

Mechanisms of leukaemogenesis downstream of activated cytokine receptors

Supervisors: A/Prof Richard D'Andrea and Dr Michelle Perugini

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

Activating mutations in FLT3 are the most common lesions in Acute Myeloid Leukaemia (AML). Constitutive activation by acquired mutation occurs in approximately 30-35% of AML cases. The most common mutation is an internal tandem duplication (ITD) in the intracellular, juxtamembrane (JM) domain which occurs in approximately 20-25% of patients.

In addition, approximately 7% of patients have point mutations in aspartic acid residue 835 in the kinase domain or mutations in the JM region. Elucidation of key downstream effectors of FLT3 may allow identification of other targets for treatment of FLT3 leukaemias.

We have previously described an activating trans-membrane mutation (GMR V449E) in the common receptor signaling subunit ($h\beta_c$) for GM-CSF, IL-3 and IL-5 which is a potent inducer of myeloid leukemia in mice. With a view to identifying critical common events induced by the activated FLT3 and GMR mutants we undertaken a comparison of signaling events and transcriptional regulation downstream of the GMR mutant, V449E, to those induced by the FLT3 activated mutants in the FDB1 cell line.

We have identified several signaling pathways (e.g. Jak/STAT, p44/42 Map Kinase), and regulated transcription factors (e.g. β -catenin, C/EBP α , Gadd45 α) that are used by all mutant receptors. Projects will be available that look in further detail at how these downstream molecules are important in contributing to the leukaemic phenotype.

1. M. Perugini, A.L. Brown, D.G. Salerno, G.W Booker, C. Stojkoski, T.R Hercus, A.F Lopez, M.L Hibbs, T.J. Gonda, and R.J. D'Andrea. 2010, "Alternative modes of GM-CSF receptor activation revealed using activated mutants of the common β -subunit" *Blood*, 115(16):3346-53, IF. 10.432
2. Angel F Lopez, Timothy R Hercus, Paul Ekert, Dene R Littler, Mark Guthridge, Daniel Thomas, Hayley S Ramshaw, Frank Stomski, Michelle Perugini, Richard D'Andrea, Michele Grimbaldston, and Michael W Parker, 2010, "Molecular basis of Cytokine Receptor Activation" *IUBMB Life Journal* (ahead of print).
3. Brown, A.L., Peters, M., D'Andrea, R.J., Gonda, T.J. 2004. Constitutive mutants of the GM-CSF receptor reveal multiple pathways leading to myeloid cell survival, proliferation, and granulocyte-macrophage differentiation. *Blood*. 103:507-16
4. McCormack MP, Gonda TJ. Myeloproliferative disorder and leukaemia in mice induced by different classes of constitutive mutants of the human IL-3/IL-5/GM-CSF receptor common beta subunit. *Oncogene*. 1999;18:7190-7199

Regulation of GADD45A in AML

Supervisors: A/Prof Richard D'Andrea and Dr Michelle Perugini

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

GADD45A is a member of the Growth Arrest and DNA Damage Inducible gene family and has been implicated as a tumour suppressor in many cancers. Gadd45a down-regulation is an important marker of tumour progression, and we observe Gadd45a down-regulation in several classes of AML and in association with common receptor mutations in AML.

We have identified methylation of the GADD45A promoter as an important predictor of survival and we have a number of ongoing projects to establish the mechanisms of down-regulation of Gadd45a and its role in AML. Specifically, these include;

- 1) studying epigenetic modification at the Gadd45a locus, in particular CpG methylation of the Gadd45a promoter in AML.
- 2) characterisation of the mechanism associated with down-regulation of Gadd45a in AML with mutations in the FLT3 receptor
- 3) using in vitro and in vivo models to characterise the co-operation of loss of Gadd45a with other oncogenic lesions such as activating mutations in FLT3 receptors or translocations involving the MLL gene
- 4) the ability of Gadd45a to induce DNA demethylation and activation of tumour suppressor genes in AML cells.

