CELL PHENOTYPE AND CYTOKINE REGULATION OF ERYTHROPOIESIS IN MOUSE FETAL SPLEEN

TAN KEAI SINN

FPSK(p) 2015 28

CELL PHENOTYPE AND CYTOKINE REGULATION OF ERYTHROPOIESIS IN MOUSE FETAL SPLEEN

By

TAN KEAI SINN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

September 2015

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

CELL PHENOTYPE AND CYTOKINE REGULATION OF ERYTHROPOIESIS IN MOUSE FETAL SPLEEN

By

TAN KEAI SINN

September 2015

Chair: Lai Mei I, PhD
Faculty: Medicine and Health Sciences

Erythropoiesis and its regulation have been extensively studied in the yolk sac, fetal liver and bone marrow, but not on the regulation of spleen erythropoiesis in the mouse embryo. Fetal spleen was reportedly a major hematopoietic site prior to initiation of bone marrow hematopoiesis. Morphologic analysis suggested erythropoietic activity in fetal spleen, but it remained unclear how erythropoiesis was regulated. To address this question, flow cytometric analysis was performed and the number of spleen erythroid cells was found to increase 18.6-fold from 16.5 days post-coitum (dpc) to 19.5 dpc. Flow cytometric analysis was carried out to further characterize fetal spleen cells. Among $CD45^{-}Ter119^{-}$ non-hematopoietic cells at 16.5 dpc fetal spleen, 9.87±1.12% were DLK-1-expressing cells, 0.32±0.14% were microvessels, 31.09±17.75% were endothelial cells and 62.01±23.03% were unclassified cells. Whereas at 19.5 dpc fetal spleen, 2.00±0.38% were DLK-1-expressing cells, 0.96±0.36% were microvessels, 57.75±18.34% were endothelial cells and 38.82±17.88% were unclassified cells. Real-time PCR was carried out to investigate whether those fetal spleen cells express erythropoietic cytokines such as *stem cell factor* (*Scf*), *insulin growth factor 1* (*Igf1*), *interleukin-3* (*Il-3*) and *erythropoietin* (*Epo*) messenger RNAs (mRNAs). Of these erythropoietic cytokines, at 16.5 dpc whole spleen cells, both *Scf* and *Igf1* mRNAs were highly expressed, while *Epo* and *Il-3* mRNAs were not. Among erythropoietic cytokines, SCF and IGF-1 proteins were primarily expressed in hematopoietic, endothelial and mesenchymal-like fetal spleen cells. Further examination of the expression of SCF receptor (c-Kit) and the IGF-1 receptor (IGF-1R) on spleen erythroid cells performed by flow cytometric analysis shows that most of the $c\text{-}Kit^{+}$ and $IGF\text{-}1R^{+}$ cells were expressed on burst forming unit-erythroid (BFU-E) and colony forming unit-erythroid (CFU-E) equivalent cells. Cultures treated with SCF and/or IGF-1R inhibitors showed significantly decreased $CD45^{-}c\text{-}Kit^{-}CD71^{+/-}Ter119^{+}$ erythroid cells and down-regulated *Gata1*, *Klf1* and *β-major globin* expression. Administration of these inhibitors to pregnant mice significantly decreased the number of $CD45^{-}c\text{-}Kit^{-}CD71^{+/-}Ter119^{+}$ cells and down-regulated *β-major globin* gene expression in embryos derived from these mice. We conclude that fetal spleen is a site where erythropoietic activity takes place and spleen endothelial and mesenchymal-like cells primarily accelerate erythropoietic activity through SCF and IGF-1 secretion.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

FENOTIP SEL DAN PENGAWALATURAN SITOKIN DALAM ERITROPOIESIS LIMPA FETAL TIKUS

Oleh

TAN KEAI SINN

September 2015

Pengerusi: Lai Mei I, PhD
Fakulti: Perubatan dan Sains Kesihatan

Erythropoiesis dan pengawalan aktiviti erythropoietic dalam kantung kuning hati fetus dan tulang sum-sum telah dikaji secara meluas. Sebaliknya, kajian berkaitan dengan pengawalan aktivit erythropoietic dalam limpa embrio tetikus masih terhad. Limpa fetus merupakan tapak hematopoietik utama sebelum hematopoiesis berlaku dalam tulang sum-sum. Analisis morfologi mencadangkan aktiviti erythropoietic berlaku dalam limpa fetus, tetapi faktor kawalan erythropoiesis masih tidak diketahui secara jelas. Untuk menjawab pertanyaan ini, analisis aliran cytometric telah dijalankan dan jumlah sel erythroid limpa didapati meningkat 18.6 kali ganda dari hari 16.5 selepas persetubuhan ke hari 19.5 selepas persetubuhan dalam limpa fetus. Seterusnya, analisis aliran cytometric dijalankan untuk mencirikan sel-sel limpa fetus. Antara sel-sel bukan hematopoietic $CD45^{-}Ter119^{-}$ dalam limpa pada hari 16.5 selepas persetubuhan, 9.87 ± 1.12% adalah sel mengungkapkan DLK-1, 0.32 ± 0.14% adalah mikro vesel, 31,09 ± 17.75% adalah sel-sel endothelial dan 62.01 ± 23,03% adalah sel-sel yang tidak dapat dikelaskan. Manakala pada hari 19.5 selepas persetubuhan, 2.00 ± 0.38% adalah sel mengungkapkan DLK-1, 0.96 ± 0.36% adalah mikro vesel, 57.75 ± 18.34% adalah sel-sel endothelial dan 38,82 ± 17,88% adalah sel-sel yang tidak dapat dikelaskan. Kuantitatif reaksi berantai polimerease (qRT-PCR) dijalankan untuk menyiasat sama ada sel-sel limpa fetus menghasilkan sitokin erythropoietic seperti *Scf*, *Igf1*, *Il-3* dan *Epo* RNA pengutus (mRNA). Pada hari 16.5 selepas persetubuhan, sel limpa mengungkapkan *Scf* dan *Igf1* mRNAs, tetapi tidak mengungkapkan *Epo* dan *Il-3* mRNAs. Antara sitokin erythropoietic yang dinyatakan, hematopoietic sel, sel-sel endothelial dan sel seperti mesenchymal mengungkapkan SCF dan IGF-1. Seterusnya, analisis aliran cytometric dijalankan untuk mengkaji expresi SCF reseptor (c-Kit) dan IGF-1 reseptor (IGF-1R) pada sel-sel erythroid dalam limpa. Antara sel erythroid populasi, BFU-E dan CFU-E sel bersamaan ekspres paling banyak $c\text{-}Kit^{+}$ dan $IGF\text{-}1R^{+}$. Dalam kajian berfungsi *in vitro*, kultur sel dirawat dengan perencat SCF dan/atau perencat IGF-1R. Hasilnya menunjukkan penurunan ketara pada $CD45^{-}c\text{-}Kit^{-}CD71^{+/-}Ter119^{+}$ sel erythroid and penurunan bagi gen erythroid *Gata1*, *Klf1* dan *β-globin*. Manakala dalam kajian fungsional *in vivo*, perencat tersebut disuntikkan ke dalam tikus hamil, embrio diperolehi ndaripada tikus tersebut. Bilangan $CD45^{-}c\text{-}Kit^{-}CD71^{+/-}Ter119^{+}$ sel erythroid meurun dengan ketara dan ungkapan *β-globin* juga menurun. Kesimpulannya, limpa fetus merupakan tapak di mana aktiviti erythropoietic berlaku, dan sel-sel endothelial dan sel seperti mesenchymal meningkatkan aktiviti erythropoietic melalui rembesan SCF dan IGF-1.

ACKNOWLEDGEMENTS

First of all, I would like to thank to my committee chair, Dr Lai Mei I, for giving me this precious chance to work on this research project. I sincerely thank to her guidance and continuous support throughout my graduate studies, her patience, motivation, enthusiasm and immense knowledge. Without her guidance and persistent help this dissertation would not have been possible. Beside my committee chair, I would like to express the deepest appreciation to my committee members, Prof Elizabeth George and Associate Prof Syahrilnizam Abdullah, whose provide valuable guidance, constructive comments and endless support.

A thank you to Prof Dr Daisuke Sugiyama, for giving me an opportunity to join his group in Kyushu University, Japan. He provided me the chance to involve on diverse exciting projects. He continually and convincingly conveyed a spirit of adventure in regard to research and scholarship, and an excitement in regard to teaching. Throughout this study, my financial support was provided by the MyPhD scholarship from Ministry of Higher Education (MOHE), Malaysia and the Tokyo Biochemical Research Foundation, Japan

I sincerely thank to Drs Kasem Kulkeaw and Tomoko Inoue for discussion and technical support. I would particular thank to Mss. Yuka Tanaka, Wai Feng Lim and Chiyo Mizuochi-Yanagi who helped me in many ways. I thank to the Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labor and Welfare, the Japan Society for the Promotion of Science, the Ichiro Kanehara Foundation for the Promotion of Medical Sciences and Medical Care, the Institute of Molecular Embryology and Genetics, and the International Research Fund for Subsidy of Kyushu University School of Medicine Alumni for grant support.

Finally words alone cannot express the gratitude I owe my family who has always given me encouragement and assistance.

I certify that a Thesis Examination Committee has met on 28 September 2015 to conduct the final examination of Tan Keai Sinn on her thesis entitled Cell Phenotype and Cytokine Regulation of Erythropoiesis in Mouse Fetal Spleen in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.
Members of the Thesis Examination Committee were as follows:

Thilakavathy Karuppiah, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Cheah Yoke Kqueen, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Maha bt Abdullah, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Seiji Okada, PhD
Professor
Division of Hematopoiesis, Centre for Aids Research
Kumamoto University
Japan
(External Examiner)

_____
ZULKARNAIN ZAINAL, PhD
Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Lai Mei I, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Elizabeth George, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Syahrilnizam Abdullah, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Daisuke Sugiyama, PhD
Professor
Faculty of Medical Sciences
Kyushu University
(Member)

_____
BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Tan Keai Sinn (GS32811)

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____

Name of Chairman of Supervisory Committee:
Lai Mei I, PhD

Signature: _____

Name of Member of Supervisory Committee:
Elizabeth George, PhD

Signature: _____

Name of Member of Supervisory Committee:
Syahrilnizam Abdullah, PhD

Signature: _____

Name of Member of Supervisory Committee:
Daisuke Sugiyama, PhD

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AGM region	Aorta-gonad-mesonephros region
APC	Allophycocyanin
B cells	B-lineage cells
BFU-E	Burst forming unit-erythroid
BM	Bone marrow
CDK	Cyclin-dependent kinases
cDNA	complementary deoxyribonucleic acid
CFU-E	Colony forming unit-erythroid
CFU-G	Colony forming unit-granulocyte
CFU-GM	Colony forming unit-granulocyte macrophage
CFU-M	Colony forming unit-macrophage
CIK/KIP	CDK interacting protein/Kinase inhibitory protein
CLP	Common lymphoid progenitors
CMP	Common myeloid progenitors
CSF	Colony stimulating factor
DLK-1	Delta-like 1 homolog
DMSO	Dimethyl sulfoxide
dpc	day post-coitum
EB	Erythroblast
ELISA	Enzyme-linked immunosorbent assay
EPO/ *Epo*	Erythropoietin
EpoR	Erythropoietin receptor
FACS	Fluorescence-activated cell sorting
FBS	Fetal bovine serum

FITC	Fluorescein isothiocyanate
Gata1	GATA-binding factor 1
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Granulocyte-macrophage progenitors
H&E	Hematoxylin & eosin
H_2O_2	Hydrogen peroxide
HOX	Homeobox gene
HRP	Horseradish peroxidise
HSCs	Hematopoietic stem cells
IGF-1/*Igf1*	Insulin growth factor 1
IgG	Immunoglobulin G
IHC	Immunohistochemistry
ILs	Interleukins
Klf1	Krüppel-like factor 1
LYVE-1	lymphatic vessel endothelial hyaluronan receptor 1
MAPK	Mitogen-activated protein kinase
M-CSF	Macrophage colony-stimulating factor
MEM	Minimum Essential Medium
MEP	Megakaryocyte-erythroid progenitor
mRNA	messenger ribonucleic acid
NCAE	Naphthol AS-D chloroacetate esterase
NCBI	National Center for Biotechnology Information
NK cells	Natural killer cells
non-Tg	non-transgenic
Ns	Newly segmented somite

O.C.T	Optimum cutting temperature
OC	Otic capsule
P	Presomitic mesoderm
PBS	Phosphate-buffered saline
PE	Phycoerythrin
PECAM-1	Platelet endothelial cell adhesion molecule 1
PFA	Paraformadehyde
PPP	Picropodophyllin
Pro-B cells	B-lymphocytes precursor cells
p-Sp	para-aortic splanchnopleura
qRT-PCR	Quantitative real-time polymerase chain reaction
Rpm	revolutions per minute
RQ	Relative Quantification
RT	Reverse Transcription
S	Somite
SCF/*scf*	Stem cell factor
SD	Standard deviation
SFM	Serum free medium
T cells	T-lineage cells
Th2 lymphocytes	T helper type 2 lymphocytes
TSA	Thyramide Signal Amplification
β- major globin	beta-major globin

CHAPTER 1

INTRODUCTION

In the mouse embryo, erythropoiesis has been extensively studied in the yolk sac, liver, spleen and bone marrow (BM). The process of generating mature red blood cells is known as erythropoiesis, however, they do not necessarily have to derive from hematopoietic stem cells (HSCs) (e.g., primitive erythropoiesis emerges prior to HSCs), nor do they need to give rise to enucleated red blood cells (e.g., the non-mammalian species circulating red blood) (McGrath and Palis, 2008). Primitive erythroid cells support growth from embryo to fetus; whereas definitive erythroid cells support growth from fetus until birth (Baron et al., 2012). During mouse embryogenesis, primitive erythropoiesis occurs in the yolk sac from 7.5-8.5 days post-coitum (dpc), and erythroid progenitor cells mature in the circulation and enucleate between 14.5-16.5 dpc (Kingsley et al., 2004; Palis et al., 1999). Definitive erythropoiesis arises in the yolk sac at 9.0 dpc and then shifts to fetal liver, fetal spleen and bone marrow (BM) (Bertrand et al., 2005; Houssaint, 1981).

Fetal liver functions as the primary organ for expansion and maturation of erythroid cell at 12.5-14.5 dpc prior to spleen hematopoiesis (Ayres-Silva et al., 2011; Ema, 2000). Between 14.5-15.5 dpc, fetal liver becomes a less favorable environment for hematopoiesis, as the liver begins to change from a primarily hematopoietic to a metabolic function (Guo, 2009). At this time, hematopoiesis is likely take place in the spleen. At 12.5 and 14.5 dpc, hematopoietic cells in the spleen are mainly myeloid and erythroid cells only start being the predominat cells produced at a later stage. Also, the fetal spleen at 12.5 and 14.5 dpc explants cultured *in vitro* reportedly can produce hematopoietic cells, suggesting that hematopoietic stem/progenitor cells colonize fetal spleen, which likely fills the hematopoietic "gap" between fetal liver and BM (Godin et al., 1999; Sasaki and Matsumura, 1987). Spleen at 13.5-15.5 dpc reportedly composed primarily of myeloid and erythroid cells (Desanti et al., 2008). The spleen also reportedly becomes erythropoietic between 16.0 dpc and 17.0 dpc until around the first week of postnatal life through microscopic observation (Djaldetti et al., 1972; Sasaki and Matsumura, 1988). In another study, the spleen reportedly is a site of active myelopoiesis during late embryonic and perinatal stages, and gradually becomes a site of lymphopoiesis after postnatal week one (Ohno et al., 1993).there are no reports which quantitates the number of erythroid and myeloid lineages in the fetal spleen presently. Hence, it is hypothesized that the spleen could possess similar function as fetal liver in which it contains a unique microenvironment to support expansion of erythroid cells.

