LUND UNIVERSITY

Genetic modeling of the Hippo pathway in hematopoietic stem cells

Jansson, Lina

2012

Link to publication

Citation for published version (APA):
Jansson, L. (2012). *Genetic modeling of the Hippo pathway in hematopoietic stem cells*. Lund University: Faculty of Medicine.

Total number of authors:
1

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

GENETIC MODELING OF THE HIPPO PATHWAY IN HEMATOPOIETIC STEM CELLS

LUNDS UNIVERSITET

Lina Jansson

ISBN 978-91-8687-85-7

ISSN 1652-8220

Copyright © Lina Jansson, 2012

Lund University, Faculty of Medicine Doctoral Dissertation Series 2012:23

Printed by: Media-Tryck, Lund 2012

Good tests kill flawed theories;

we remain alive to guess again

- *Karl Popper*

(1902-1994)

ABSTRACT

Hematopoiesis is the process of blood formation from a limited pool of hematopoietic stem cells (HSCs). These rare stem cells can both self-renew to maintain the HSC pool, and differentiate to continuously replenish lost blood cells. The mechanisms of HSC regulation are not fully known. The aim of this thesis was to study the role of the Hippo signaling pathway in HSCs. The Hippo pathway is a newly discovered signaling pathway, which regulates organ size in *Drosophila*. Hippo signaling has further been implicated in regulation of mammalian stem cells. In Article I we developed a new way of modeling genetic changes by combining genetic engineering of murine ES cells with blastocyst complementation. This approach avoids the cost and time constraints associated with the creation of standard transgenic mouse strains while taking advantage of the sophisticated site-directed manipulations that are possible in ES cells. In Article II we studied YAP1, the downstream effector in the Hippo pathway. We created a transgenic model with inducible YAP1 expression exclusively within the hematopoietic system using the blastocyst complementation approach developed in article I. When investigating the effect of overexpressing YAP1 in HSCs we detected no effect on HSC function during steady state or regenerative stress. This is contrast to effects seen in other tissue stem cells and suggests tissue specific functions of YAP1 in regulation of stem cells. In Article III we investigated a knockout model for the other Hippo effector Taz. Adult mice deficient in Taz display no changes in hematopoietic parameters but are born below mendelian ratios. Taz thus seems dispensable for adult hematopoiesis but may influence embryonic development.

Taken together, using both novel and traditional genetic engineering approaches in mice, we have taken the first steps to understand the role of the Hippo pathway in hematopoiesis.

TABLE OF CONTENTS

ABSTRACT 5
TABLE OF CONTENTS 7
ARTICLES INCLUDED IN THE THESIS 9
ABBREVIATIONS 10
BACKGROUND 13
Stem cells 13
Definition and concepts 13
Pluripotency - Embryonic stem cells 14
Multipotency - Adult stem cells 17
Cues governing stem cells – the niche 19
Stem cell fitness and competition 20
Hematopoietic stem cells 22
Hematopoiesis 22
The function of the blood and immune system 22
Discovery of the hematopoietic stem cell 23
The hematopoietic hierarchy 24
Hematopoiesis during development 26
The hematopoietic stem cell niche 27
Regulation of hematopoietic stem cells 29
Functional assays for HSCs 30
The Hippo pathway 32
Discovery of the Hippo pathway 32
The Hippo signaling cascade 32
Hippo signaling redundancy - Wwtr1 35

The role of Hippo signaling in disease and cancer *35*
Hippo signaling in stem cells *38*
A Hippo in hematopoiesis? *38*
Genetic modeling 39
Gain of function models *39*
Loss of function models *40*
AIM OF THE THESIS 42
SUMMARY OF THE RESULTS 43
Article I 43
Article II 43
Article III 44
GENERAL DISCUSSION 45
Genetic modeling of hematopoiesis 45
The Hippo pathway in hematopoiesis 47
Solid versus liquid tissues *48*
Yap and Wwtr1 specificity *49*
Characteristics of fetal liver HSCs *49*
CONCLUSIONS 51
ACKNOWLEDGEMENTS 53
POPULÄRVETENSKAPLIG SAMMANFATTNING 54
REFERENCES 57

ARTICLES INCLUDED IN THE THESIS

Article I **W41/W41 blastocyst complementation: a system for genetic modeling of hematopoiesis**.
Jansson, L. and Larsson, J (2010). Blood 115(1): 47-50.

