

Transcription Factors in Leukaemogenesis

Supervisors: A/Prof Richard D'Andrea and Dr Anna Brown.

Email: richard.dandrea@health.sa.gov.au , anna.brown@health.sa.gov.au

We currently have projects focussed on the transcription factor, Kruppel like factor 5 (KLF5) with the aim of understanding the role of this gene in normal blood cell development and how its function may be modified in acute myeloid leukaemia (AML).

KLF5 was identified by us in a gene expression microarray study as a potential myeloid tumour suppressor gene¹ and our data shows that it has reduced expression in AML and that it has tumour suppressor activity when expressed in human AML cell lines.

Further projects in this area are currently investigating expression, methylation and mutation of the *KLF5* gene in a large panel of AML patient samples. We have also generated a conditional loss-of-function mouse model to study *KLF5* function in blood cell development. Projects will be available that aim to identify and verify target genes of *KLF5* in blood cells (by microarray and chromatin immunoprecipitation) and to study the regulation of expression of the *KLF5* gene itself in normal and leukaemic blood cells.

Key References:

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Molecular Characterisation of Diamond Blackfan Anemia

Supervisors: A/Prof. Richard D'Andrea, Dr. Sarah Bray, Dr. Michelle Perugini.

Email: richard.dandrea@health.sa.gov.au , sarah.bray@adelaide.edu.au , michelle.perugini@health.sa.gov.au

Diamond Blackfan Anemia (DBA) is a bone marrow failure syndrome characterised by haploinsufficiency for a number of ribosomal proteins associated with specific red cell and congenital defects. DBA is one of an emerging group of disorders known as 'ribosomopathies'. The mechanism of tissue specificity in DBA remains unclear.

We aim to understand how decreased expression of these ubiquitously expressed ribosomal proteins translates to defective erythropoiesis, causing a distinct erythroid phenotype.

To achieve this we aim to identify key genes and proteins in erythroid progenitors that are expressed at altered levels in cells with the ribosomal protein (RPS/RPL) defects, and contribute to the disease phenotype. For this we will make use of proteomic technologies, global gene expression profiling and pathway analyses which will allow us to compare the complement of proteins, genes, pathways and regulatory networks in cells with and without the ribosomal protein defect. Key genes and proteins identified in our studies will subsequently be tested in established experimental models to assess their contribution to the defective erythropoiesis.

Key DBA references:

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Molecular mechanisms of Polycythemia Vera – identification and characterisation of novel candidate genes.

Supervisors: A/Prof. Richard D'Andrea, Ms Carolyn Butcher.

Email: Richard.dandrea@health.sa.gov.au, Carolyn.butcher@health.sa.gov.au

The Philadelphia chromosome negative myeloid proliferative neoplasms (MPNs) including Polycythemia vera (PV), Essential Thrombocythemia (ET) and Primary myelofibrosis (PMF), are clonal stem cell disorders characterised by an excess of mature cells of one or more myeloid lineages and an associated risk of progression to myelofibrosis and leukemic transformation. The MPN-specific somatic mutation in *JAK2* (JAK2V617F) is found in greater than 95% of PV, 50% of ET and 50% of IMF patients. Numerous other changes have been identified in common between MPN and *de novo* acute myeloid leukemia (AML), including somatic mutations of *TET2*, *ASXL1*, *CBL*, *IKZF1*, *EZH2*, *IDH1*, *IDH2*, *RUNX1* and *DNMT3A* consistent with a model for MPN involving a pre-clinical unstable hematopoietic phenotype and disease progression that is associated with further mutation in a select group of progression genes. We have used several genetic approaches with our MPN patient cohort to identify changes associated with disease initiation and progression to AML. Most recently we have conducted a targeted exon capture and Next-gen sequence screening approach for 15 MPN patients and 657 genes prioritised as potential candidates from a number of sources including the Catalogue of Somatic Mutations in Cancer (COSMIC) database. This study has identified several novel somatic gene variants that highlight novel pathways as potentially important in MPN pathogenesis. The project will initially focus on determining the significance of these gene variants in MPN and AML by screening larger MPN and AML patient cohorts followed by functional characterisation using retroviral expression of gene variants to examine growth and differentiation responses *in vitro*. Candidate gene mutations that show effects in these assays will be further tested *in vivo* in a murine bone marrow reconstitution model in order to assess their engraftment and transformation potential.

Key references:

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Dissecting the TRIB3 function in MLL AML

Supervisors: A/Prof Richard D'Andrea and Dr Chung Hoow Kok

Email: richard.dandrea@health.sa.gov.au

AML is associated with a number of chromosome translocations which produce transcription factor fusion proteins that disrupt the normal myeloid differentiation program. The most frequent chromosome translocations are t(15;17) PML-RARA, t(8;21) AML-ETO, inv(16) CBF β -MYH11 and the 11q23 MLL-fusion AML. Among these AML translocations, the MLL group has the worst prognostic outcome. Recently, we have identified the mRNA for TRIB3, a negative regulator of NF-kappaB, is lower in MLL AML compared to AML with good prognostic outcome (e.g. PML-RARA subgroup). TRIB3 function is largely uncharacterised in AML. We will use several molecular

biology and bioinformatics approaches to interrogate TRIB3 function in AML cell lines, patient samples and using in vivo models

β -catenin activation and target gene identification in Acute Myeloid Leukaemia (AML)

Supervisors: A/Prof Richard D'Andrea, Dr Michelle Perugini, Dr Hayley Ramshaw

Email: richard.dandrea@health.sa.gov.au , michelle.perugini@health.sa.gov.au , hayley.ramshaw@health.sa.gov.au

β -catenin is a central regulator of growth and self-renewal in multiple cell types, and mutations that cause activation of β -catenin activity have been found in many solid tumours (e.g. colorectal, lung, ovarian, breast).

Self-renewal is a critical property of cancer stem cells that contributes to disease relapse and targeting self-renewal regulators is an important new approach in cancer treatment. β -catenin is a transcriptional co-activator that is central to transmission of canonical Wnt signalling.

Over the past several years evidence has been emerging that β -catenin protein stabilisation, which is essential for its transcriptional regulatory activity, has important roles in self-renewal of normal haemopoietic stem cells as well as leukaemic stem cells.

The mechanism associated with β -catenin stabilisation in haemopoietic cells is not well understood. We have shown β -catenin regulation downstream of receptor signalling in AML and we will use several approaches to dissect the pathways associated with this. The transcription factor DNA-binding partners and direct targets genes of β -catenin are also poorly defined. We will characterise the function of direct target genes and partner binding proteins for β -catenin, as well as the associated signalling pathways activated, in acute myeloid leukaemia.