Regulation of the mouse fetal hematopoietic niche has been identified as a key extrinsic component of the hematopoietic environment (Sugiyama et al., 2011a). Particularly, extrinsic regulation through cytokine secretion, cell-cell interactions and cell-extracellular matrix activity is required for survival, self-renewal, proliferation and differentiation of hematopoietic cells into multiple lineages (Watt and Hogan, 2000). Several cytokines, such as erythropoietin (EPO), stem cell factor (SCF), insulin-like growth factor 1 (IGF-1), interleukin 3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF), are needed for optimal development and terminal differentiation of erythroid cells (Emerson et al., 1989; Goodman et al., 1985 ; Muta et

al., 1994; Umemura et al., 1989). Binding of Epo to its receptor, EpoR, which is expressed on the surface of erythroid progenitors, is particularly critical for these activities (Koury and Bondurant, 1992; Palis, 2014). SCF, a c-Kit ligand, is required for growth of burst-forming unit-erythroids (BFU-Es) under serum-free conditions (Dai et al., 1991). Also, the formation of erythrocyte colony-forming units (CFU-Es) requires synergistic SCF and EPO activity (Wu et al., 1997), whereas, IGF-1 stimulates proliferation of BM and peripheral blood erythroid progenitor cells (Miyagawa et al., 2000).

Work done between the years 1965 and 1980 clarified that specific stromal elements underlie skewing of hematopoietic cells lineage development (Lowy et al., 1970; Wolf and Trentin, 1968). A study done by Wolf and Trentin revealed that following administration of BM cells into the spleen of lethally irradiated recipients, between the junction of marrow stroma and spleen, most of the erythroid colonies are on the splenic side; while myeloid colonies predominated on the BM side(Wolf and Trentin, 1968). Another study showed that at 14.5 dpc, fetal spleen stromal cells drive macrophage and B cell commitment (Bertrand et al., 2006).Hematopoietic niche regulation in the placenta and liver of mouse embryo has been reported recently but there is no report on the regulation of spleen niche cells (Sasaki et al., 2010; Sugiyama et al., 2011a; Sugiyama et al., 2011b).

Generation of red blood cells from hematopoietic stem cells or embryonic stem cells may represent an important new source for blood transfusion. Prior to blood transfusion, establishment of an efficient way to produce sufficient erythrocytes *in vitro* is required. Studies on the spleen and its niche regulation would be able to guide the development of novel therapy that can complement current research trend. Nevertheless, the fetal spleen hematopoiesis and its regulation remains unclear particularly which hematopoietic lineages is predominant in the spleen at 16.5 dpc and how the spleen niche cells regulate the development of spleen hematopoiesis (Godin et al., 1999). Also, there are only a few studies regarding the interaction of spleen hematopoietic cells with spleen non-hematopoeitic cells, suggesting an importance of this study for a better understanding on hematopoietic embryology

This study aims to explore the niche regulation of the fetal spleen hematopoiesis. Hematopoietic cell types were characterized and identified that erythropoesis dominantly takes place in the spleenat both 16.5 dpc and 19.5 dpc. To investigate extrinsic factors regulating fetal spleen erythropoiesis, the expression of cytokine secretion by 16.5 dpc fetal spleen cells were examined in sorted hematopoietic, endothelial and unclassified (or mesenchymal-like) cells on erythropoiesis. In this study, SCF and IGF-1 are reportedly the primary erythropoietic cytokines expressed in fetal spleen. Finally, *in vitro* and *in vivo* analyses using inhibitors of SCF and IGF-1R revealed that both are crucial factors that accelerate spleen erythropoiesis at 16.5 dpc. Taken together, these findings represent a step towards understanding the development of erythropoiesis during mouse embryogenesis and regulation of its niche.

The general objective for this study was:

- To identify niche regulation of the mouse fetal spleen during hematopoietic cell development at 16.5 dpc and 19.5 dpc.

The specific objectives for this study were:

- To characterize hematopoietic cells that predominantly takes place in the mouse spleen
- To identify non-hematopoietic cells or niche cells of the mouse spleen
- To screen erythropoietic cytokines that is expressed by the spleen cells
- To compare the microenvironment of fetal spleen and liver
- To investigate the effects of inhibitors of SCF and/or IGF-1R on erythroid cell development *in vitro* and *in vivo.*

REFERENCES

Adimy, M., Crauste, F., Ruan, S., 2006. Periodic oscillations in leukopoiesis models with two delays. Journal of theoretical biology 242, 288-299.

Adu, E.K., Yeboah, S., 2000. The efficacy of the vaginal plug formation after mating for pregnancy diagnosis, and embyonic resorption in utero in the greater cane rat (Thryonomys swinderianus, Temminck). Tropical animal health and production 32, 1-10.

Alharbi, R.A., Pettengell, R., Pandha, H.S., Morgan, R., 2013. The role of HOX genes in normal hematopoiesis and acute leukemia. Leukemia 27, 1000-1008.

Alvarez-Silva, M., Belo-Diabangouaya, P., Salaun, J., Dieterlen-Lievre, F., 2003. Mouse placenta is a major hematopoietic organ. Development 130, 5437-5444.

Anagnostou, A., Liu, Z., Steiner, M., Chin, K., Lee, E.S., Kessimian, N., Noguchi, C.T., 1994. Erythropoietin receptor mRNA expression in human endothelial cells. Proc Natl Acad Sci U S A 91, 3974-3978.

Anderson, D.M., Lyman, S.D., Baird, A., Wignall, J.M., Eisenman, J., Rauch, C., March, C.J., Boswell, H.S., Gimpel, S.D., Cosman, D., et al., 1990. Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. Cell 63, 235-243.

Anthony, B.A., Link, D.C., 2014. Regulation of hematopoietic stem cells by bone marrow stromal cells. Trends Immunol 35, 32-37.

Arcasoy, M.O., Jiang, X., 2005. Co-operative signalling mechanisms required for erythroid precursor expansion in response to erythropoietin and stem cell factor. British journal of haematology 130, 121-129.

Arinobu, Y., Mizuno, S., Chong, Y., Shigematsu, H., Iino, T., Iwasaki, H., Graf, T., Mayfield, R., Chan, S., Kastner, P., Akashi, K., 2007. Reciprocal activation of GATA-1 and PU.1 marks initial specification of hematopoietic stem cells into myeloerythroid and myelolymphoid lineages. Cell Stem Cell 1, 416-427.

Armitage, J.O., 1998. Emerging applications of recombinant human granulocyte-macrophage colony-stimulating factor. Blood 92, 4491-4508.

Ayres-Silva, J.D., Manso, P.P.D., Madeira, M.R.D., Pelajo-Machado, M., Lenzi, H.L., 2011. Sequential morphological characteristics of murine fetal liver hematopoietic microenvironment in Swiss Webster mice. Cell and Tissue Research 344, 455-469.

Bagamery, K., Kvell, K., Landau, R., Graham, J., 2005. Flow cytometric analysis of CD41-labeled platelets isolated by the rapid, one-step OptiPrep method from human blood. Cytometry. Part A : the journal of the International Society for Analytical Cytology 65, 84-87.

Baron, M.H., Isern, J., Fraser, S.T., 2012. The embryonic origins of erythropoiesis in mammals. Blood 119, 4828-4837.

Bartel, D.P., 2009. MicroRNAs: target recognition and regulatory functions. Cell 136, 215-233.

Bauer, R., Plieschnig, J.A., Finkes, T., Riegler, B., Hermann, M., Schneider, W.J., 2013. The developing chicken yolk sac acquires nutrient transport competence by an orchestrated differentiation process of its endodermal epithelial cells. The Journal of biological chemistry 288, 1088-1098.

Baumgarth, N., Roederer, M., 2000. A practical approach to multicolor flow cytometry for immunophenotyping. Journal of immunological methods 243, 77-97.

Bertrand, J.Y., Desanti, G.E., Lo-Man, R., Leclerc, C., Cumano, A., Golub, R., 2006. Fetal spleen stroma drives macrophage commitment. Development 133, 3619-3628.

Bertrand, J.Y., Giroux, S., Golub, R., Klaine, M., Jalil, A., Boucontet, L., Godin, I., Cumano, A., 2005. Characterization of purified intraembryonic hematopoietic stem cells as a tool to define their site of origin. Proceedings of the National Academy of Sciences of the United States of America 102, 134-139.

Bianco, P., Riminucci, M., Gronthos, S., Robey, P.G., 2001. Bone marrow stromal stem cells: nature, biology, and potential applications. Stem cells 19, 180-192.

Bochner, B.S., Sterbinsky, S.A., Bickel, C.A., Werfel, S., Wein, M., Newman, W., 1994. Differences between human eosinophils and neutrophils in the function and expression of sialic acid-containing counterligands for E-selectin. J Immunol 152, 774-782.

Bodi, E., Hurtado, S.P., Carvalho, M.A., Borojevic, R., Carvalho, A.C., 2004. Gap junctions in hematopoietic stroma control proliferation and differentiation of blood cell precursors. An Acad Bras Cienc 76, 743-756.

Boisset, J.C., Clapes, T., Van Der Linden, R., Dzierzak, E., Robin, C., 2013. Integrin alphaIIb (CD41) plays a role in the maintenance of hematopoietic stem cell activity in the mouse embryonic aorta. Biology open 2, 525-532.

Broudy, V.C., Kovach, N.L., Bennett, L.G., Lin, N., Jacobsen, F.W., Kidd, P.G., 1994. Human umbilical vein endothelial cells display high-affinity c-kit receptors and produce a soluble form of the c-kit receptor. Blood 83, 2145-2152.

Calvi, L.M., Adams, G.B., Weibrecht, K.W., Weber, J.M., Olson, D.P., Knight, M.C., Martin, R.P., Schipani, E., Divieti, P., Bringhurst, F.R., Milner, L.A., Kronenberg, H.M., Scadden, D.T., 2003. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 425, 841-846.

Cardell, S., Sander, B., Moller, G., 1991. Helper interleukins are produced by both CD4 and CD8 splenic T cells after mitogen stimulation. European journal of immunology 21, 2495-2500.

Carlyle, J.R., Mesci, A., Ljutic, B., Belanger, S., Tai, L.H., Rousselle, E., Troke, A.D., Proteau, M.F., Makrigiannis, A.P., 2006. Molecular and genetic basis for strain-dependent NK1.1 alloreactivity of mouse NK cells. J Immunol 176, 7511-7524.

Carter, R.H., Wang, Y., Brooks, S., 2002. Role of CD19 signal transduction in B cell biology. Immunol Res 26, 45-54.

Caselli, A., Olson, T.S., Otsuru, S., Chen, X., Hofmann, T.J., Nah, H.D., Grisendi, G., Paolucci, P., Dominici, M., Horwitz, E.M., 2013. IGF-1-mediated osteoblastic niche expansion enhances long-term hematopoietic stem cell engraftment after murine bone marrow transplantation. Stem cells 31, 2193-2204.

Cecchini, M.G., Dominguez, M.G., Mocci, S., Wetterwald, A., Felix, R., Fleisch, H., Chisholm, O., Hofstetter, W., Pollard, J.W., Stanley, E.R., 1994. Role of colony stimulating factor-1 in the establishment and regulation of tissue macrophages during postnatal development of the mouse. Development 120, 1357-1372.

Cesta, M.F., 2006. Normal structure, function, and histology of the spleen. Toxicologic pathology 34, 455-465.

Chen, K., Liu, J., Heck, S., Chasis, J.A., An, X., Mohandas, N., 2009. Resolving the distinct stages in erythroid differentiation based on dynamic changes in membrane protein expression during erythropoiesis. Proc Natl Acad Sci U S A 106, 17413-17418.

Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J., Rutter, W.J., 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18, 5294-5299.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical biochemistry 162, 156-159.

Chou, S., Lodish, H.F., 2010. Fetal liver hepatic progenitors are supportive stromal cells for hematopoietic stem cells. Proceedings of the National Academy of Sciences of the United States of America 107, 7799-7804.

Chow, K.N., Starostik, P., Dean, D.C., 1996. The Rb family contains a conserved cyclin-dependent-kinase-regulated transcriptional repressor motif. Molecular and cellular biology 16, 7173-7181.

Christensen, J.E., Andreasen, S.O., Christensen, J.P., Thomsen, A.R., 2001. CD11b expression as a marker to distinguish between recently activated effector CD8(+) T cells and memory cells. Int Immunol 13, 593-600.

Christensen, J.L., Wright, D.E., Wagers, A.J., Weissman, I.L., 2004. Circulation and chemotaxis of fetal hematopoietic stem cells. PLoS biology 2, E75.

Clark, R., Strasser, J., McCabe, S., Robbins, K., Jardieu, P., 1993. Insulin-like growth factor-1 stimulation of lymphopoiesis. The Journal of clinical investigation 92, 540-548.

Cluitmans, F.H., Esendam, B.H., Veenhof, W.F., Landegent, J.E., Willemze, R., Falkenburg, J.H., 1997. The role of cytokines and hematopoietic growth factors in

the autocrine/paracrine regulation of inducible hematopoiesis. Annals of hematology 75, 27-31.

Coffman, R.L., Weissman, I.L., 1981. B220: a B cell-specific member of th T200 glycoprotein family. Nature 289, 681-683.

Crawford, L.W., Foley, J.F., and Elmore, S.A, 2010. Histology atlas of the developing mouse hepatobiliary system with emphasis on embryonic days 9.5-18.5. Toxicologic Patholology 38, 872-906.

Dai, C.H., Krantz, S.B., Zsebo, K.M., 1991. Human burst-forming units-erythroid need direct interaction with stem cell factor for further development. Blood 78, 2493-2497.

Daley, J.M., Thomay, A.A., Connolly, M.D., Reichner, J.S., Albina, J.E., 2008. Use of Ly6G-specific monoclonal antibody to deplete neutrophils in mice. J Leukoc Biol 83, 64-70.

Dao, M.A., Taylor, N., Nolta, J.A., 1998. Reduction in levels of the cyclin-dependent kinase inhibitor p27(kip-1) coupled with transforming growth factor beta neutralization induces cell-cycle entry and increases retroviral transduction of primitive human hematopoietic cells. Proceedings of the National Academy of Sciences of the United States of America 95, 13006-13011.

Desanti, G.E., Cumano, A., Golub, R., 2008. Identification of CD4int progenitors in mouse fetal spleen, a source of resident lymphoid cells. Journal of leukocyte biology 83, 1145-1154.

Djaldetti, M., Bessler, H., Rifkind, R.A., 1972. Hematopoiesis in the embryonic mouse spleen: an electron microscopic study. Blood 39, 826-841.

Doulatov, S., Notta, F., Laurenti, E., Dick, J.E., 2012. Hematopoiesis: a human perspective. Cell Stem Cell 10, 120-136.

Dzierzak, E., Medvinsky, A., de Bruijn, M., 1998. Qualitative and quantitative aspects of haematopoietic cell development in the mammalian embryo. Immunology today 19, 228-236.

El-Nefiawy, N., Abdel-Hakim, K., Yamashita, A., Kanayama, N., 2002. Embryonic macrophages of early rat yolk sac: Immunohistochemistry and ultrastructure with reference to endodermal cell layer. Immunology and cell biology 80, 441-447.

Elliott, S., 2008. Erythropoiesis-stimulating agents and other methods to enhance oxygen transport. British journal of pharmacology 154, 529-541.

Ema, H., and Nakauchi, H, 2000. Expansion of hematopoietic stem cells in the developing liver of a mouse embryo. Blood 95, 2284-2288.

Emerson, S.G., Thomas, S., Ferrara, J.L., Greenstein, J.L., 1989. Developmental regulation of erythropoiesis by hematopoietic growth factors: analysis on populations of BFU-E from bone marrow, peripheral blood, and fetal liver. Blood 74, 49-55.

Emes, R.D., Goodstadt, L., Winter, E.E., Ponting, C.P., 2003. Comparison of the genomes of human and mouse lays the foundation of genome zoology. Human molecular genetics 12, 701-709.