Article II **Normal hematopoietic stem cell function in mice with enforced expression of the Hippo signaling effector YAP1**.
Jansson L. and Larsson, J. (2012) PLoS ONE 7(2): e32013.

Article III **The role of Taz in fetal and adult hematopoiesis.**
Jansson L. and Larsson, J. Manuscript (2012).

ABBREVIATIONS

AGM	-	Aorta-Gonad-Mesonephros
ALL	-	Acute Lymphoblastic Leukemia
aPKC	-	atypical Protein Kinase C
CFU-S	-	Colony Forming Unit Spleen
CLP	-	Common Lymphoid Progenitor
CMP	-	Common Myeloid Progenitor
Crb	-	Crumbs
Dco	-	Discs overgrown
dHCS	-	definitive Hematopoietic Stem Cell
Dlg	-	Discs large
Dpp	-	Decapentaplegic
Ds	-	Dachsous
Ex	-	Expanded
EX.Y	-	Embryonic day X.Y
EC cells	-	Embryonal Carcinoma cells
ES cells	-	Embryonic Stem cells
FGF	-	Fibroblast Growth Factor
Fj	-	Four-jointed
FL	-	Fetal Liver

GMP	-	Granulocyte Macrophage Progenitor
Hpo	-	Hippo
HSC	-	Hematopoietic Stem Cell
HSPC	-	Hematopoietic Stem and Progenitor Cell
ICM	-	Inner Cell Mass
iPS	-	induced Pluripotent Stem cell
Kbr	-	Kibra
KO	-	Knock Out
LATS	-	Large Tumor Suppressor
Lgl	-	Lethal giant larvae
MEP	-	Megakaryocyte Erythrocyte Progenitor
Mer	-	Merlin
MST	-	Mammalian Ste20-like kinase
MSC	-	Mesenchymal Stem Cell
NF2	-	Neurofibromatosis 2
NSC	-	Neural Stem Cell
RNAi	-	Ribo Nucleic Acid interference
ROS	-	Reactive Oxygen Species
Sav	-	Salvador
SCF	-	Stem Cell Factor
Scrib	-	Scribble
siRNAs	-	small interfering RNAs
TEAD	-	TEA Domain family of transcription factors
TGF-β	-	Transforming Growth Factor β

TPO	-	Thrombopoietin
YAP1	-	Yes-associated protein 1
Yki	-	Yorkie
Wg	-	Wingless
Wts	-	Warts
Wwtr1	-	WW domain Transcription factor 1

BACKGROUND

STEM CELLS

Definition and concepts

Stem cells are unspecialized cells present in all multicellular organisms set apart by their potential for generating multiple other specialized cell types. The criteria used to define stem cells are self-renewal, i.e. the indefinite production of a daughter cell with the same potential, and maturation into specialized cells. In the strictest sense of the definition the stem cells of the early embryo are able to give rise to all tissues in an organism including all extra-embryonic tissues and is therefore said to be totipotent from the latin word "***totus***" meaning "*whole*" (Cauffman et al., 2009; Pan et al., 2002; Rossant, 1976; Suwinska et al., 2008; Tarkowski and Rossant, 1976; Tarkowski and Wroblewska, 1967).

In practical terms the definition of a stem cell is represented by its functional abilities; the ability to rescue an organism deficient in stem cells by self-renewing and generating daughter cells throughout a lifetime. The idea of the *stemness* of a *cell* as a deterministic characteristic was turned on its head with the advent of the cloning of Dolly the sheep (Estrov, 2009). Removing the haploid DNA from an egg, and replacing it with the genetic material from a mature differentiated cell resulted in the birth of Dolly, healthy and normal (Campbell et al., 1996). This raises the question of what actually confers potency to a cell and how permanent the presence or absence of stemness in a cell can be. Despite Dolly not being the only example of the plasticity of cell potency (we will discuss other examples

later) the basic definition of a stem cell, self-renewal and differentiation, remains the same.

