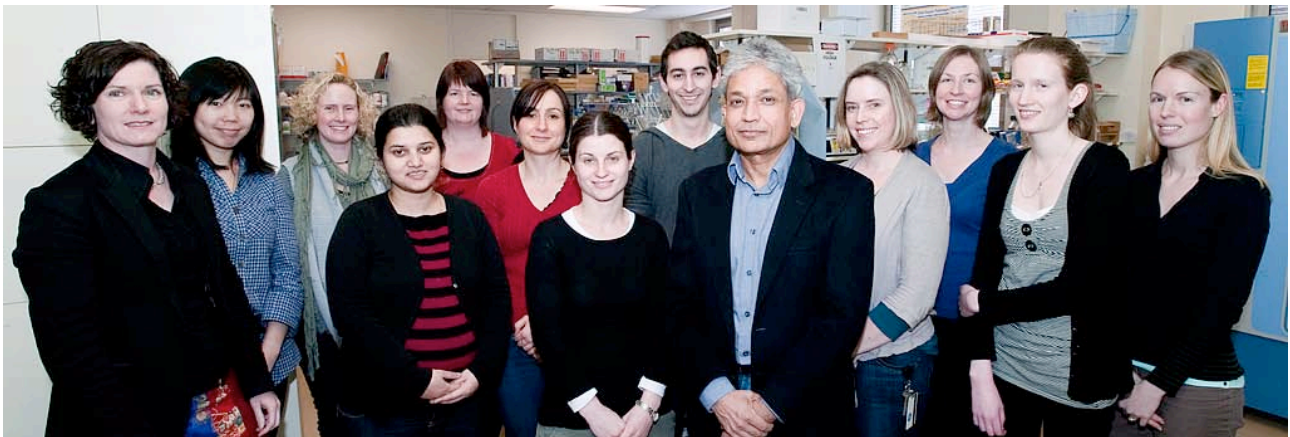
Molecular Regulation Laboratory

Prof. Sharad Kumar
Centre for Cancer Biology
SA Pathology
Frome Rd

Supervisors:

Primary: Professor Sharad Kumar

Others: Dr Loretta Dorstyn; Dr Donna Denton; Dr Natasha Boase; Dr Hazel Dalton;
Dr Jantina Manning



From left to right: Donna Denton, Layla Zhu, Natalie Foot, Sonia Shalini, Natasha Boase, Claire Wilson, Jantina Manning, Joey Puccini, Sharad Kumar, Hazel Dalton, Kathryn Mills, Shannon Nicolson, Loretta Dorstyn.

Contact person:

Professor Sharad Kumar OR please see below for individual project contacts.

Sharad.kumar@health.sa.gov.au

(08) 8222 3604

Research interests:

The Molecular Regulation Laboratory provides outstanding opportunities for training of graduate students (Honours and PhD). Our broad research focus is on cellular and molecular biology of disease, with an emphasis on cancer biology. Our two major interests are (1) the study of programmed cell death of normal and cancer cells and (2) understanding the regulation of cellular homeostasis by ubiquitination.

Millions of cells in the human body die every minute as part of normal homeostasis by a special process termed apoptosis. Apoptotic cell death plays a fundamental role in cell and tissue homeostasis and too little or too much of it can lead to many human diseases including cancer. Given the essential role of cell death in normal functioning of the human body, deciphering the

mechanisms of apoptosis is essential for understanding disease processes and to design effective treatment strategies for diseases which arise due to inappropriate apoptosis. We study the mechanisms and regulation of cell death in normal homeostasis and during animal development, with a particular emphasis on the roles of the cell death and survival machinery in cancer.

Ubiquitination (attachment of ubiquitin to a target protein) is a common type of protein modification that is involved in the regulation of protein stability, degradation, localisation and trafficking. Ubiquitination is a major regulator of many ion channels, receptors and transporters. We are studying the functions of a group of ubiquitin-protein ligating enzymes (Nedd4 family of ubiquitin ligases), which are implicated in the ubiquitination of a number of proteins mentioned above. We use a variety of molecular, cellular and gene knockout approaches to study the physiological functions of these enzymes and establish their roles in human diseases.

Eligible students will be able to apply for a RAH Research Committee Honours Scholarship or a top-up Postgraduate Scholarship.

For more information about our laboratory, please visit our website <http://www.centreforcancerbiology.org.au/kumar.htm>

Projects offered for 2012:

(1) The role of Nedd4-2 in hypertension and the regulation of sodium channels

(Contact: Dr Natasha Boase, natasha.boase@health.sa.gov.au and Dr Jantina Manning, jantina.manning@health.sa.gov.au)

Aberrations in the ubiquitin system underpin the pathogenesis of many diseases including cancer, neurodegenerative disorders and channelopathies. The Nedd4 and related ubiquitin ligases (E3s) are required for the ubiquitination of numerous cellular targets involved in processes such as transcription, stability and trafficking of plasma membrane proteins, and the degradation of misfolded proteins. We have shown that Nedd4-2 E3 ubiquitinates the epithelial sodium channel (ENaC). ENaC is required for sodium absorption across a range of epithelial tissues such as the lungs, colon and kidney and is an important regulator of blood sodium concentration and blood pressure. Ubiquitination of ENaC by Nedd4-2 leads to its internalisation and degradation. Our current focus is to characterise the mechanisms of regulation of ENaC by Nedd4-2 in vivo by using Nedd4-2 gene knockout mice. We are also studying the role of Nedd4-2 in regulating additional novel targets. In this project the students will use a range of molecular, cellular and animal techniques.