Eshghi, S., Vogelezang, M.G., Hynes, R.O., Griffith, L.G., Lodish, H.F., 2007. Alpha4beta1 integrin and erythropoietin mediate temporally distinct steps in erythropoiesis: integrins in red cell development. The Journal of cell biology 177, 871-880.

Fairchild Benyo, D., Conrad, K.P., 1999. Expression of the erythropoietin receptor by trophoblast cellsin the human placenta. Biology of reproduction 60, 861-870.

Ferkowicz, M.J., Yoder, M.C., 2005. Blood island formation: longstanding observations and modern interpretations. Experimental hematology 33, 1041-1047.

Ferret-Bernard, S., Sai, P., Bach, J.M., 2004. In vitro induction of inhibitory macrophage differentiation by granulocyte-macrophage colony-stimulating factor, stem cell factor and interferon-gamma from lineage phenotypes-negative c-kit-positive murine hematopoietic progenitor cells. Immunology letters 91, 221-227.

Filonzi, E.L., Zoellner, H., Stanton, H., Hamilton, J.A., 1993. Cytokine regulation of granulocyte-macrophage colony stimulating factor and macrophage colony-stimulating factor production in human arterial smooth muscle cells. Atherosclerosis 99, 241-252.

Fischer, A.H., Jacobson, K.A., Rose, J., Zeller, R., 2008a. Fixation and permeabilization of cells and tissues. CSH protocols 2008, pdb top36.

Fischer, A.H., Jacobson, K.A., Rose, J., Zeller, R., 2008b. Hematoxylin and eosin staining of tissue and cell sections. CSH protocols 2008, pdb prot4986.

Fleming, T.J., Fleming, M.L., Malek, T.R., 1993. Selective expression of Ly-6G on myeloid lineage cells in mouse bone marrow. RB6-8C5 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. J Immunol 151, 2399-2408.

Fornas, O., Domingo, J.C., Marin, P., Petriz, J., 2002. Flow cytometric-based isolation of nucleated erythroid cells during maturation: an approach to cell surface antigen studies. Cytometry 50, 305-312.

Fraser, S.T., Baron, M.H., 2009. Embryonic fates for extraembryonic lineages: New perspectives. Journal of cellular biochemistry 107, 586-591.

Fujio, K., Evarts, R.P., Hu, Z., Marsden, E.R., Thorgeirsson, S.S., 1994. Expression of stem cell factor and its receptor, c-kit, during liver regeneration from putative stem cells in adult rat. Laboratory investigation; a journal of technical methods and pathology 70, 511-516.

Gahmberg, C.G., Autero, M., Hermonen, J., 1988. Major O-glycosylated sialoglycoproteins of human hematopoietic cells: differentiation antigens with poorly understood functions. Journal of cellular biochemistry 37, 91-105.

Geissler, E.N., McFarland, E.C., Russell, E.S., 1981. Analysis of pleiotropism at the dominant white-spotting (W) locus of the house mouse: a description of ten new W alleles. Genetics 97, 337-361.

Gekas, C., Dieterlen-Lievre, F., Orkin, S.H., Mikkola, H.K., 2005. The placenta is a niche for hematopoietic stem cells. Developmental cell 8, 365-375.

Giampaolo, A., Sterpetti, P., Bulgarini, D., Samoggia, P., Pelosi, E., Valtieri, M., Peschle, C., 1994. Key functional role and lineage-specific expression of selected HOXB genes in purified hematopoietic progenitor differentiation. Blood 84, 3637-3647.

Gibbons, S.J., Rich, A., Distad, M.A., Miller, S.M., Schmalz, P.F., Szurszewski, J.H., Sha, L., Blume-Jensen, P., Farrugia, G., 2003. Kit/stem cell factor receptor-induced phosphatidylinositol 3'-kinase signalling is not required for normal development and function of interstitial cells of Cajal in mouse gastrointestinal tract. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 15, 643-653.

Giuliani, M., Fleury, M., Vernochet, A., Ketroussi, F., Clay, D., Azzarone, B., Lataillade, J.J., Durrbach, A., 2011. Long-lasting inhibitory effects of fetal liver mesenchymal stem cells on T-lymphocyte proliferation. PLoS One 6, e19988.

Godin, I., Garcia-Porrero, J.A., Dieterlen-Lievre, F., Cumano, A., 1999. Stem cell emergence and hemopoietic activity are incompatible in mouse intraembryonic sites. J Exp Med 190, 43-52.

Goodman, J.W., Hall, E.A., Miller, K.L., Shinpock, S.G., 1985 Interleukin 3 promotes erythroid burst formation in "serum-free" cultures without detectable erythropoietin. Proceedings of the National Academy of Sciences of the United States of America 82, 3291-3295.

Green, M.C., 1967. A defect of the splanchnic mesoderm caused by the mutant gene dominant hemimelia in the mouse. Developmental biology 15, 62-89.

Gregory, C.J., Eaves, A.C., 1977. Human marrow cells capable of erythropoietic differentiation in vitro: definition of three erythroid colony responses. Blood 49, 855-864.

Gregory, T., Yu, C., Ma, A., Orkin, S.H., Blobel, G.A., Weiss, M.J., 1999. GATA-1 and erythropoietin cooperate to promote erythroid cell survival by regulating bcl-xL expression. Blood 94, 87-96.

Guo, Y., Zhang, X., Huang, J., Zeng, Y., Liu, W., Geng, C., Li, K.W., Yang, D., Wu, S., Wei, H., Han, Z., Qian, X., Jiang, Y., He, F., 2009. Relationships between hematopoiesis and hepatogenesis in the midtrimester fetal liver characterized by dynamic transcriptomic and proteomic profiles. PLoS One 4, e7641.

Guo, Y., Zhang, X., Huang, J., Zeng, Y., Liu, W., Geng, C., Li, K.W., Yang, D., Wu, S., Wei, H., Han, Z., Qian, X., Jiang, Y., and He, F, 2009. Relationships between hematopoiesis and hepatogenesis in the midtrimester fetal liver characterized by dynamic transcriptomic and proteomic profiles. PLoS One 4, e7641.

Haar, J.L., Ackerman, G.A., 1971. Ultrastructural changes in mouse yolk sac associated with the initiation of vitelline circulation. The Anatomical record 170, 437-455.

Hall, B.K., Miyake, T., 1992. The membranous skeleton: the role of cell condensations in vertebrate skeletogenesis. Anatomy and embryology 186, 107-124.

Hayden, M.S., Ghosh, S., 2011. NF-kappaB in immunobiology. Cell Res 21, 223-244.

Heberlein, C., Friel, J., Laker, C., von Laer, D., Bergholz, U., Bogel, M., Ashman, L.K., Klingler, K., Ostertag, W., 1999. Downregulation of c-kit (stem cell factor receptor) in transformed hematopoietic precursor cells by stroma cells. Blood 93, 554-563.

Heinrich, M.C., Dooley, D.C., Freed, A.C., Band, L., Hoatlin, M.E., Keeble, W.W., Peters, S.T., Silvey, K.V., Ey, F.S., Kabat, D., et al., 1993. Constitutive expression of steel factor gene by human stromal cells. Blood 82, 771-783.

Henwood, H., 2010. Microscopic Quality Control of Hematoxylin and Eosin – Know your Histology, in: Kumar, G.L., Kiernan, J.A. (Eds.), Special Stains and H & E Second Edition. Dako North America, Carpinteria, California, pp. 135-140.

Hewitson, T.D., Wigg, B., Becker, G.J., 2010. Tissue preparation for histochemistry: fixation, embedding, and antigen retrieval for light microscopy. Methods Mol Biol 611, 3-18.

Hinton, R., Petvises, S., O'Neill, H., 2011. Myelopoiesis related to perinatal spleen. Immunol Cell Biol 89, 689-695.

Hogan, B.M., Layton, J.E., Pyati, U.J., Nutt, S.L., Hayman, J.W., Varma, S., Heath, J.K., Kimelman, D., Lieschke, G.J., 2006. Specification of the primitive myeloid precursor pool requires signaling through Alk8 in zebrafish. Current biology : CB 16, 506-511.

Houlihan, D.D., Mabuchi, Y., Morikawa, S., Niibe, K., Araki, D., Suzuki, S., Okano, H., Matsuzaki, Y., 2012. Isolation of mouse mesenchymal stem cells on the basis of expression of Sca-1 and PDGFR-alpha. Nat Protoc 7, 2103-2111.

Houssaint, E., 1981. Differentiation of the mouse hepatic primordium. II. Extrinsic origin of the haemopoietic cell line. Cell Differ 10, 243-252.

Howard, R.B., Christensen, A.K., Gibbs, F.A., Pesch, L.A., 1967. The enzymatic preparation of isolated intact parenchymal cells from rat liver. The Journal of cell biology 35, 675-684.

Huang, H., Zettergren, L.D., Auerbach, R., 1994. In vitro differentiation of B cells and myeloid cells from the early mouse embryo and its extraembryonic yolk sac. Experimental hematology 22, 19-25.

Ihle, J.N., 1992. Interleukin-3 and hematopoiesis. Chemical immunology 51, 65-106.

Inoue, T., Sugiyama, D., Kurita, R., Oikawa, T., Kulkeaw, K., Kawano, H., Miura, Y., Okada, M., Suehiro, Y., Takahashi, A., Marumoto, T., Inoue, H., Komatsu, N., Tani, K., 2011. APOA-1 is a novel marker of erythroid cell maturation from hematopoietic stem cells in mice and humans. Stem Cell Rev 7, 43-52.

Jacobsen, F.W., Veiby, O.P., Skjonsberg, C., Jacobsen, S.E., 1993. Novel role of interleukin 7 in myelopoiesis: stimulation of primitive murine hematopoietic progenitor cells. J Exp Med 178, 1777-1782.

Jelkmann, W., 2004. Molecular biology of erythropoietin. Internal medicine 43, 649-659.

Ji, P., Jayapal, S.R., Lodish, H.F., 2008. Enucleation of cultured mouse fetal erythroblasts requires Rac GTPases and mDia2. Nature cell biology 10, 314-321.

Johnson, G.B., Raven, P.H., 2002. Chapter 7 Cell-cell interaction, Biology, 6/e McGraw-Hill, pp. 123-140.

Junk, A.K., Mammis, A., Savitz, S.I., Singh, M., Roth, S., Malhotra, S., Rosenbaum, P.S., Cerami, A., Brines, M., Rosenbaum, D.M., 2002. Erythropoietin administration protects retinal neurons from acute ischemia-reperfusion injury. Proceedings of the National Academy of Sciences of the United States of America 99, 10659-10664.

Karsenty, G., 1999. The genetic transformation of bone biology. Genes & development 13, 3037-3051.

Kassel, O., Schmidlin, F., Duvernelle, C., Gasser, B., Massard, G., Frossard, N., 1999. Human bronchial smooth muscle cells in culture produce stem cell factor. The European respiratory journal 13, 951-954.

Kawai, K., Tsuno, N.H., Matsuhashi, M., Kitayama, J., Osada, T., Yamada, J., Tsuchiya, T., Yoneyama, S., Watanabe, T., Takahashi, K., Nagawa, H., 2005. CD11b-mediated migratory property of peripheral blood B cells. The Journal of allergy and clinical immunology 116, 192-197.

Kawakami, T., Galli, S.J., 2002. Regulation of mast-cell and basophil function and survival by IgE. Nature reviews. Immunology 2, 773-786.

Khanolkar, A., Burden, S.J., Hansen, B., Wilson, A.R., Philipps, G.J., Hill, H.R., 2013. Evaluation of CCR3 as a basophil activation marker. Am J Clin Pathol 140, 293-300.

Kikly, K.K., Bochner, B.S., Freeman, S.D., Tan, K.B., Gallagher, K.T., D'Alessio K, J., Holmes, S.D., Abrahamson, J.A., Erickson-Miller, C.L., Murdock, P.R., Tachimoto, H., Schleimer, R.P., White, J.R., 2000. Identification of SAF-2, a

novel siglec expressed on eosinophils, mast cells, and basophils. J Allergy Clin Immunol 105, 1093-1100.

Kim, Y.K., Nakagawa, N., Nakano, K., Sulakvelidze, I., Dolovich, J., Denburg, J., 1997. Stem cell factor in nasal polyposis and allergic rhinitis: increased expression by structural cells is suppressed by in vivo topical corticosteroids. The Journal of allergy and clinical immunology 100, 389-399.

Kina, T., Ikuta, K., Takayama, E., Wada, K., Majumdar, A.S., Weissman, I.L., Katsura, Y., 2000. The monoclonal antibody TER-119 recognizes a molecule associated with glycophorin A and specifically marks the late stages of murine erythroid lineage. British journal of haematology 109, 280-287.

Kingsley, P.D., Malik, J., Fantauzzo, K.A., Palis, J., 2004. Yolk sac-derived primitive erythroblasts enucleate during mammalian embryogenesis. Blood 104, 19-25.

Kitamura, T., Sato, N., Arai, K., Miyajima, A., 1991. Expression cloning of the human IL-3 receptor cDNA reveals a shared beta subunit for the human IL-3 and GM-CSF receptors. Cell 66, 1165-1174.

Kodama, H., Nose, M., Niida, S., Nishikawa, S., Nishikawa, S., 1994. Involvement of the c-kit receptor in the adhesion of hematopoietic stem cells to stromal cells. Experimental hematology 22, 979-984.

Komiya, A., Nagase, H., Okugawa, S., Ota, Y., Suzukawa, M., Kawakami, A., Sekiya, T., Matsushima, K., Ohta, K., Hirai, K., Yamamoto, K., Yamaguchi, M., 2006. Expression and function of toll-like receptors in human basophils. Int Arch Allergy Immunol 140 Suppl 1, 23-27.

Kopp, H.G., Avecilla, S.T., Hooper, A.T., Rafii, S., 2005. The bone marrow vascular niche: home of HSC differentiation and mobilization. Physiology (Bethesda) 20, 349-356.

Kostura, M.J., Tocci, M.J., Limjuco, G., Chin, J., Cameron, P., Hillman, A.G., Chartrain, N.A., Schmidt, J.A., 1989. Identification of a monocyte specific pre-interleukin 1 beta convertase activity. Proceedings of the National Academy of Sciences of the United States of America 86, 5227-5231.

Koulnis, M., Pop, R., Porpiglia, E., Shearstone, J.R., Hidalgo, D., Socolovsky, M., 2011. Identification and analysis of mouse erythroid progenitors using the CD71/TER119 flow-cytometric assay. J Vis Exp e2809, 1-6.

Koury, M.J., Bondurant, M.C., 1992. The molecular mechanism of erythropoietin action. European Journal of Biochemistry / FEBS 210, 649-663.

Lanotte, M., Allen, T.D., Dexter, T.M., 1981. Histochemical and ultrastructural characteristics of a cell line from human bone-marrow stroma. Journal of Cell Science 50, 281-297.

Laron, Z., 2001. Insulin-like growth factor 1 (IGF-1): a growth hormone. Molecular Pathology 54, 311-316.

Larsson, J., Karlsson, S., 2005. The role of Smad signaling in hematopoiesis. Oncogene 24, 5676-5692.

Lawrence, H.J., Sauvageau, G., Humphries, R.K., Largman, C., 1996. The role of HOX homeobox genes in normal and leukemic hematopoiesis. Stem Cells 14, 281-291.

Lee, L.K., Ueno, M., Van Handel, B., Mikkola, H.K., 2010. Placenta as a newly identified source of hematopoietic stem cells. Current Opinion in Hematology 17, 313-318.

Lenox, L.E., Perry, J.M., Paulson, R.F., 2005, BMP4 and Madh5 regulate the erythroid response to acute aneemia. Blood 105, 2741-2748.

Lesley, J., Hyman, R., Schulte, R., Trotter, J., 1984. Expression of transferrin receptor on murine hematopoietic progenitors. Cellular Immunology 83, 14-25.