1. M. Perugini, C.H. Kok, A.L. Brown, C.R. Wilkinson, D.G. Salerno, S.M. Young, S.M. Diakiw, I.D. Lewis, T.J. Gonda, and R.J. D'Andrea 2009, "Repression of Gadd45a by activated FLT3 and GM-CSF receptor mutants contributes to growth, survival and blocked differentiation". *Leukemia*, 23(4):729-38, IF. 6.924

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 hypothesise that the action of β -catenin in myeloid cells may not be solely through TCF/LEF family members (DNA binding members of the canonical Wnt signalling pathway) and propose here to analyse specific gene targets identified by chromatin immunoprecipitation and high-throughput sequencing (ChIP-seq) using β -catenin antibodies. This will characterise the function of the direct target genes and partner binding proteins for β -catenin in myeloid leukaemia.

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 researching two particular transcription factors, Kruppel like factor 5 (KLF5) and β -catenin with the aim of understanding exactly how they malfunction to contribute to AML. *KLF5* was identified by us in a gene expression microarray study as a potential myeloid tumour suppressor gene.

We have generated data that shows 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 will involve looking at expression, methylation and mutation of the gene in a large panel of AML patient samples, studying loss of function effects in a mouse conditional knockout model and identification of target genes in AML.

Over the past several years evidence has been emerging that Wnt signaling and a major downstream effector of this signalling, β -catenin, have important roles in normal haemopoietic stem cell self renewal and development of myeloid leukaemia. However the mechanisms for this and the key downstream targets are yet to be completely understood.

Several studies have now shown that β -catenin protein is commonly stabilised in AML leading to constitutive transcriptional activator activity. We currently have projects looking at how β -catenin stabilisation contributes to AML. This include β -catenin ChIP-seq to identify β -catenin target genes, profiling of β catenin stability and activity in our local panel of AML patient samples and studying the requirement of β -catenin for AML stem cell self-renewal in mouse conditional knockout models.

1. Brown, A. L., Wilkinson, C.R., Waterman S.R., Kok, C.H., Salerno, D.G., Diakiw, S.M., Scott, H.S., Tyskin, A., Goodall, G.J., Solomon, P.J., Gonda, T.J. and D'Andrea, R.J. Genetic regulators of myelopoiesis and leukemic signaling identified by gene profiling and linear modeling. *J. Leuk. Biol.* 80(2):433-47 (2006)
2. Mikesch, J.H., Steffen, B., Berdel, W.E., Serve, H., and Muller-Tidow, C. (2007). The emerging role of Wnt signaling in the pathogenesis of acute myeloid leukemia. *Leukemia* 21, 1638-1647.

Molecular Characterisation of Diamond Blackfan Anemia

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

Email: richard.dandrea@health.sa.gov.au michelle.perugini@health.sa.gov.au , carolyn.butcher@health.sa.gov.au

Diamond Blackfan Anemia (DBA) is characterised by haploinsufficiency for a number of ribosomal proteins associated with specific red cell and skeletal defects. The mechanism of tissue specificity in this disease remains unclear.

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

To achieve this we aim to identify key proteins in erythroblasts that are produced at reduced levels in cells with mutant ribosomal proteins (RPS/RPL), and contribute to the disease phenotype. For this we will make use of proteomic technologies which allow us to compare the complement of proteins produced in cells with and without the ribosomal protein defect. These proteins will subsequently be tested in established experimental models to assess their contribution to the defective erythropoiesis.

Screening for compounds that induce differentiation or cell death of AML cells.

Supervisors: A/Prof Richard D'Andrea and Dr Mark Guthridge.

Email: richard.dandrea@health.sa.gov.au , mark.guthridge@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 der(11q23) MLL-fusions. The presence of these readily identifiable karyotypic abnormalities may define features of the AML that will provide for selective therapy. This is clearly the case with AML carrying the t(15:17) translocation which can be successfully treated with ATRA.