(2) Regulation of animal growth by Nedd4

(Contact: Dr Natasha Boase)

In a collaborative study with Prof Yang (University of Iowa) we have recently found that the loss of Nedd4 in mice results in reduced IGF-1 and insulin signalling, reduced growth and neonatal lethality. Nedd4-deficient cells show reduced mitogenic activity. This appears to be due to increased levels of the adaptor protein Grb10 resulting in IGF-1R mislocalization and inhibition of

IGF-1 and insulin signalling. We are now studying the mechanism of Grb10 regulation by Nedd4. There is evidence to suggest that Nedd4 has additional cellular targets. Thus, we are analysing additional phenotypes that may be associated with the knockout of Nedd4. In this project the students will use animal studies, immunohistochemistry, biochemical cellular and molecular techniques to decipher the mechanisms by which Nedd4 controls animal growth and development.

(3) Regulation of iron homeostasis by the Nedd4-interacting proteins, Ndfip1 and Ndfip2

(Contact: Dr Hazel Dalton, hazel.dalton@health.sa.gov.au)

Iron homeostasis is a highly regulated process which, if perturbed, leads to a number of disease states such as haemochromatosis or anaemia. Our recent work shows that Ndfips regulate the divalent metal ion transporter DMT1, the primary non-heme iron transporter in mammals. DMT1 interacts with both Ndfip1 and Ndfip2, and this promotes DMT1 ubiquitination and degradation by the Nedd4-family ubiquitin ligase, WWP2. Furthermore the Ndfip1 knockout mice show increased hepatic iron deposition, indicating an essential function of Ndfip1 in iron homeostasis. Our current focus is to further characterise WWP2 and Ndfip knockout mice to provide additional understanding of this novel mechanism of regulating iron transport. Students will carry out iron feeding experiments, tissue analysis for iron deposition and a range of cellular and molecular studies to delineate the regulation of DMT1 by Ndfips and WWP2.

(4) The role of caspase-2 in apoptosis, cell cycle and tumor suppression

(Contact: Dr Loretta Dorstyn, loretta.dorstyn@health.sa.gov.au)

Our ongoing work has made a landmark discovery that the lack of caspase-2 enhances the ability of cells to transform readily and that caspase-2 deficiency increases the potential of tumourigenesis *in vivo*. Using a mouse model of tumorigenesis, the E μ -Myc lymphoma model, we found that the loss of even a single allele of caspase-2 resulted in accelerated tumourigenesis, and this was further enhanced in caspase-2^{-/-} mice. The caspase-2^{-/-} cells show increased growth rates, a defective apoptotic response to cell cycle checkpoint regulation, a defective DNA damage response and abnormal cycling following γ -irradiation. These data show that loss of caspase-2 results in an increased ability of cells to acquire a transformed phenotype and become malignant, indicating that caspase-2 is a tumour suppressor protein. In this project the prospective students will test whether caspase-2 can function as a tumour suppressor in other mouse models of tumorigenesis. The study will involve cellular and molecular techniques to dissect out the mechanism(s) by which caspase-2 acts to suppress tumour development and to identify target(s) of caspase-2 that mediate its tumour suppressor function.

(5) Cell death regulation during animal development

(Contact: Dr Donna Denton, donna.denton@health.sa.gov.au)

We have been utilising *Drosophila* as an *in vivo* model to dissect out the mechanisms of developmentally programmed cell death (PCD). Our ongoing studies have led to several seminal findings, including the discovery of the key canonical pathway of PCD involving the caspase Dronc, and the adaptor Ark. We have also discovered a novel potential regulator of caspase activation from a Dronc-interaction screen. This project will now characterise the role of the novel protein in caspase activation and cell death, and identify other potential regulators of caspase

activation using a range of molecular, cellular, biochemical and genetic approaches.

(6) Role of autophagy and growth arrest in cell death

(Contact: Dr Donna Denton)

In recent studies we discovered that the Dronc/Ark pathway, while essential for most PCD, is largely dispensable for developmental PCD in specific tissues. This is most obvious in the larval midgut (MG), which undergoes PCD during metamorphosis, and this process is unaffected in *dronc* and *ark* mutants. In preliminary studies we have found that the inhibition of autophagy, a caspase-independent mechanism of PCD, leads to a delay in MG removal indicating a potential role for autophagy in MG PCD. Given that the role of autophagy in cell death is a matter of extensive debate, our discovery that MG PCD can be delayed by genetically blocking autophagy provides a unique model for delineating this controversy. We hypothesise that during development PCD utilises caspase-dependent (most tissues), caspase- and autophagy-dependent (e.g. larval salivary glands), and caspase-independent but autophagy-dependent (e.g. midgut) mechanisms. In this project we will delineate the mechanism of midgut cell death by exploring the contribution of caspases and autophagy. The project also aims to define the role of growth signalling in midgut cell death, to determine how growth signals and death signals may be integrated to regulate autophagy. A range of cellular, molecular and genetic approaches will be utilized in these projects. Given the controversial role of autophagy in human disease a better understanding of the regulation of autophagy is important for future treatments of disease.

Key references:

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2. Ho LH, Taylor R, Cakouros D, Dorstyn L, Bouillet P, Kumar S (2009) A tumor suppressor function for caspase-2. **Proc. Natl. Acad. Sci. USA** 106: 5336-41.
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5. Kumar S (2009) Caspase-2 in apoptosis, DNA damage response and tumor suppression: Enigma no more? **Nature Reviews Cancer** 9: 897-903.