Ley, T.J., Hug, B., Fiering, S., Epner, E., Bender, M.A., Groudine, M., 1998. Reduced beta-globin gene expression in adult mice containing deletions of locus control region 5' HS-2 or 5' HS-3. Annals of the New York Academy of Sciences 850, 45-53.

Li, T., Wu, Y., 2011. Paracrine molecules of mesenchymal stem cells for hematopoietic stem cell niche. Bone Marrow Research 2011, 353878.

Li, Z.J., Abramov, Y., Bordner, J., Leonard, J., Medek, A., Trask, A.V., 2006. Solid-state acid-base interactions in complexes of heterocyclic bases with dicarboxylic acids: crystallography, hydrogen bond analysis, and 15N NMR spectroscopy. Journal of the American Chemical Society 128, 8199-8210.

Linenberger, M.L., Jacobson, F.W., Bennett, L.G., Broudy, V.C., Martin, F.H., Abkowitz, J.L., 1995. Stem cell factor production by human marrow stromal fibroblasts. Experimental Hematology 23, 1104-1114.

Liu, J., Mohandas, N., An, X., 2011. Membrane assembly during erythropoiesis. Current Opinion in Hematology 18, 133-138.

Liu, Y., Pop, R., Sadegh, C., Brugnara, C., Haase, V.H., Scocolovsky, M., 2006. Suppression of Fas-FasL coexpression by erythropoietin mediates erythroblast expansion during erythropoietic stress response in vivo. Blood 108, 123-133.

Lopez, A.F., Begley, C.G., Williamson, D.J., Warren, D.J., Vadas, M.A., Sanderson, C.J., 1986. Murine eosinophil differentiation factor. An eosinophil-specific colony-stimulating factor with activity for human cells. The Journal of Experimental Medicine 163, 1085-1099.

Lowy, P.H., Keighley, G., Cohen, N.S., 1970. Stimulation by serotonin of erythropoietin-dependent erythropoiesis in mice. British Journal of Haematology 19, 711-718.

Luis, T.C., Killmann, N.M., Staal, F.J., 2012. Signal transduction pathways regulating hematopoietic stem cell biology: introduction to a series of Spotlight Reviews. Leukemia 26, 86-90.

Magli, M.C., Largman, C., Lawrence, H.J., 1997. Effects of HOX homeobox genes in blood cell differentiation. Journal of Cellular Physiology 173, 168-177.

Makita, T., Hernandez-Hoyos, G., Chen, T.H., Wu, H., Rothenberg, E.V., and Sucov, H.M, 2001. A developmental transition in definitive erythropoiesis: erythropoietin expression is sequentially regulated by retinoic acid receptors and HNF4. Genes and Development 15, 889-901.

Marsee, D.K., Pinkus, G.S., Yu, H., 2010. CD71 (transferrin receptor): an effective marker for erythroid precursors in bone marrow biopsy specimens. American Journal of Clinical Pathology 134, 429-435.

Matsuoka, S., Tsuji, K., Hisakawa, H., Xu, M., Ebihara, Y., Ishii, T., Sugiyama, D., Manabe, A., Tanaka, R., Ikeda, Y., Asano, S., Nakahata, T., 2001. Generation of definitive hematopoietic stem cells from murine early yolk sac and paraaortic splanchnopleures by aorta-gonad-mesonephros region-derived stromal cells. Blood 98, 6-12.

Maxwell, P.H., Osmond, M.K., Pugh, C.W., Heryet, A., Nicholls, L.G., Tan, C.C., Doe, B.G., Ferguson, D.J., Johnson, M.H., Ratcliffe, P.J., 1993. Identification of the renal erythropoietin-producing cells using transgenic mice. Kidney International 44, 1149-1162.

McGrath, K., Palis, J., 2008. Ontogeny of erythropoiesis in the mammalian embryo. Curr Top Dev Biol 82, 1-22.

McKnight, A.J., Macfarlane, A.J., Dri, P., Turley, L., Willis, A.C., Gordon, S., 1996. Molecular cloning of F4/80, a murine macrophage-restricted cell surface glycoprotein with homology to the G-protein-linked transmembrane 7 hormone receptor family. J Biol Chem 271, 486-489.

Mebius, R.E., Kraal, G., 2005. Structure and function of the spleen. Nature reviews. Immunology 5, 606-616.

Medvinsky, A., Dzierzak, E., 1996. Definitive hematopoiesis is autonomously initiated by the AGM region. Cell 86, 897-906.

Medvinsky, A.L., Gan, O.I., Semenova, M.L., Samoylina, N.L., 1996. Development of day-8 colony-forming unit-spleen hematopoietic progenitors during early murine embryogenesis: spatial and temporal mapping. Blood 87, 557-566.

Metcalf, D., 1993. Hematopoietic regulators: redundancy or subtlety? Blood 82, 3515-3523.

Metcalf, D., 2008. Hematopoietic cytokines. Blood 111, 485-491.

Mikkola, H.K., Orkin, S.H., 2006. The journey of developing hematopoietic stem cells. Development 133, 3733-3744.

Miller, J.L., 2002. Hemoglobin switching and modulation: genes, cells, and signals. Current opinion in hematology 9, 87-92.

Miyagawa, S., Kobayashi, M., Konishi, N., Sato, T., Ueda, K., 2000. Insulin and insulin-like growth factor I support the proliferation of erythroid progenitor cells in bone marrow through the sharing of receptors. British journal of haematology 109, 555-562.

Mizuochi, C., Fraser, S.T., Biasch, K., Horio, Y., Kikushige, Y., Tani, K., Akashi, K., Tavian, M., Sugiyama, D., 2012. Intra-aortic clusters undergo endothelial to hematopoietic phenotypic transition during early embryogenesis. PLoS One 7, e35763.

Morrison, S.J., Hemmati, H.D., Wandycz, A.M., and Weissman, I.L, 1995. The purification and characterization of fetal liver hematopoietic stem cells. Proceedings of the National Academy of Sciences of the United States of America 92, 10302-10306.

Mucklow, J.C., 1986. The fate of drugs in pregnancy. Clinics in obstetrics and gynaecology 13, 161-175.

Muller, A.M., Medvinsky, A., Strouboulis, J., Grosveld, F., Dzierzak, E., 1994. Development of hematopoietic stem cell activity in the mouse embryo. Immunity 1, 291-301.

Munugalavadla, V., Kapur, R., 2005. Role of c-Kit and erythropoietin receptor in erythropoiesis. Critical reviews in oncology/hematology 54, 63-75.

Muta, K., Krantz, S.B., Bondurant, M.C., Wickrema, A., 1994. Distinct roles of erythropoietin, insulin-like growth factor I, and stem cell factor in the development of erythroid progenitor cells. The Journal of clinical investigation 94, 34-43.

Na, Y.J., Baek, H.S., Ahn, S.M., Shin, H.J., Chang, I.S., Hwang, J.S., 2007. [4-t-butylphenyl]-N-(4-imidazol-1-yl phenyl)sulfonamide (ISCK03) inhibits SCF/c-kit signaling in 501mel human melanoma cells and abolishes melanin production in mice and brownish guinea pigs. Biochemical pharmacology 74, 780-786.

Nakano, T., Kodama, H., Honjo, T., 1994. Generation of lymphohematopoietic cells from embryonic stem cells in culture. Science 265, 1098-1101.

Nandurkar, H.H., Robb, L., Begley, C.G., 1998. The role of IL-II in hematopoiesis as revealed by a targeted mutation of its receptor. Stem cells 16 Suppl 2, 53-65.

Neiva, K., Sun, Y.X., Taichman, R.S., 2005. The role of osteoblasts in regulating hematopoietic stem cell activity and tumor metastasis. Braz J Med Biol Res 38, 1449-1454.

Neubauer, K., Wilfling, T., Ritzel, A., Ramadori, G., 2000. Platelet-endothelial cell adhesion molecule-1 gene expression in liver sinusoidal endothelial cells during liver injury and repair. Journal of Hepatology 32, 921-932.

Nocka, K., Majumder, S., Chabot, B., Ray, P., Cervone, M., Bernstein, A., Besmer, P., 1989. Expression of c-kit gene products in known cellular targets of W mutations in normal and W mutant mice--evidence for an impaired c-kit kinase in mutant mice. Genes & development 3, 816-826.

Nuez, B., Michalovich, D., Bygrave, A., Ploemacher, R., Grosveld, F., 1995. Defective haematopoiesis in fetal liver resulting from inactivation of the EKLF gene. Nature 375, 316-318.

Ohls, R.K., Li, Y., Trautman, M.S., Christensen, R.D., 1994. Erythropoietin production by macrophages from preterm infants: implications regarding the cause of the anemia of prematurity. Pediatric research 35, 169-170.

Ohno, H., Ogawa, M., Nishikawa, S., Hayashi, S., Kunisada, T., Nishikawa, S., 1993. Conditions required for myelopoiesis in murine spleen. Immunol Lett 35, 197-204.

Ohtani, K., 1999. Implication of transcription factor E2F in regulation of DNA replication. Frontiers in bioscience : a journal and virtual library 4, D793-804.

Oliveira, S.H., Taub, D.D., Nagel, J., Smith, R., Hogaboam, C.M., Berlin, A., Lukacs, N.W., 2002. Stem cell factor induces eosinophil activation and degranulation: mediator release and gene array analysis. Blood 100, 4291-4297.

Ono, E., Taniguchi, M., Higashi, N., Mita, H., Kajiwara, K., Yamaguchi, H., Tatsuno, S., Fukutomi, Y., Tanimoto, H., Sekiya, K., Oshikata, C., Tsuburai, T., Tsurikisawa, N., Otomo, M., Maeda, Y., Hasegawa, M., Miyazaki, E., Kumamoto, T., Akiyama, K., 2010. CD203c expression on human basophils is associated with asthma exacerbation. J Allergy Clin Immunol 125, 483-489 e483.

Orkin, S.H., 2000. Diversification of haematopoietic stem cells to specific lineages. Nat Rev Genet 1, 57-64.

Ottersbach, K., Dzierzak, E., 2005. The murine placenta contains hematopoietic stem cells within the vascular labyrinth region. Dev Cell 8, 377-387.

Palis, J., 2014. Primitive and definitive erythropoiesis in mammals. Front Physiol 5, 3.

Palis, J., Robertson, S., Kennedy, M., Wall, C., Keller, G., 1999. Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. Development 126, 5073-5084.

Papayannopoulou, T., Craddock, C., 1997. Homing and trafficking of hemopoietic progenitor cells. Acta Haematol 97, 97-104.

Patterson, K.D., Drysdale, T.A., Krieg, P.A., 2000. Embryonic origins of spleen asymmetry. Development 127, 167-175.

Perfetto, S.P., Chattopadhyay, P.K., Lamoreaux, L., Nguyen, R., Ambrozak, D., Koup, R.A., Roederer, M., 2006. Amine reactive dyes: an effective tool to discriminate live and dead cells in polychromatic flow cytometry. Journal of immunological methods 313, 199-208.

Peterson, H., Abu Dawud, R., Garg, A., Wang, Y., Vilo, J., Xenarios, I., Adjaye, J., 2013. Qualitative modeling identifies IL-11 as a novel regulator in maintaining self-renewal in human pluripotent stem cells. Front Physiol 4, 303.

Petzold, K.M., Spagnoli, F.M., 2012. A system for ex vivo culturing of embryonic pancreas. Journal of visualized experiments : JoVE, e3979.

Pietras, E.M., Warr, M.R., Passegue, E., 2011. Cell cycle regulation in hematopoietic stem cells. J Cell Biol 195, 709-720.

Pinho, S., Lacombe, J., Hanoun, M., Mizoguchi, T., Bruns, I., Kunisaki, Y., Frenette, P.S., 2013. PDGFRalpha and CD51 mark human nestin+ sphere-forming mesenchymal stem cells capable of hematopoietic progenitor cell expansion. The Journal of experimental medicine 210, 1351-1367.

Potocnik, A.J., Brakebusch, C., Fassler, R., 2000. Fetal and adult hematopoietic stem cells require beta1 integrin function for colonizing fetal liver, spleen, and bone marrow. Immunity 12, 653-663.

Prosper, F., Verfaillie, C.M., 2001. Regulation of hematopoiesis through adhesion receptors. Journal of leukocyte biology 69, 307-316.

Radcliff, G., Jaroszeski, M.J., 1998. Basics of flow cytometry. Methods in molecular biology 91, 1-24.

Rio, D.C., Ares, M., Jr., Hannon, G.J., Nilsen, T.W., 2010. Removal of DNA from RNA. Cold Spring Harbor protocols 2010, pdb prot5443.

Robin, C., Ottersbach, K., Durand, C., Peeters, M., Vanes, L., Tybulewicz, V., Dzierzak, E., 2006. An unexpected role for IL-3 in the embryonic development of hematopoietic stem cells. Developmental cell 11, 171-180.

Rolink, A., ten Boekel, E., Melchers, F., Fearon, D.T., Krop, I., Andersson, J., 1996. A subpopulation of B220+ cells in murine bone marrow does not express CD19 and contains natural killer cell progenitors. The Journal of experimental medicine 183, 187-194.

Rose, S., Misharin, A., Perlman, H., 2012. A novel Ly6C/Ly6G-based strategy to analyze the mouse splenic myeloid compartment. Cytometry A 81, 343-350.

Ross, M.H., Pawlina, W., 2010. Chapter 10 Blood, in: Taylor, C. (Ed.), Histology: A Text and Atlas, with Correlated Cell and Molecular Biology, 6th edition. Lippincott William & Wilkins, Philadelphia, pp. 268-308.

Rossant, J., Cross, J.C., 2001. Placental development: lessons from mouse mutants. Nature reviews. Genetics 2, 538-548.

Rudolph, N.S., Ohlsson-Wilhelm, B.M., Leary, J.F., Rowley, P.T., 1985. Single-cell analysis of the relationship among transferrin receptors, proliferation, and cell cycle phase in K562 cells. Cytometry 6, 151-158.

Rurak, D.W., Wright, M.R., Axelson, J.E., 1991. Drug disposition and effects in the fetus. Journal of developmental physiology 15, 33-44.

Russell, E.S., 1979. Hereditary anemias of the mouse: a review for geneticists. Advances in genetics 20, 357-459.

Ryan, M.J., Sigmund, C.D., 2002. Use of transgenic and knockout strategies in mice. Seminars in nephrology 22, 154-160.

Sadahira, Y., Yasuda, T., Yoshino, T., Manabe, T., Takeishi, T., Kobayashi, Y., Ebe, Y., Naito, M., 2000. Impaired splenic erythropoiesis in phlebotomized mice injected with CL2MDP-liposome: an experimental model for studying the role of stromal macrophages in erythropoiesis. Journal of leukocyte biology 68, 464-470.

Sangfelt, O., Erickson, S., Einhorn, S., Grander, D., 1997. Induction of Cip/Kip and Ink4 cyclin dependent kinase inhibitors by interferon-alpha in hematopoietic cell lines. Oncogene 14, 415-423.

Sargent, P.J., Farnaud, S., Evans, R.W., 2005. Structure/function overview of proteins involved in iron storage and transport. Current medicinal chemistry 12, 2683-2693.

Sasaki, K., Matsumura, G., 1987. Hemopoietic cells in the liver and spleen of the embryonic and early postnatal mouse: a karyometrical observation. The Anatomical record 219, 378-383.

Sasaki, K., Matsumura, G., 1988. Spleen lymphocytes and haemopoiesis in the mouse embryo. Journal of anatomy 160, 27-37.

Sasaki, T., Mizuochi, C., Horio, Y., Nakao, K., Akashi, K., Sugiyama, D., 2010. Regulation of hematopoietic cell clusters in the placental niche through SCF/Kit signaling in embryonic mouse. Development 137, 3941-3952.

Schuettpelz, L.G., Borgerding, J.N., Christopher, M.J., Gopalan, P.K., Romine, M.P., Herman, A.C., Woloszynek, J.R., Greenbaum, A.M., Link, D.C., 2014. G-CSF regulates hematopoietic stem cell activity, in part, through activation of Toll-like receptor signaling. Leukemia 28, 1851-1860.

