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Investigation of the Role and Mechanism of Beta-Catenin Activation in Acute Myeloid Leukaemia

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Abbreviations

AML	Acute myeloid leukaemia
AML-ETO	AML1-Eight-Twenty One oncoprotein fusion
APC	Adenomatous polyposis coli
βc	Common beta-subunit
BCL-2	B-cell lymphoma 2
BM	Bone marrow
ChIP	Chromatin immunoprecipitation
ChEA	ChIP enrichment analysis
GSEA	Gene set enrichment analysis
CISH	Cytokine-inducible SH2-containing protein
CK1α	Casein kinase 1 alpha
CML	Chronic myeloid leukaemia
CSC	Cancer stem cell
CSF2RB	Colony stimulating factor 2 receptor beta
EGR-1	Early growth response protein 1
ERK	Extracellular regulated kinase
FAB	Frech-American-British
FACS	Fluorescence activated cell sorting
FCS	Foetal calf serum
FDM	Factor-dependent myeloid
FDR	False discovery rate
FRA-1	Fos-related antigen 1
GFP	Green fluorescent protein
GSK3β	Glycogen synthase kinase 3 beta
GM	Granulocyte-macrophage
GM-CSF	Granulocyte-macrophage colony stimulating factor
GMP	Granulocyte-macrophage progenitor

HSC	Haematopoietic stem cell
HOX	Homeobox
IL-3	Interleukin-3
IL-3R	Interleukin-3 receptor
IL-3Rα	Interleukin-3 receptor alpha-subunit
IL-5	Interleukin-5
ITD	Internal tandem duplication
JAK	Janus kinase
LEF	Lymphoid enhancer factor
LIMMA	Linear models for microarray data analysis
LSC	Leukaemic stem cell
mAb	Monoclonal antibody
MIG	MSCV-IRES-GFP
MLL	Mixed-lineage leukaemia
miR	Micro RNA
MSigDB	Molecular signatures database
PI3K	Phosphoinositol 3 kinase
PIM1	Proviral integration site for Moloney-murine leukaemia virus 1
PML-RARα	Promyelocytic leukaemia-retinoid acid receptor alpha
PTGS2	Prostaglandin-endoperoxide synthase 2
QRT-PCR	Quantitative real-time polymerase chain reaction
RUNX1	Runt-related transcription factor 1
STAT	Signal transducer and activator of transcription
TCF	T-cell factor
WHO	World Health Organization
Wnt	Wingless-type
WT	Wild-type

Abstract

Aberrant activation of β-catenin is a common event in Acute Myeloid Leukaemia (AML), and accumulating evidence indicates this pathway plays a critical role in the establishment and maintenance of myeloid neoplasms. In AML, increased β-catenin signalling has been associated with activating mutations in the FLT3 receptor, and the oncogenic AML1-ETO and PML-RARα translocation products. In the absence of these lesions, however, it remains unclear which mechanisms may activate β-catenin in AML more broadly. Here we have explored a potential role for the multipotent haematopoietic cytokine, interleukin-3 (IL-3) in the regulation of β-catenin signalling in myeloid and leukaemic cells.

We show that IL-3 can induce the dose dependent stabilisation of β-catenin in a myeloid model of Hox oncogenesis, and that β-catenin is required for IL-3 driven colony formation and growth. Enforced expression of β-catenin in this system allows cell survival at sub-optimal concentrations of IL-3 which may contribute to leukaemic transformation by providing a survival advantage to blast cells in the haematopoietic niche.

We also demonstrate that IL-3 can promote β-catenin activation in the IL-3 dependent human erythroleukaemia cell line, TF-1.8, and in primary AML cells. Furthermore, Affymetrix gene expression analysis of bone marrow cells from four AML patients treated ± IL-3 revealed a strong correlation between the IL-3 induced signature and Wnt/β-catenin gene networks. Interestingly, the IL-3 receptor alpha subunit (IL-3Rα) has been previously shown to be overexpressed in AML leukaemic stem cells and progenitors compared to normal counterparts, and elevated levels of IL-3Rα are associated with poor prognosis and overall survival.

Consistent with the regulation of β-catenin by IL-3, we show that a neutralising monoclonal antibody (7G3) which targets IL-3Rα, inhibits IL-3 mediated activation of β-catenin in TF-1.8 and primary AML cells. Modified versions of 7G3 are currently undergoing clinical trials for patients with AML, and our data indicates that this therapy may be more effective for patients with elevated levels of oncogenic β-catenin.

As previous studies have demonstrated that cytokines can induce the inhibitory phosphorylation of GSK3β via activation of the PI3K/AKT pathway, we have also made use of pharmacological PI3K and AKT small molecule inhibitors to determine the importance of this axis in the IL-3 mediated regulation of β-catenin.

On the whole, the work in this thesis reveals a novel mechanism which may contribute to β-catenin activation in AML, and provides further insight into the amplitude of IL-3 signalling in normal and malignant haematopoiesis**.**

Table of Contents

List of Figures

List of Tables

List of Appendices