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Investigation of KLF5 Function in Normal Haemopoiesis

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Abbreviations

ACK	Ammonium, Chloride, Potassium
AML	acute myeloid leukaemia
AngII	angiotensin II
APC	adenomatous polyposis coli
APL	acute promyelocytic leukaemia
ATF3	activating transcription factor 3
Atg	*Autophagy*
BM	Bone marrow
C/EBP	CCAAT-enhancer-binding proteins
CBC	complete blood counts
CFU	erythroid progenitor
CFU Assay	colony-forming unit assays
CFU-E/BFU-E	erythroid colony forming cells
CHO	Chinese hamster ovary
CLP	common lymphoid progenitors
CMP	common myeloid progenitors
Dpc	days postcoitum
DSS	dextran sodium sulphate
Egr-1	epidermal growth factor receptor
EKLF	Erythroid Krüppel-like factor
EKLFTAD2	transactivation domain 2 of KLF1
ER	estrogen receptor

ESCC	esophageal squamous cell cancer
ESCs	embryonic stem cells
FASN	fatty acid enzyme
FGF-BP	fibroblast growth factor binding protein 1
FISH	fluorescent in situ hybridization
FS	forward scatter
G-CSF	Granulocyte-colony stimulating factor
GM	Granulocyte-Macrophage
GMP	granulocyte-macrophage progenitors
Hct	Haematocrit
HE	haematoxylin and eosin
Hgb	Haemoglobin
HRP	horseradish peroxidase
HSC	haemopoietic stem cells
HSPC	haemopoietic stem and progenitor cell
IBD	inflammatory bowel disease
ICM	inner cell mass
JNK	c-Jun N-terminal kinase
Klf5-KO	*Klf5* knockout
KLFE	KLF-binding element
KLFs	*Krüppel-like factors*
KO	knockout
LOH	Loss-of-heterozygosity

LT-HSC	long-term HSC
MAPK	Ras-mitogen-activated protein kinase
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MEP	megakaryocyte-erythrocyte progenitor
MkP	megakaryocyte progenitor
MPO	myeloperoxidase
MPP	potent progenitor
PAGE	SDS-Polyacrylamide Gel Electrophoresis
PAH	pulmonary arterial hypertension
PASMC	pulmonary artery smooth muscle cell
PB	Peripheral blood
PCR	Polymerase Chain Reaction
PE	primitive endoderm
PKC	lisophosphatidic acid and non-canonical Wnt signalling
preCFU	early erythroid progenitor
preGM	primitive granulocyte-macrophage progenitors
preMEGE	primitive erythroid/megakaryocyte progenitor
QIMR	Queensland Institute of Medical Research
QPCR	Quantitative reverse transcription PCR
Rb	*Retinoblastoma*
RBC	red blood cell

SDS	Sodium Dodecyl Sulfate
SID	Sin3a-interacting domain
SM	Sample Media
Socs3	*suppressor of cytokine signalling-3*
SS	side scatter
ST-HSC	short-term HSC
TAC	Tris Ammonium Chloride
TAD	transactivation domain
TAZ	PDZ-binding motif
TE	trophectoderm
TFs	transcription factors
VSMCs	vascular smooth muscle cells
WBC	white blood cell
YAP	Yes-associated protein

Abstract

Krüppel-like factor 5 (KLF5) is a zinc-finger transcription factor known to have regulatory roles in the growth and differentiation of many adult tissues. In humans, *KLF5* is located at 13q21-22, which is frequently lost in multiple tumour types, including tumours of the breast, endometrium, ovary and prostate where it is associated with loss of *KLF5* expression. Little is known about the potential role of KLF5 in the haemopoietic system. Previous work by us and others has shown that KLF5 has a functional role in induction of differentiation of the myeloid compartment. In acute myeloid leukaemia (AML), our group has previously show that *KLF5* expression is reduced relatively to normal $CD34^{+}$ cells and that reduction of expression is associated with hypermethylation in intron 1. We also found that hypermethylation of *KLF5* was associated with poor outcome, identifying KLF5 as an important target for further investigation in haemopoiesis and particularly the myeloid compartment. To extend the functional analysis of *Klf5*, an *in vivo* gene-ablation model was generated. As non-conditional *Klf5* knockout mice (KO) die at embryonic day 8.5, pan-haemopoietic *Klf5* conditional gene KO mice were generated by crossing $Klf5^{fl/fl}$ mice with *Vav-cre* transgenic mice. The $Klf5^{fl/fl}Vav\text{-}cre^{+/-}$ and $Klf5^{fl/fl}$ mice were analysed at 3, 9 and 12 month of age for defects in steady state haemopoiesis. Peripheral blood (PB) analysis of 9 and 12 month old mice revealed that $Klf5^{fl/fl}Vav\text{-}cre^{+/-}$ animals displayed significantly higher values for total white blood cell (WBC) count. To further characterise the changes in blood cell populations, flow cytometry was used with a range of different lineage antibody markers. The peripheral blood data indicated a decrease in the granulocytes of $Klf5^{fl/fl}Vav\text{-}cre^{+/-}$ mice as well as an increase in the T-cell compartment.

Interestingly, we also showed that the *Klf5*$^{fl/fl}$*Vav-cre*$^{+/-}$ mice have increased numbers of blood and bone marrow eosinophils compared to *Klf5*$^{fl/f}$ mice. Consistently, we found significantly increased numbers of eosinophils in the lungs of *Klf5*$^{fl/fl}$*Vav-cre*$^{+/-}$ mice compared to *Klf5*$^{fl/fl}$ mice. The spleen weight of *Klf5*$^{fl/fl}$*Vav-cre*$^{+/-}$ mice was significantly higher compared to *Klf5*$^{fl/fl}$ mice. In addition, clonal assays conducted from the spleen of 9 and 12 month old mice showed a significant increase in colony number in the *Klf5*$^{fl/fl}$*Vav-cre*$^{+/-}$ mice compared to *Klf5*$^{fl/fl}$ mice. This correlated with the flow cytometry data which showed a significant increase in haemopoietic stem cell (HSC) populations; short-term HSC (ST-HSC) and multipotent progenitor (MPP) in the spleen of *Klf5*$^{fl/fl}$*Vav-cre*$^{+/-}$ mice. In summary, this study revealed multiple functional roles for Klf5 in haemopoiesis. Firstly, these studies demonstrated that as predicted losing *Klf5* leads to alterations in the development of the myeloid compartment. Secondly, we showed that the stem cell compartments (ST-HSC and MPP) were significantly increased in the spleen of *Klf5*$^{fl/fl}$*Vav-cre*$^{+/-}$ mice compared to *Klf5*$^{fl/fl}$ mice, which also correlated with extra-medullary splenic haemopoiesis and increased spleen size. Finally, the increase in T-cells for the *Klf5*$^{fl/fl}$*Vav-cre*$^{+/-}$ mice suggests a possible previously unidentified functional role for Klf5 outside the myeloid compartment.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Nur Hezrin Shahrin and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. With respect to my relative contribution in each of the experimental Chapters, I certify that I carried out all of the experimentation described except where duly noted in the text.

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