Schwanhausser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., Chen, W., Selbach, M., 2011. Global quantification of mammalian gene expression control. Nature 473, 337-342.

Shi, J., Ikeda, K., Maeda, Y., Shinagawa, K., Ohtsuka, A., Yamamura, H., Tanimoto, M., 2008. Identification of CD123+ myeloid dendritic cells as an early-stage immature subset with strong tumoristatic potential. Cancer Lett 270, 19-29.

Shiozawa, Y., Jung, Y., Ziegler, A.M., Pedersen, E.A., Wang, J., Wang, Z., Song, J., Wang, J., Lee, C.H., Sud, S., Pienta, K.J., Krebsbach, P.H., Taichman, R.S., 2010. Erythropoietin couples hematopoiesis with bone formation. PLoS One 5, e10853.

Sieff, C.A., Niemeyer, C.M., Mentzer, S.J., Faller, D.V., 1988. Interleukin-1, tumor necrosis factor, and the production of colony-stimulating factors by cultured mesenchymal cells. Blood 72, 1316-1323.

Singh-Jasuja, H., Thiolat, A., Ribon, M., Boissier, M.C., Bessis, N., Rammensee, H.G., Decker, P., 2013. The mouse dendritic cell marker CD11c is down-regulated upon cell activation through Toll-like receptor triggering. Immunobiology 218, 28-39.

Smith, C.A., Rennick, D.M., 1986. Characterization of a murine lymphokine distinct from interleukin 2 and interleukin 3 (IL-3) possessing a T-cell growth factor activity and a mast-cell growth factor activity that synergizes with IL-3. Proceedings of the National Academy of Sciences of the United States of America 83, 1857-1861.

Smith, L., 2013. Chapter 7 Hematopoieisis, in: Rodak, B.F., Fritsma, G.A., Keohane, E.M. (Eds.), Hematology: Clinical Principles and Applications. Elsevier Health Sciences, Missouri, pp. 66-85.

Soon, L., Flechner, L., Gutkind, J.S., Wang, L.H., Baserga, R., Pierce, J.H., Li, W., 1999. Insulin-like growth factor I synergizes with interleukin 4 for hematopoietic cell proliferation independent of insulin receptor substrate expression. Mol Cell Biol 19, 3816-3828.

Speck, N., Peeters, M., Dzierzak, E., 2002. Developement of the Vertebrate Hematopoietic System, in: Rossant, J., Tam, P.P.L. (Eds.), Mouse Development: Patterning, Morphogenesis, and Organogenesis. Academic Press, California, pp. 191-206.

Spiekermann, K., Roesler, J., Emmendoerffer, A., Elsner, J., Welte, K., 1997. Functional features of neutrophils induced by G-CSF and GM-CSF treatment: differential effects and clinical implications. Leukemia 11, 466-478.

Steinman, R., Yaroslavskiy, B., Goff, J.P., Alber, S.M., Watkins, S.C., 2004. Cdk-inhibitors and exit from quiescence in primitive haematopoietic cell subsets. British journal of haematology 124, 358-365.

Stromberg, T., Ekman, S., Girnita, L., Dimberg, L.Y., Larsson, O., Axelson, M., Lennartsson, J., Hellman, U., Carlson, K., Osterborg, A., Vanderkerken, K., Nilsson, K., Jernberg-Wiklund, H., 2006. IGF-1 receptor tyrosine kinase inhibition by the cyclolignan PPP induces G2/M-phase accumulation and apoptosis in multiple myeloma cells. Blood 107, 669-678.

Stuart, C.A., Meehan, R.T., Neale, L.S., Cintron, N.M., Furlanetto, R.W., 1991. Insulin-like growth factor-I binds selectively to human peripheral blood monocytes and B-lymphocytes. The Journal of clinical endocrinology and metabolism 72, 1117-1122.

Sugiyama, D., Inoue-Yokoo, T., Fraser, S.T., Kulkeaw, K., Mizuochi, C., Horio, Y., 2011a. Embryonic regulation of the mouse hematopoietic niche. ScientificWorldJournal 11, 1770-1780.

Sugiyama, D., Kulkeaw, K., Mizuochi, C., 2013. TGF-beta-1 up-regulates extra-cellular matrix production in mouse hepatoblasts. Mechanisms of Development 130, 195-206.

Sugiyama, D., Kulkeaw, K., Mizuochi, C., Horio, Y., Okayama, S., 2011b. Hepatoblasts comprise a niche for fetal liver erythropoiesis through cytokine production. Biochemical and Biophysical Research Communications 410, 301-306.

Suire, C., Brouard, N., Hirschi, K., Simmons, P.J., 2012. Isolation of the stromal-vascular fraction of mouse bone marrow markedly enhances the yield of clonogenic stromal progenitors. Blood 119, e86-95.

Swain, A., Inoue, T., Tan, K.S., Nakanishi, Y., Sugiyama, D., 2014. Intrinsic and extrinsic regulation of mammalian hematopoiesis in the fetal liver. Histol Histopathol.

Tacke, F., Alvarez, D., Kaplan, T.J., Jakubzick, C., Spanbroek, R., Llodra, J., Garin, A., Liu, J.H., Mack, M., van Rooijen, N., Lira, S.A., Habenicht, A.J., Randolph, G.J., 2007. Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. Journal of Clinical Investigation 117, 185-194.

Takacs, P., Green, K.L., Nikaeo, A., Kauma, S.W., 2003. Increased vascular endothelial cell production of interleukin-6 in severe preeclampsia. American journal of obstetrics and gynecology 188, 740-744.

Takaku, T., Malide, D., Chen, J., Calado, R.T., Kajigaya, S., Young, N.S., 2010. Hematopoiesis in 3 dimensions: human and murine bone marrow architecture visualized by confocal microscopy. Blood 116, e41-55.

Tallack, M.R., Magor, G.W., Dartigues, B., Sun, L., Huang, S., Fittock, J.M., Fry, S.V., Glazov, E.A., Bailey, T.L., Perkins, A.C., 2012. Novel roles for KLF1 in erythropoiesis revealed by mRNA-seq. Genome research 22, 2385-2398.

Tam, P.P., 1981. The control of somitogenesis in mouse embryos. Journal of embryology and experimental morphology 65 Suppl, 103-128.

Tan, J.C., Nocka, K., Ray, P., Traktman, P., Besmer, P., 1990. The dominant W42 spotting phenotype results from a missense mutation in the c-kit receptor kinase. Science 247, 209-212.

Tanaka, Y., Inoue-Yokoo, T., Kulkeaw, K., Yanagi-Mizuochi, C., Shirasawa, S., Nakanishi, Y., Sugiyama, D., 2015. Embryonic Hematopoietic Progenitor Cells Reside in Muscle before Bone Marrow Hematopoiesis. PLoS One 10, e0138621.

Tavian, M., Peault, B., 2005. Analysis of hematopoietic development during human embryonic ontogenesis. Methods in molecular medicine 105, 413-424.

Taylor, E., Taoudi, S., Medvinsky, A., 2010. Hematopoietic stem cell activity in the aorta-gonad-mesonephros region enhances after mid-day 11 of mouse development. The International journal of developmental biology 54, 1055-1060.

Thorsteinsdottir, U., Sauvageau, G., Hough, M.R., Dragowska, W., Lansdorp, P.M., Lawrence, H.J., Largman, C., Humphries, R.K., 1997. Overexpression of HOXA10 in murine hematopoietic cells perturbs both myeloid and lymphoid differentiation and leads to acute myeloid leukemia. Molecular and cellular biology 17, 495-505.

Tian, Q., Stepaniants, S.B., Mao, M., Weng, L., Feetham, M.C., Doyle, M.J., Yi, E.C., Dai, H., Thorsson, V., Eng, J., Goodlett, D., Berger, J.P., Gunter, B., Linseley, P.S., Stoughton, R.B., Aebersold, R., Collins, S.J., Hanlon, W.A., Hood, L.E., 2004. Integrated genomic and proteomic analyses of gene expression in Mammalian cells. Mol Cell Proteomics 3, 960-969.

Tober, J., Koniski, A., McGrath, K.E., Vemishetti, R., Emerson, R., de Mesy-Bentley, K.K., Waugh, R., Palis, J., 2007. The megakaryocyte lineage originates from hemangioblast precursors and is an integral component both of primitive and of definitive hematopoiesis. Blood 109, 1433-1441.

Traver, D., Miyamoto, T., Christensen, J., Iwasaki-Arai, J., Akashi, K., and Weissman, I.L, 2001. Fetal liver myelopoiesis occurs through distinct, prospectively isolatable progenitor subsets. Blood 98, 627-635.

Udupa, K.B., Reissmann, K.R., 1979. In vivo erythropoietin requirements of regenerating erythroid progenitors (BFU-e, CFU-e) in bone marrow of mice. Blood 53, 1164-1171.

Umemura, T., al-Khatti, A., Donahue, R.E., Papayannopoulou, T., Stamatoyannopoulos, G., 1989. Effects of interleukin-3 and erythropoietin on in vivo erythropoiesis and F-cell formation in primates. Blood 74, 1571-1576.

van Agthoven, A., Goridis, C., Naquet, P., Pierres, A., Pierres, M., 1984. Structural characteristics of the mouse transferrin receptor. European journal of biochemistry / FEBS 140, 433-440.

Van Dyken, S.J., Locksley, R.M., 2013. Interleukin-4- and interleukin-13-mediated alternatively activated macrophages: roles in homeostasis and disease. Annual review of immunology 31, 317-343.

Verfaillie, C.M., 1998. Adhesion receptors as regulators of the hematopoietic process. Blood 92, 2609-2612.

Wang, K., Wei, G., Liu, D., 2012. CD19: a biomarker for B cell development, lymphoma diagnosis and therapy. Exp Hematol Oncol 1, 36.

Wang, X., Hisha, H., Taketani, S., Adachi, Y., Li, Q., Cui, W., Cui, Y., Wang, J., Song, C., Mizokami, T., Okazaki, S., Li, Q., Fan, T., Fan, H., Lian, Z., Gershwin, M.E., Ikehara, S., 2006. Characterization of mesenchymal stem cells isolated from mouse fetal bone marrow. Stem cells 24, 482-493.

Wang, X.Y., Gelfanov, V., Sun, H.B., Tsai, S., Yang, Y.C., 1999. Distinct actions of interleukin-9 and interleukin-4 on a hematopoietic stem cell line, EMLC1. Exp Hematol 27, 139-146.

Watson, E.D., Cross, J.C., 2005. Development of structures and transport functions in the mouse placenta. Physiology (Bethesda) 20, 180-193.

Watt, F.M., Hogan, B.L.M., 2000. Out of Eden: Stem cells and their niches. Science 287, 1427-1430.

Weaver, J.L., Broud, D.D., McKinnon, K., Germolec, D.R., 2002. Serial phenotypic analysis of mouse peripheral blood leukocytes. Toxicology mechanisms and methods 12, 95-118.

Weinstein, R., Riordan, M.A., Wenc, K., Kreczko, S., Zhou, M., Dainiak, N., 1989a. Dual role of fibronectin in hematopoietic differentiation. Blood 73, 111-116.

Weinstein, Y., Morishita, K., Cleveland, J.L., Ihle, J.N., 1989b. Interleukin 3 (IL-3) induces transcription from nonrearranged T cell receptor gamma loci in IL-3-dependent cell lines. The Journal of experimental medicine 169, 2059-2071.

Willems, E., Mateizel, I., Kemp, C., Cauffman, G., Sermon, K., Leyns, L., 2006. Selection of reference genes in mouse embryos and in differentiating human and mouse ES cells. The International journal of developmental biology 50, 627-635.

Wolber, F.M., Leonard, E., Michael, S., Orschell-Traycoff, C.M., Yoder, M.C., Srour, E.F., 2002. Roles of spleen and liver in development of the murine hematopoietic system. Exp Hematol 30, 1010-1019.

Wolf, N.S., Trentin, J.J., 1968. Hemopoietic colony studies. V. Effect of hemopoietic organ stroma on differentiation of pluripotent stem cells. The Journal of experimental medicine 127, 205-214.

Wu, H., Klingmuller, U., Acurio, A., Hsiao, J.G., Lodish, H.F., 1997. Functional interaction of erythropoietin and stem cell factor receptors is essential for erythroid colony formation. Proceedings of the National Academy of Sciences of the United States of America 94, 1806-1810.

Wu, H., Liu, X., Jaenisch, R., Lodish, H.F., 1995. Generation of committed erythroid BFU-E and CFU-E progenitors does not require erythropoietin or the erythropoietin receptor. Cell 83, 59-67.

Yanai, N., Matsuya, Y., Obinata, M., 1989. Spleen stromal cell lines selectively support erythroid colony formation. Blood 74, 2391-2397.

Yanai, N., Satoh, T., Obinata, M., 1991. Endothelial cells create a hematopoietic inductive microenvironment preferential to erythropoiesis in the mouse spleen. Cell structure and function 16, 87-93.

Yossef, R., Gur, C., Shemesh, A., Guttman, O., Hadad, U., Nedvetzki, S., Miletic, A., Nalbandyan, K., Cerwenka, A., Jonjic, S., Mandelboim, O., Porgador, A., 2015. Targeting natural killer cell reactivity by employing antibody to NKp46: implications for type 1 diabetes. PLoS One 10, e0118936.

Yu, W., Niu, W., Wang, S., Chen, X., Sun, B.O., Wang, F., Sun, Y., 2015. Co-culture with endometrial stromal cells enhances the differentiation of human embryonic stem cells into endometrium-like cells. Exp Ther Med 10, 43-50.

Zhang, C.C., Kaba, M., Ge, G., Xie, K., Tong, W., Hug, C., Lodish, H.F., 2006. Angiopoietin-like proteins stimulate ex vivo expansion of hematopoietic stem cells. Nat Med 12, 240-245.

Zhang, C.C., Lodish, H.F., 2004. Insulin-like growth factor 2 expressed in a novel fetal liver cell population is a growth factor for hematopoietic stem cells. Blood 103, 2513-2521.

Zhang, J.M., An, J., 2007. Cytokines, inflammation, and pain. International anesthesiology clinics 45, 27-37.

Zimmermann, F., Rich, I.N., 1997. Mammalian homeobox B6 expression can be correlated with erythropoietin production sites and erythropoiesis during development, but not with hematopoietic or nonhematopoietic stem cell populations. Blood 89, 2723-2735.

Zsebo, K.M., Williams, D.A., Geissler, E.N., Broudy, V.C., Martin, F.H., Atkins, H.L., Hsu, R.Y., Birkett, N.C., Okino, K.H., Murdock, D.C., et al., 1990. Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. Cell 63, 213-224.

APPENDICES

APPENDIX A

Guidelines for Laboratory Animals of Kyushu University.

Regulation for Animal Experiments at Kyushu University

October 1, 2005

Regulation No. 14, 2005

(Basis)

Article 1

This Regulation covers the proper and safe performance of Animal Experiments and Related Activities in Kyushu University (the "University") from the standpoints of science, animal welfare, environmental conservation. It is based on the Law for the Humane Treatment and Management of Animals (Law No. 105, 1973), the Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain (Notice No. 88 of Ministry of Environment, 2006), the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions (Notice No. 71 of the Ministry of Education, Culture, Sports, Science and Technology, 2006) and other related laws and regulations.

(Definitions)

Article 2

The following terms used in this Regulation are defined below:

(1) Animal Experiments and Related Activities

Use of animals for education, testing, research and development, manufacture of biological products, or other scientific purposes.

(2) Laboratory Animals

Animals of mammalian, avian, or reptilian species cared for or managed in animal facilities or laboratories for use in Animal Experiments and Related Activities (including animals in transport to or from animal facilities or laboratories).

(3) Animal Experiment Protocol

Protocol for the conduct of Animal Experiments and Related Activities.

(4) Animal Experiment Researcher

Person performing Animal Experiments and Related Activities.