We have used an approach to understanding better the contribution of the aberrant fusion-products to AML pathogenesis and identify selective therapies. To identify unique features associated with particular translocations and to define overlapping pathways involved in AML we examined gene expression signatures associated with the major PML-RARa, AML-ETO, CBF β -MYH11 and MLL gene fusions.

We compared the gene expression patterns of 76 AML patients (N Engl J Med. 350:1617-28) with translocation events categorized as t(15;17), t(8;21), inv(16) or der(11q23) and determined the gene expression changes, relative to normal bone marrow. We generated translocation-specific gene signatures, which were used to query the drug gene expression profiles via the Connectivity Map identifying small molecules or FDA-approved drugs associated with reversal of the signature.

This has suggested a number of potential treatments selective for these sub-types of AML. Projects are available to test the effectiveness of these candidate therapies on AML cell lines, patient samples and using in vivo models.

Proteomic analysis of chemotherapy response in Acute Myeloid Leukaemia.

Supervisors: A/Prof Richard D'Andrea, Dr Ian Lewis, and Dr Michelle Perugini

Email: richard.dandrea@health.sa.gov.au , ian.lewis@health.sa.gov.au , michelle.perugini@health.sa.gov.au

In AML, several factors have been identified that confer a good or poor prognosis. These include age, white blood cell count, the presence or absence of a preceding haematologic disorder, response to induction chemotherapy and the cytogenetic profile. Cytogenetics is the most important prognostic marker, but there remains heterogeneity within specific cytogenetic subtypes. No cytogenetic abnormality, or a normal karyotype, at diagnosis accounts for 40 – 50% of AML cases at diagnosis but within this group specific molecular mutations such as FLT3, NPM1 or CEBP-A mutations confer unfavourable or favourable outcomes.

Response to induction chemotherapy has been found to be a negative prognostic indicator. Gene expression signatures predictive of in vivo drug resistance have been identified. However, changes in gene expression do not necessarily correlate with protein levels within the cell. Therefore it is of interest to determine if there is a change in protein expression levels in patients depending on their response to chemotherapy. Proteomics offers the ability to identify changes in expression level and post-translational modification of a vast number of proteins in patient samples.

We predict different protein expression profiles will be observed in patients that respond to chemotherapy compared to patients that do not respond to chemotherapy. Protein profiles of patients that respond or did not respond to induction chemotherapy will be compared and proteins that are differentially expressed or modified between groups will be identified.

Identification of specific changes in protein expression may lead to the development of specific therapies targeting these abnormal proteins.

Molecular characterisation of Polycythemia Vera

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

Email: richard.dandrea@health.sa.gov.au , carolyn.butcher@health.sa.gov.au

Myeloproliferative neoplasms (MPN), especially polycythemia vera (PV), are associated with somatic mutations of JAK2 and although murine in vivo studies indicate that their acquisition is sufficient for development of MPN, it is evident from human studies that additional genes are involved.

A number of other genes have now been identified with a role in progression but the nature of a pre-JAK2 event remains undetermined. We have used a number of genetic approaches with our MPN patient cohort to identify changes associated with disease initiation.

These include candidate-gene sequencing from granulocyte and BFU-e DNA, exon capture followed by Next-gen sequencing and genome-wide paired SNP-array analysis (Affymetrix Genome-Wide Human array 6.0) of granulocyte samples and corresponding germ-line DNA to identify genomic regions of acquired LOH and copy number variation.

This analysis has highlighted changes in chromatin-associated factors as potentially important in disease initiation. We have also shown acquisition of RUNX1 and JAK2 mutations in divergent clones in a PV patient who developed acute leukemia, consistent with a model for MPN involving a pre-clinical unstable hematopoietic phenotype and progression associated with further mutation in a select group of progression genes. Projects associated in this area are focussed on identifying initiating molecular lesions in MPN.