(5) Principal Investigator

The Animal Experiment Researcher who is responsible for all activities concerning the Animal Experiments and Related Activities.

(6) Facility for Care and Management

Facility used to constantly care for or manage Laboratory Animals and to perform Animal Experiments and Related Activities (excluding Laboratory, mentioned in the next item).

(7) Laboratory

Laboratory where the Animal Experiments and Related Activities (including temporary management, up to 48 h) are performed on Laboratory Animals.

(8) Dean

Chief of the Faculty conducting Animal Experiments and Related Activities or managing Facility for Care and Management and Laboratory.

(9) Dean of the Faculty

Chief of the Faculty where Animal Experiment Researcher, Principal Investigator and animal technician who is in charge of care and management of laboratory animals belong.

(The President)

Article 3

The President of the University unifies the proper and safe performance of Animal Experiments and Related Activities in the University.

(Institutional Animal Care and Use Committee)

Article 4

1. The Institutional Animal Care and Use Committee (the "Committee") defined in the regulations of Kyushu University Deans and Directors Meeting (Regulation No. 14, 2004) deliberates the matters of Animal Experiments and Related Activities.

2. Membership of Committee, Committee Meeting and relevant particulars were based on the rules of Kyushu University Institutional Animal Care and Use Committee (Rule No. 195, 2004; the "Committee Rules").

(Supervisor)

Article 5

1. The Supervisors (the "Supervisor") are appointed by the President to manage Animal Experiments and Related Activities. The Supervisor shall be a member of teaching staff of the University with knowledge and experience related to Laboratory Animals.

2. The Supervisor shall assist the President to execute the following missions.

(1) Conduct of the proper performance of Animal Experiments and Related Activities in the University.

(2) Supervision for keeping the legitimateness and promoting the safety management of Animal Experiments and Related Activities to the Dean and the Faculty Supervisors who based on the Paragraph 1 of the Article 8 of this Regulation in charge of Animal Experiments and Related Activities.

(3) Coordination for keeping the legitimateness and promoting the safety management of Animal Experiments and Related Activities in the University.

3. The term of office of the Supervisor shall be two (2) years, and can be reappointed.

4. The President may appoint the Assistant Supervisor (the "Assistant Supervisor") to assist the Supervisor's missions.

5. The Assistant Supervisor is appointed by the President from a member of teaching staff of the University with knowledge and experience related to Laboratory Animals, based on the recommendation of the Supervisor.

(Responsibilities of the Dean)

Article 6

The Dean shall take measure to the facilities, equipment and organization for conducting the proper and safe performance of Animal Experiments and Related Activities.

(Institutional Animal Care and Use Committee in the Faculty)

Article 7

1. The Institutional Animal Care and Use Committee in the Faculty (the "Faculty Committee") defined in the Paragraph 1 of the Article 4 of the Committee Rules deliberate the Animal Experiments and Related Activities, the Facility for Care and Management and the Laboratory in the Faculty.

2. Membership of Committee and relevant particulars were based on the rules set up in

each Faculty.

(Supervisor in the Faculty)

Article 8

1. The Supervisor in the Faculty (the "Faculty Supervisor") is appointed by the Dean. The Faculty Supervisor shall be a Professor, Associate Professor or Lecturer of the Faculty with knowledge and experience related to Laboratory Animals.

2. The Faculty Supervisor shall assist the Dean to execute the following missions.

(1) Conduct of the proper performance of Animal Experiments and Related Activities in the Faculty.

(2) Supervision for keeping the legitimateness and promoting the safety management of Animal Experiments and Related Activities to the Animal Experiment Researchers and animal technicians (the "Animal Experiment Researcher, etc.") in charge of Animal Experiments and Related Activities.

(3) Coordination for keeping the legitimateness and promoting the safety management of Animal Experiments and Related Activities in the Faculty.

(Education and Training)

Article 9

The President provides education and training for the Animal Experiment Researcher the "Education and Training", etc. before starting the Animal Experiments and Related Activities.

(Registration of the Animal Experiment Researcher)

Article 10

1. The Animal Experiment Researcher, etc. shall apply for the registration of the Animal Experiment Researcher to the Dean of the Faculty.

2. The Dean of the Faculty shall register the applicant as the Animal Experiment Researcher after confirming that the applicant attending the education and training in the previous paragraph.

3. The Dean of the Faculty shall report the names of registrants to the President.

(Medical Examination)

Article 11

The President provides a medical examination to the registrants based on the previous Article the “Medical Examination”.

(Review Procedure of Animal Experiments and Related Activities)

Article 12

1. When conducting Animal Experiments and Related Activities, the Principal Investigator shall draft and submit an Animal Experiment Protocol to the President through the Dean of the Faculty, and receive approval before beginning the Animal Experiment and Related Activities.

2. In cases where changes are made to an approved Animal Experiment Protocol in the previous paragraph, the Principal Investigator shall submit an application form for the alteration of the protocol to the President through the Dean of the Faculty, and receive approval.

3. When submitting the applications in the previous two paragraphs, the Dean of the Faculty shall request that the Faculty Committee pre-reviews the protocol and report the decision to the President.

4. When accepting the report in the previous paragraph, the President shall request that the Committee reviews the protocol and then decide whether or not approve.

(Safety Management)

Article 13

The President shall consider the following particulars, when conducting the Animal Experiments and Related Activities that need special safety management.

(1) In cases of animal experiments involving materials that may pose a physical or chemical risk or that involve pathogens, and affecting the safety and health of humans or the surrounding environment, the President shall secure appropriate facilities or equipment necessary for the safety and health of the Animal Experiment Researcher.

(2) The President shall ensure maintenance of the facilities and equipment and conduct necessary health management such as quarantine to prevent Laboratory Animals suffering injuries unrelated to the objective of an animal experiment or from contracting a disease.

(3) When conducting the Animal Experiments and Related Activities using the Laboratory Animals are genetically modified animals or affecting the ecological systems, the President shall secure appropriate facilities necessary for preventing the genetic modified animals from escaping, and follow the related regulations of the University.

(Responsibility of the Animal Experiment Researcher)

Article 14

1. The Animal Experiment Researcher shall take the items below into consideration for drafting and conducting the Animal Experiments and Related Activities.

(1) When drafting the Animal Experiments and Related Activities, the Animal Experiment Researcher shall consider the application of alternative methods that do not require the use of Laboratory Animals within limits that allow scientific objectives to be achieved.

(2) Consideration of the selection of Laboratory Animals species appropriate for the purpose of Animal Experiments and Related Activities and the use of as few Laboratory Animals as possible within limits that allow scientific objectives to be achieved, and the genetic and microbiological quality.

(3) Consideration of the use of appropriate anesthetics and analgesics and the application of methods that do not distress the Laboratory Animals or subject them to pain within limits required for use.

(4) Selecting proper procedure of euthanasia to cause as little pain as possible, when completing the experiment.

(5) Taking proper measures with carcasses of Laboratory Animals related waste, so as not to have any adverse influence on the environment.

(6) Consideration of the prevention of the safety of the surrounding humans or animals as well as he Animal Experiment Researcher, and any adverse influence on the environment, in cases of animal experiments as may require special attention to safety management (those involving materials that may pose a physical or chemical risk or that involve pathogens).

(7) Conducting Animal Experiments and Related Activities using the facility and laboratory including equipment maintained appropriately.

2. The Animal Experiment Researcher shall request animal technicians to conduct the previous items, as required.

(Responsibility of the Principal Investigator)

Article 15

The Principal Investigator shall be a teaching staff in the University and ensure that the Animal Experiment Researcher perform the responsibility based on the previous Article 14.

(Report for completion and results of Animal Experiment and Related Activities)

Article 16

1. The Principal Investigator shall report the results of Animal Experiments and Related Activities to the President through the Dean in the Faculty after the completion or cancellation of Animal Experiments and Related Activities.

2. The President shall report the results to the Committee.

3. The Committee shall advise to the reports as required.

(Care and Management of Laboratory Animals)

Article 17

The Animal Experiment Researcher, etc. shall supply the Laboratory Animals with food and water, as appropriate to the physiology, ecology, and behavior of the animals, endeavor to preserve the health and safety of Laboratory Animals and provide them with appropriate treatment as required.

(Application and approval for establishing, changing or closing the Facility for Care and Management and the Laboratory)

Article 18

1. The Dean shall submit an application for an establishment and shall request the approval of the President for establishing a Facility for the Care and Management or Laboratory (the "Facilities").

2. The Dean shall submit an application for a changing the Facilities and shall request the approval of the President for the changing.

3. The President shall ask the Committee to review the application and then the President shall approve or deny the establishment or change of the Facilities concerned.

4. In the event that Facilities are closed, the Dean shall notice the closing to the President.

(Person in charge of the Facilities)

Article 18-2

1. A Person in charge of the Facilities shall be appointed.

2. A Person in charge of establishing Facilities shall be responsible for management and maintenance of the Facilities.

(Retention and report of records)

Article 18-3

1. The Principal Investigator shall prepare and retain record books related to Laboratory Animal sources, rearing history, history of disease, and rearing environment.

2. The Person in charge of the Facilities shall submit a report annually about the species of the Laboratory Animals that cared and managed, and their numbers, etc. to the Faculty Committee.

3 The Faculty Committee shall collect the reports and submit a report annually about the species of the Laboratory Animals that cared and managed in the Faculty, and their numbers, etc. to the Committee.

(Measure to Accidents)

Article 19

1. In cases in which the infection, the environment pollution or other accident is occurred in the Animal Experiments and Related Activities, the Animal Experiment Researcher etc. shall inform the Dean as soon as possible.

2. If the Dean receives such a notification, the Dean shall take necessary measures; promptly report the details and handling of the matter to the President.

(Self-inspection, Assessment, and Verification)

Article 20

1. The President requires that the Committee conducts inspections and assessments to determine whether the situation regarding the Animal Experiments and Related Activities in the University complies with related ordinances and regulations etc. of the University.

2. The Committee shall implement self-inspections and assessments and shall report its findings to the President.

3. The Committee requires that the Faculty Committee conducts self-inspections and assessments to determine whether the situation regarding the Animal Experiments and Related Activities in the Faculty and shall report its findings to the Committee.

4. The President shall endeavour to have the results of self-inspections and assessments verified by persons or agencies outside the University.

(Public Disclosure of Information)

Article 21

The President shall publicly disclose information on the conduct of Animal Experiments and Related Activities at the University every academic year.

(Exclusion of Application)

Article 22

These rules shall not be applied to the care or maintenance of Laboratory Animals (which are limited to animals generally considered to be industrial live stock) for the purpose of care management education, testing and research, or breed improvement in stockbreeding. Neither of these rules shall be applied to the care or maintenance of Laboratory Animals for the purpose of ecological observation.

(Miscellaneous provisions)

Article 23

1. The President shall provide necessary rules regarding the proper conduct of Animal Experiments and Related Activities covered by provisions in this Regulation through deliberateness of the Committee.

2. The Dean shall provide necessary rules regarding the proper conduct of Animal Experiments and Related Activities in the Faculty not covered by provisions in this Regulation.

Additional Provisions

1. The Regulation shall come into force on October 1, 2005.

2. Regulations for Prevention of Epidemic Hemorrhagic Fever at Kyushu University (Regulation No. 84, 2004) shall be abolished.

Additional Provisions (Regulation No. 33, 2006)

1. The Regulation shall come into force on January1, 2007.

2. Animal Experiment Protocol approved and conducted on the preceding day of the effective date of this Regulation shall be approved under the revised Regulation.

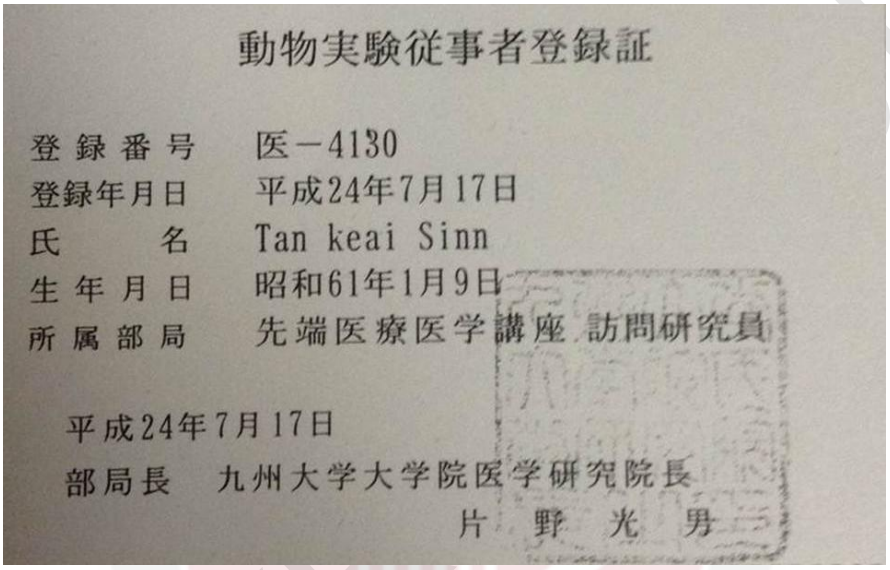
Additional Provisions (Regulation No. 159, 2006)
The Regulation shall come into force on April1, 2007.

Additional Provisions (Regulation No. 72, 2007)
The Regulation shall come into force on April1, 2008.
Additional Provisions (Regulation No. 8, 2008)
The Regulation shall come into force on July18, 2008.
Additional Provisions (Regulation No. 37, 2009)
The Regulation shall come into force on November1, 2009.
In case of conflict between the English translation of this Regulation and the Japanese original, the latter shall prevail.

APPENDIX B

Signed in of person engaged in animal experiments in Kyushu University

The approval for animal handling is given after attended lecture in handling animals on 17th July 2012 by the Dean of Faculty of Medical Research, Kyushu University.



動物実験従事者登録証

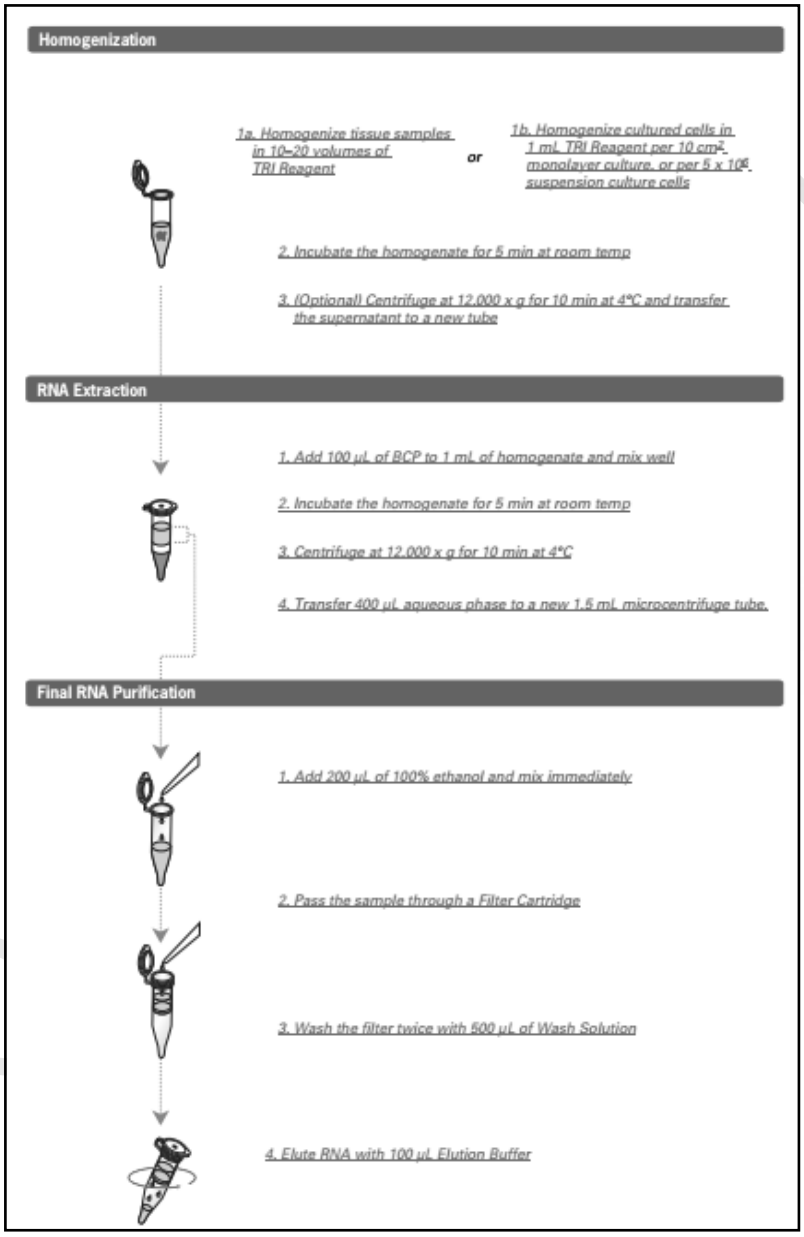
登録番号　医－4130
登録年月日　平成24年7月17日
氏名　Tan keai Sinn
生年月日　昭和61年1月9日
所属部局　先端医療医学講座 訪問研究員

平成24年7月17日
部局長　九州大学大学院医学研究院長
片野光男

APPENDIX C

Total RNA Isolation Procedure

Figure below shows RiboPure™ Procedure Overview



APPENDIX D

Components to prepare 4% paraformaldehyde (PFA) and 30% sucrose

i. Components to prepare 4% paraformaldehyde (PFA).
The 4% PFA was prepared as indicated the table below. The 4% PFA was further diluted with PBS to 2% paraformaldehyde for tissue fixation purpose.

Components	Volume/Mass
Paraformaldehyde, PFA(Wako Pure Chemical Industries)	0.8 g
5 M NaOH Sodium hydroxide solution volumetric, 5.0 M NaOH (5.0N) (Sigma-Aldrich, St. Louis, MO)	32 µL
6 N HCl Hydrochloric acid solution volumetric, 6 M HCl (6N) (Sigma-Aldrich)	18.7 µL
PBS	20 mL

ii. Components to prepare 30% sucrose in PBS.
The 30% sucrose was prepared as indicated the table below. The solution was filtered and stored after being prepared.

Components	Volume/Mass
Sucrose (Wako Pure Chemical Industries)	150 g
10X PBS	50 mL
MiliQ	up to 500 mL

APPENDIX E

Components to prepare 1% BSA

The 1% BSA was prepared using PBS as indicated the table below. The solution was kept in cool room overnight. On the next day, the solution was filtered using Minisart® NML Syringe Filters 17598 (Sartorius, Goettingen, Germany).

Components	Volume/Mass
Bovine Serum Albumin, BSA (Sigma-Aldrich)	5 g
10X PBS	50 mL

APPENDIX F

Components to prepare 0.05% Triton-X100, 1.5% H_2O_2 in PBS and 0.5% blocking buffer

i. Components to prepare 0.05% Triton-X100 in PBS.
The 0.05% Triton-X100 was prepared in PBS as indicated the table below.

Components	Volume/Mass
Polyoxyethylene(8) Octylphenyl Ether, Triton-X100 (Wako Pure Chemical Industries)	150 µL
10X PBS	300 mL

ii. Components to prepare 1.5% H_2O_2 in PBS.
The 1.5 % H_2O_2 was prepared in PBS as indicated the table below.

Components	Volume/Mass
Hydrogen peroxide, H2O2((including in TSA system kit)	150 µL
10X PBS	950 µL

iii. Components to prepare 0.5% blocking buffer.
The 0.5% blocking buffer was prepared in PBS as indicated the table below.

Components	Volume/Mass
Blocking reagent (including in TSA system kit)	0.05g
0.05% Triton-X100 in PBS	950 µL

APPENDIX G

Supplemental material 1

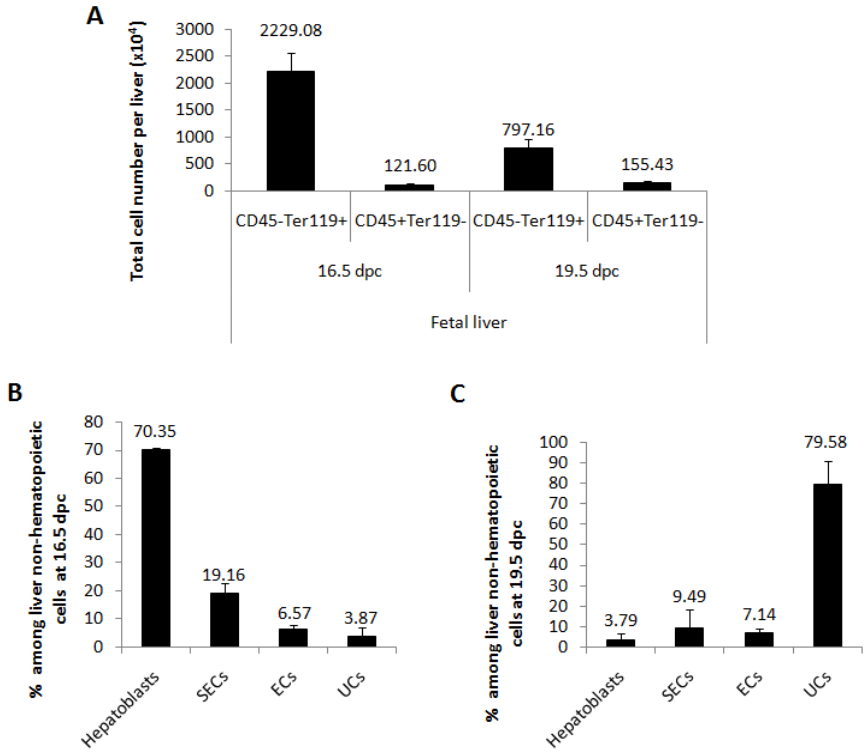


Figure S1. Characterization of fetal liver hematopoietic cells.
(A) Graph showing total number of $CD45^{-}Ter119^{+}$ and $CD45^{+}Ter119^{-}$ cells per liver at both 16.5 dpc and 19.5 dpc (n=3). (B, C) Graphs showing the percentage of fetal liver cells among non-hematopoietic cells at 16.5 dpc and 19.5 dpc. $CD45^{-}Ter119^{-}DLK\text{-}1^{+}$defines hepatoblasts; (2) $CD45^{-}Ter119^{-}CD31^{+}LYVE\text{-}1^{+}$ defines sinusoidal endothelial cells; (3) $CD45^{-}Ter119^{-}CD31^{+}LYVE\text{-}1^{-}$ defines endothelial cells (ECs); and (4) $CD45^{-}Ter119^{-}CD31^{-}LYVE\text{-}1^{-}$ defines unclassified cells (UCs) (n=3). Data are means ± standard deviation (SD).

APPENDIX H

Supplemental material 2

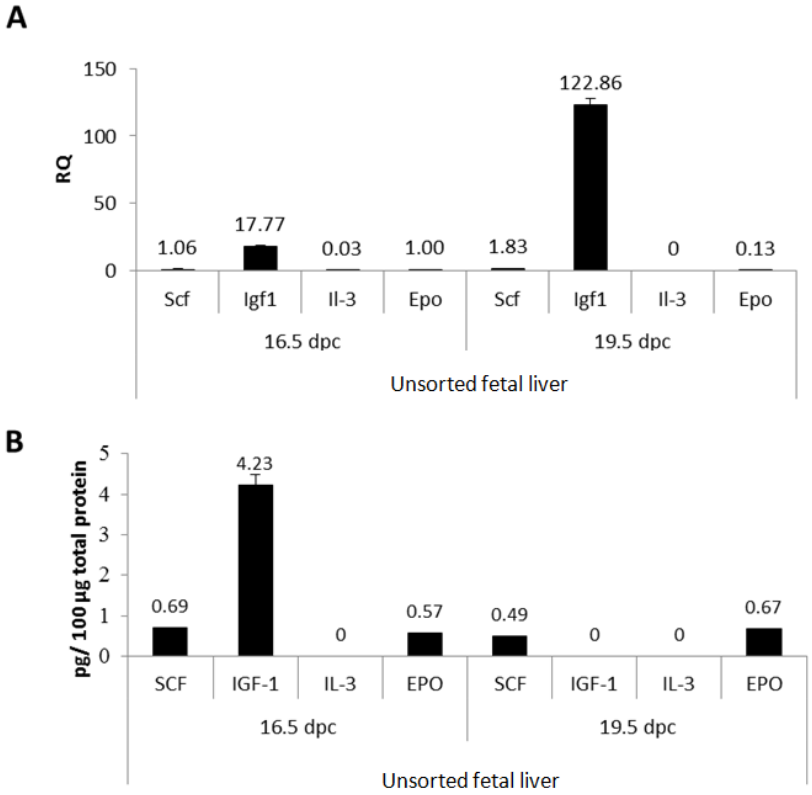


Figure S2. Expression of cytokine mRNA and protein in unsorted fetal liver at 16.5 dpc and 19.5 dpc.
(A) Relative expression (RQ) of *stem cell factor (Scf), insulin-like growth factor1 (Igf1), interleukin-3 (Il-3)* and *erythropoietin (Epo)* mRNAs were examined in unsorted fetal liver at 16.5 dpc and 19.5 dpc by quantitative real-time polymerase chain reaction (qRT-PCR). Epo at 16.5 dpc unsorted fetal liver served as controls. (B) Amounts of SCF, IGF-1, IL-3 and EPO protein per 100 µg of total protein in unsorted fetal liver at both 16.5 dpc and 19.5 dpc.

APPENDIX I

Supplemental material 3

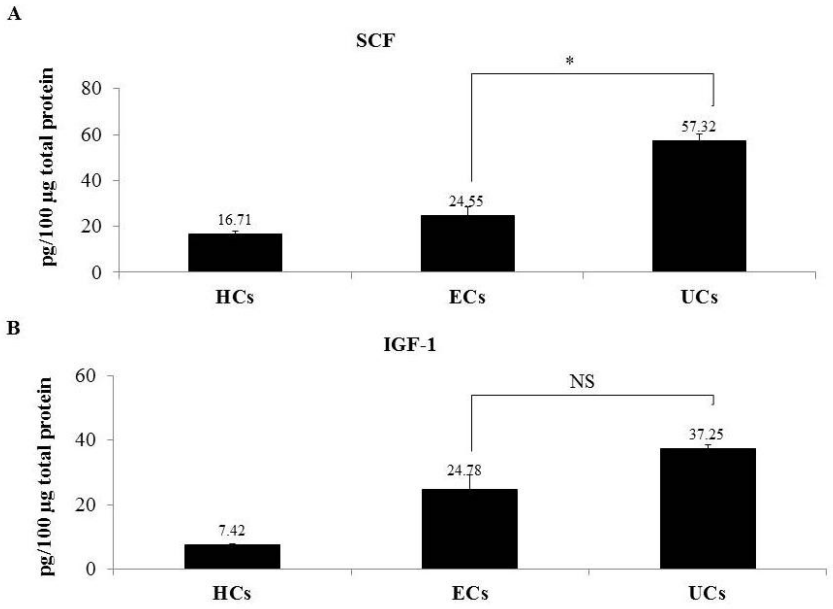


Figure S3. Expression of SCF and IGF-1 in sorted fetal spleen cells.
(A, B) Amounts of SCF and IGF-1 protein per 100 µg of total protein in hematopoietic cells (HCs), endothelial cells (ECs) and unclassified cells (UCs) (n=3). Data are means ± standard deviation (SD). NS, not significant. *, $P < 0.05$.

APPENDIX J

Supplemental material 4

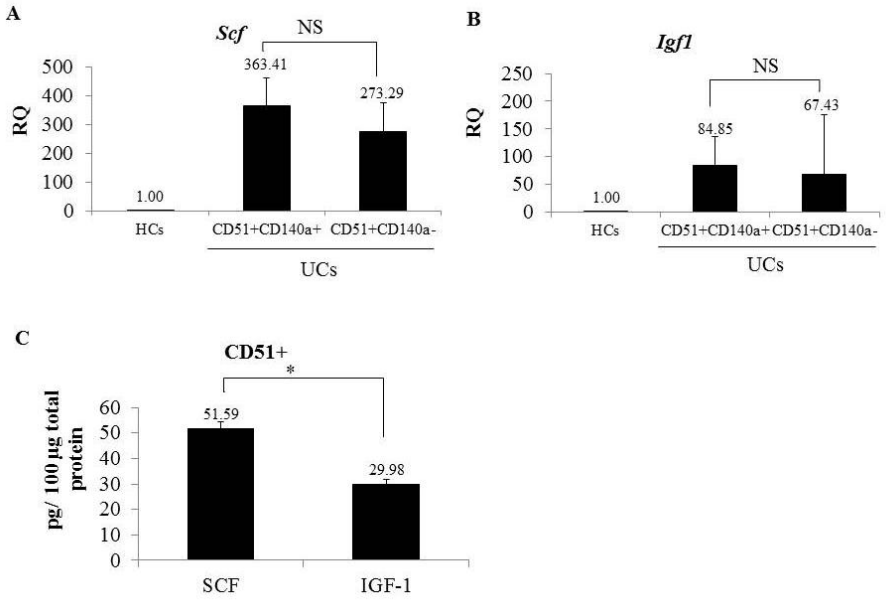


Figure S4. Cytokine expression in $CD51^+$ and $CD51^+CD140a^{+/-}$ cells among unclassified cells (UCs).

(A, B) Relative *Scf* and *Igf1* expression (RQ) was assessed by quantitative real-time PCR in hematopoietic cells (HCs) and $CD51^+CD140a^{+/-}$cells among UCs. HCs served as controls. *Scf* and *Igf1* expression was comparable in $CD51^+CD140a^{+/-}$cells compared to HCs (n=3). (C) Amounts of SCF and IGF-1 protein per 100 µg of total protein in $CD51^+$ cells (n=3). Data are means ± standard deviation (SD). NS, not significant. *, $P < 0.05$.

APPENDIX K

Supplemental material 5

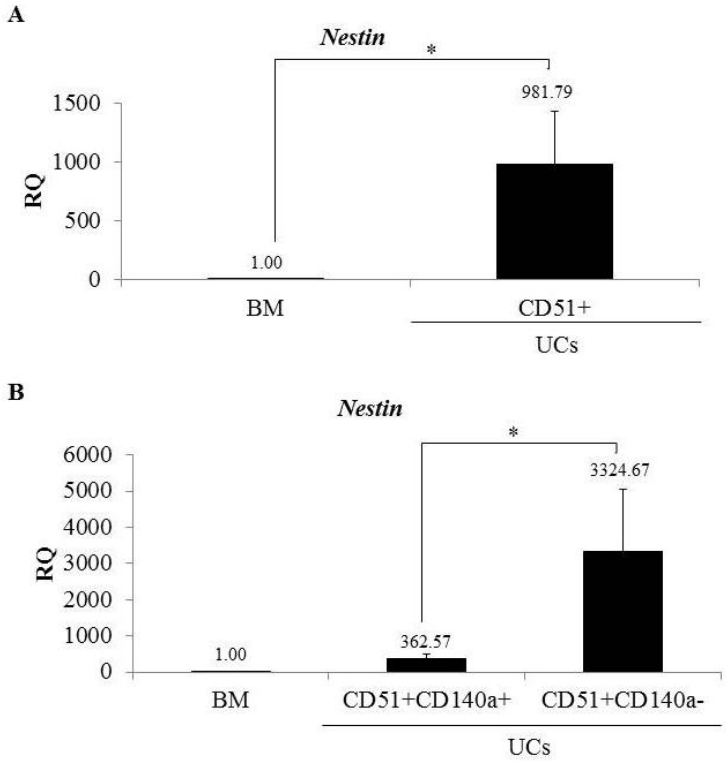


Figure S5. *Nestin* expression in $CD51^+$ and $CD51^+CD140a^{+/-}$ cells among unclassified cells (UCs).
(A) Relative *Nestin* expression (RQ) was examined by quantitative real-time polymerase chain reaction (qRT-PCR) in control unsorted BM cells and in $CD51^+$ cells. *Nestin* was expressed abundantly in $CD51^+$ cells (n=3). (B) *Nestin* expression was examined by qRT-PCR in unsorted BM cells, $CD51^+CD140a^+$ and $CD51^+CD140a^-$ cells. (n=3). *Nestin* expression was highest in $CD51^+CD140a^-$ cells (n=3). Data are means ± standard deviation (SD). *, $P < 0.05$.

BIODATA OF STUDENT

Tan Keai Sinn was born on January 9, 1986 in Selangor. She is the youngest daughter of Mr. Tan Ah See and Mrs. Chia Chai Yah. She obtained her early education in Sekolah Rendah Jenis Kebangsaan (Cina) Yoke Kuan, Sekinchan, Selangor from 1992 to 1998. She then continues her higher education in Sekolah Menengah Kebangsaan Yoke Kuan, Sekinchan. She passed her Penilaian Menengah Rendah (PMR) examination in 2001 and Sijil Pelajaran Malaysia (SPM) in 2003. She was active in co-curriculum activities since primary school. She represented Selangor state in volleyball competitions from 1997 to 2008. In 2004, she was offered to continue her study in Kolej Matrikulasi Pahang (KMPh), Gambang, Kuantan.

Soon after that, she completed her tertiary education with a second upper class honors in Bachelor of Science (Honors) Biomedical Science in Universiti Putra Malaysia, Serdang, Selangor in 2008. In the same year, she was employed as a junior underwriter by an insurance company; however, she decided to continue her studies at Master level in the field of Genetics in June 2009, and completed in December 2011 under sponsorship of Mini Budget and graduate research fellowship (GRF). In February 2012, she continues her studies at PhD level in the field of Molecular Medicine.

From July 2012 until July 2014, she carried out some research at Prof. Dr. Sugiyama's laboratory in Kyushu University, Japan. During her stays in Japan, she successfully obtains a scholarship from the Tokyo Biochemical Research Foundation, Japan as to support her living expenses there. She also received a grant from the International Research Fund for Subsidy of Kyushu University School of Medicine Alumni for the project "Identification and niche regulation of fetal spleen cells". In addition, she is a recipient of MyPhD scholarship from Ministry of Higher Education (MOHE), Malaysia. Her current research is focused on the development of hematopoietic cells during mouse embryogenesis.

LIST OF PUBLICATIONS

Publications:

i **Tan KS**, Inoue T, Kulkeaw K, Tanaka Y, Lai MI, Sugiyama D. Localized SCF and IGF-1 secretion enhances erythropoiesis in the spleen of murine embryos. Biology Open. 2015 April; 4(5):596-607.

ii Kodiappan R, **Tan KS**, Lai JY, Chin FW, Chan SC, Veerakumarasivam A. MicroRNA: Epigenetic Modifiers in Regenerative Medicine. Regenerative Research. 2014 June; 3(1):29-40.

iii Tanaka Y, Kulkeaw K, Inoue T, **Tan KS**, Nakanishi Y, Shirasawa S, Sugiyama D. Dok2 Likely Down-regulates *Klf1* in Mouse Erythroleukemia Cells. Anticancer Research.. 2014 August; 34(8):4561-7.

iv Swain A, Inoue T, **Tan KS**, Nakanishi Y, Sugiyama D. Intrinsic and Extrinsic Regulation of Mammalian Hematopoiesis in the Fetal Liver. Histology and Histopathology. 2014 September; 29(9):1077-82.

v **Tan KS**, Tamura K, Lai MI, Veerakumarasivam A, Nakanishi Y, Ogawa M, Sugiyama D. Molecular Pathways Governing Development of Vascular Endothelial Cells from ES/iPS Cells. Stem cell review and report. 2013 October; 9(5):586-98.

vi Lim WF, Inoue T, **Tan KS**, Lai MI, Sugiyama D. Hematopoietic cell differentiation from embryonic and induced pluripotent stem cells. Stem Cell Research and Therapy. 2013 June; 4(3):71.

vii **Tan KS**, Chan SC, Abdullah S, Rosli R, Veerakumarasivam A. Inhibition of MiR-21 Mitigates the Aggressive Bladder Cancer Phenotype. 4th Malaysian Tissue Engineering and Regenerative Medicine Scientific Meetings. Regenerative Research. Supplement. 37-38. 2012.

viii Lai JY, Chan SC, **Tan KS**, Kodiappan R, Abdullah S, Rosli R, Veerakumarasivam A. The Phenotypic Effect of MiR-141 Modulation on the Aggressive Bladder Cancer Phenotype. 4th Malaysian Tissue Engineering and Regenerative Medicine Scientific Meetings. Regenerative Research. Supplement. 36-37. 2012.

ix Fauziah O, Che Nor Zarida CS, **Tan KS**, Au LF, Saidi M, Idris B, Rusnah M, Asmah R, Mohd Azam Khan GK, Azfar Rizal A. Biocompatibility of Gentamicin-Coated Hydroxyapatite (Ha) with Osteoblast. Medical Journal of Malaysia. Vol. 65, No 10, pp. 87-89. October, 2010.

Proceedings:

i. **Tan KS**, Kulkeaw K, Inoue T, Tanaka Y, Kojima N, Swain A, Madtookung M, Yonehara A, Nakanishi Y, Lai MI, Sugiyama D. Lypodystrophy-related molecule likely upregulates HSC development at the AGM region in mice. The 77th Annual Meeting of the Japanese Society of Hematology. 16-18 October 2015. Kanazawa, Japan. (Poster Presentation).

ii. Madtookung M, Kulkeaw K, Inoue T, Tanaka Y, Kojima N, Swain A, **Tan KS**, Yonehara A, Svasti S, Sugiyama D. Target identification for thalassemia therapy using human β-thalassemic mice. The 77th Annual Meeting of the Japanese Society of Hematology. 16-18 October 2015. Kanazawa, Japan. (Poster Presentation).

iii. Kulkeaw K, Inoue T, Muennu K, Ishitani T, Tanaka Y, **Tan KS**,

Madtookung M, Swain A, Kojima N, Yonehara A, Nakanishi Y, Sugiyama D. Identification of molecular targets for granulopoiesis using lectin-based flow cytometry in zebrafish. The 77th Annual Meeting of the Japanese Society of Hematology. 16-18 October 2015. Kanazawa, Japan. (Poster Presentation).

iv. Swain A, Kulkeaw K, Inoue T, Tanaka Y, Kojima N, Madtookung M, Yonehara A, **Tan KS**, Ishitani T, Shirasawa S, Nakanishi Y, Sugiyama D. Ubiquitin like protein is a novel molecule for the regulation of HSCs in the zebrafish embryo. The 77th Annual Meeting of the Japanese Society of Hematology. 16-18 October 2015. Kanazawa, Japan. (Poster Presentation).

v. Tanaka Y, Inoue T, Kulkeaw K, **Tan KS**, Kojima N, Swain A, Madtookung M, Yonehara A, Shirasawa S, Nakanishi Y, Sugiyama D. Exo-utero surgical technique enables investigation of hematopoietic homing during embryogenesis. The 77th Annual Meeting of the Japanese Society of Hematology. 16-18 October 2015. Kanazawa, Japan. (Poster Presentation).

vi. Kojima N, Kulkeaw K, Inoue T, Tanaka Y, **Tan KS**, Swain A, Madtookung M, Yonehara A, Nakanishi Y, Sugiyama D. AIF functions in erythropoiesis by regulating Klf1 gene and enucleation related genes. The 77th Annual Meeting of the Japanese Society of Hematology. 16-18 October 2015. Kanazawa, Japan. (Oral Presentation).

vii. **Tan KS**, Anagha J, Inoue T, Kulkeaw K, Mizuochi C, Tani K, Akashi K, the FANTOM consortium, Suzuki H, Forrest ARR, Hayashizaki Y, Sugiyama D. Promoterome Analysis of Transcript Specific Dynamics during hematopoietic Stem Cell Ontogeny. The 76th Annual Meeting of the Japanese Society of Hematology. 31-2 November 2014. Osaka, Japan. (Poster Presentation- *Travel Award*).

viii. Inoue T, Kulkeaw K, Muennu K, **Tan KS**, Tanaka Y, Kojima N, Swain A, Nakamichi C, Svasti S, Sugiyama D. Igfbp3 promotes erythroid differentiation via competitive binding to Igf1 in mouse fetal liver. The 76th Annual Meeting of the Japanese Society of Hematology. 31-2 November 2014. Osaka, Japan. (Oral Presentation).

ix. Tanaka Y, Kulkeaw K, Inoue T, **Tan KS**, Kojima N, Swain A, Nakamichi C, Shirawasa S, Nakanishi Y, Sugiyama D. Dok2 down-regulates Klf1 in mouse erythroleukemia cells. The 76th Annual Meeting of the Japanese Society of Hematology. 31-2 November 2014. Osaka, Japan. (Poster Presentation).

x. Kojima N, Kulkeaw K, Inoue T, Tanaka Y, **Tan KS**, Swain A, Nakamichi C, Shirawasa S, Nakanishi Y, Sugiyama D. Aifm2 down-regulates globin gene expression through binding to klf1 promoter *in vitro*. The 76th Annual Meeting of the Japanese Society of Hematology. 31-2 November 2014. Osaka, Japan. (Poster Presentation).

xi. Kulkeaw K, Inoue T, Tanaka Y, **Tan KS**, Kojima N, Swain A, Nakamichi C, Nakanishi Y, Sugiyama D. KS-13, a novel peptide antagonizes survival study of himan myeloid leukemia cells. The 76th Annual Meeting of the Japanese Society of Hematology. 31-2 November 2014. Osaka, Japan. (Oral Presentation).

xii. **Tan KS**, Inoue T, Kulkeaw K, Lim WF, Preedagasamzin S, Tanaka Y, Lai MI, Sugiyama D. Mouse embryonic spleen cells accelerate erythropoiesis through SCF and IGF-1 secretion. The 2nd Red Blood Cell Symposium. 30 October 2014. Osaka, Japan. (Oral Presentation).

xiii. **Tan KS**, Inoue T, Kulkeaw K, Lim WF, Preedagasamzin S, Tanaka Y, Lai MI, Sugiyama D. Embryonic spleen niche accelerates erythropoiesis through SCF and IGF-1 secretion. The 12th Stem Cell Symposium. 30-31 May 2014. Fukuoka, Japan. (Poster Presentation).

xiv. Kulkeaw K, Inoue T, Srinoun K, Ishitani T, Tanaka Y, **Tan KS**, Kanemaru T, Sugiyama D. Antibody-free flow cytometric analysis of zebrafish neutrophil by DNA-binding DRAQ5 dye and lectin. The 12th Stem Cell Symposium. 30-31 May 2014. Fukuoka, Japan. (Poster Presentation).

xv. **Tan KS**, Inoue T, Lim WF, Mizuochi C, Kulkeaw K, Preedagasamzin S, Tanaka Y, Lai MI, Sugiyama D. SCF and IGF-1 accelerate erythropoiesis in the spleen of mouse embryo. The 7th Takeda Science Foundation Symposium on PharmaScience. 15-18 January 2014. Osaka, Japan. (Poster Presentation).

xvi. **Tan KS**, Inoue T, Lim WF, Mizuochi C, Kulkeaw K, Preedagasamzin S, Tanaka Y, Lai MI, Sugiyama D. Spleen Niche Cells Accelerate Erythropoiesis through SCF and IGF-1 Secretion in Mouse Embryo. The 75th Annual Meeting of the Japanese Society of Hematology. 11-13 October 2013. Sapporo, Japan. (Oral Presentation).

xvii. Inoue T, Kulkeaw K, Mizuochi C, **Tan KS**, Tanaka Y, Preedagasamzin S, Sugiyama D. Twist-1 Regulates IAC-formation Through Binding to Myb and Gata2 Promoter Regions. The 75th Annual Meeting of the Japanese Society of Hematology. 11-13 October 2013. Sapporo, Japan. (Oral Presentation).

xviii. Tanaka Y, Yanagi C, Kulkeaw K, Inoue T, **Tan KS**, Preedagasamzin S, Shirasawa S, Sugiyama D. Hematopoietic Stem Cells Stand By in Muscle Before Homing to Bone Marrow. The 75th Annual Meeting of the Japanese Society of Hematology. 11-13 October 2013. Sapporo, Japan. (Oral Presentation).

xix. Kulkeaw K, Inoue T, Srinoun K, Ishitani T, Tanaka Y, **Tan KS**, Preedagasamzin S, Sugiyama D. Antibody-free Flow cytometric Analysis of Zebrafish Granulocytes by DNA-binding DRAQ-5 Dye. The 75th Annual Meeting of the Japanese Society of Hematology. 11-13 October 2013. Sapporo, Japan. (Poster Presentation).

xx. Inoue T, Mizuochi C, Kulkeaw K, **Tan KS**, Tanaka Y, Preedagasamzin S, Tanaka Y, Sugiyama D. Insulin-like Growth Factor 2, Igf2, Accelerates Mesenchymal Cell Activity in the Mouse Yolk Sac. The 75th Annual Meeting of the Japanese Society of Hematology. 11-13 October 2013. Sapporo, Japan. (Poster Presentation).

xxi. **Tan KS**, Inoue T, Lim WF, Mizuochi C, Kulkeaw K, Preedagasamzin S, Tanaka Y, Lai MI, Sugiyama D. Analysis of Endothelial Niche Cells in Fetal Spleen Erythropoiesis. The 11th Stem Cell Symposium. 17-18 May 2013. Tokyo, Japan. (Poster Presentation).

xxii. Inoue T, Mizuochi C, Kulkeaw K, **Tan KS**, Tanaka Y, Preedagasamzin S, Tanaka Y, Sugiyama D. Igf2 Accelerates Mesenchymal Stem Cells (MSCs) Proliferation in the Mouse Yolk Sac. The 11th Stem Cell Symposium. 17-18 May 2013. Tokyo, Japan. (Poster Presentation).

xxiii. Sugiyama D, Kulkeaw K, Inoue T, Mizuochi C, Lim WF , Preedagasamzin S, **Tan KS**. Hepatoblasts comprise a niche for fetal liver hematopoiesis through extracellular matrix and cytokine production. Keystone Symposia, Hematopoiesis. 14 -19 January 2013. Colorado USA.

xxiv. Sugiyama D, Kulkeaw K, Inoue T, Mizuochi C, Lim WF , Preedagasamzin S, **Tan KS**. Hepatoblasts comprise a niche for fetal liver hematopoiesis through extracellular matrix and cytokine production. Cold Spring Harbor Asia Conferences, Stem Cells and Developmental Mechanisms. 3-7 December 2012. Suzhou Dushu Lake Conference Center, China.

xxv. Veerakumarasivam A, Rosli R, Abdullah S, Chan SC, **Tan KS**. Histone demethylase KDM5B regulates cellular proliferation and invasion via the E2F/RB pathway. Invention, Research & Innovation Exhibition 2010. UPM, Malaysia. (Poster Presentation- Silver Medal).

UNIVERSITI PUTRA MALAYSIA
STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION: SECOND SEMESTER 2015/2016

TITLE OF THESIS / PROJECT REPORT:

CELL PHENOTYPE AND CYTOKINE REGULATION OF ERYTHROPOIESIS IN MOUSE FETAL SPLEEN

NAME OF STUDENT: TAN KEAI SINN

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.

2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.

3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as:

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official SecretAct 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by theorganization/institution where research was done).
☑	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for:

☐	**PATENT**	Embargo from_____ until _____ (date) (date)

Approved by:

_____
(Signature of Student)
New IC No/ Passport No.:860109105608

Date :

_____
(Signature of Chairman of Supervisory Committee)
Name: Dr. Lai Mei I

Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]