Pluripotency - Embryonic stem cells

After just a few divisions of the initial zygote, totipotency is lost. At the blastocyst stage, cells in the embryo located in the inner cell mass (**ICM**) are now specialized to give rise to embryonic tissues, whereas the hypoblast cells of the primitive extra-embryonic endoderm and the outside cells of the trophectoderm give rise to extra-embryonic tissues such as the yolk sac and the placenta (Brinster, 1974b; Chen et al., 2010; Senner and Hemberger, 2010; Tarkowski et al., 2010).

Since the cells of the inner cell mass can give rise to all the tissues of the embryo and the adult organism, they are considered pluripotent stem cells, from the latin word "***plures***" meaning "*several*". Cells from the ICM are today one of our most important tools we have for studying genetics at the organism level in mammals (Bockamp et al., 2002; Clarke, 2000).

During the 50s' and 60s' some researchers were exploring the potential of teratocarcinomas, serially transplantable tumors, in mice. Scientists at the time had noticed that there existed mouse strains with a high incidence of spontaneous testicular carcinomas, which were shown to be derived from the primordial germ cells in the testes. Furthermore, transplantation of cells isolated from such tumors produced teratomas. The teratomas contained not only differentiated cells but also undifferentiated cells that highly resembled the cells isolated from the original tumor and used in the first transplantation (Stevens and Little, 1954). It was further demonstrated that a *single* cell from a teratocarcinoma tumor, ectopically transplanted, could actually give rise to an array of different specialized tissues representing all germ layers, and these pluripotent cells were therefore aptly named embryonal carcinoma (**EC**) cells (Kleinsmith and Pierce, 1964).

Early on, researchers working in the field understood the inherent potential of being able to propagate EC cells in *in vitro* culture systems and a lot of

work was undertaken to map out factors governing the differentiation and pluripotency of these cells (Evans, 1972; Kahan and Ephrussi, 1970; Pierce and Dixon, 1959; Rosenthal et al., 1970). It was observed that EC cells in culture during the early stages of differentiation formed embryoid bodies with an outer covering of primary extra-embryonic endoderm, similar to that of an isolated ICM. With the comprehension that the differentiation pattern of EC cells actually mirrored that of normal embryonic development, and the additional findings that incorporation of EC cells into a blastocyst could contribute to chimera formation, came the realization that teratocarcinomas, although in principle malignant, can contain normal, non-cancerous, cells as well (Brinster, 1974a; Mintz and Illmensee, 1975; Papaioannou et al., 1975; Rossant, 1975). This also lead to the understanding that it was possible to isolate cells with the same *in vivo* potential as EC cells without having to go through the tumor stage (Evans and Kaufman, 1981). Cells isolated directly from the embryo and propagated in culture were termed embryonic stem (**ES**) cells to emphasize their normal embryonic origin and behavior (Martin, 1981).

ES cells are exploited today primarily in two ways in research. First, the directed differentiation of ES cells in culture serves both as a model system for gathering information on early embryonic development and cellular differentiation, and as a potential future source of material for cell replacement therapy (Odorico et al., 2001; Polak and Bishop, 2006). Specification to each tissue type presents with its own set of challenges to be able to generate functional mature cells. Additionally, all differentiation protocols share the common issue of obtaining a *pure* cell pool of differentiated cells for transplantation to be able to circumvent teratoma formation (Findikli et al., 2006; Heng et al., 2005; Hwang et al., 2006; Kaufman et al., 2001; Zhang et al., 2001). Second, since ES cells injected into blastocysts can contribute to germline chimerism, any genetic modifications that are introduced in the ES cells will also be present in the progeny and can be transmitted from one generation to another. Because of the ease of culture and genetic manipulation, murine ES cells have become the primary choice to establish models for studying genetic changes. The

many advances in genetic engineering of ES cells have paved the way for quicker and more sophisticated transgenic mouse models.

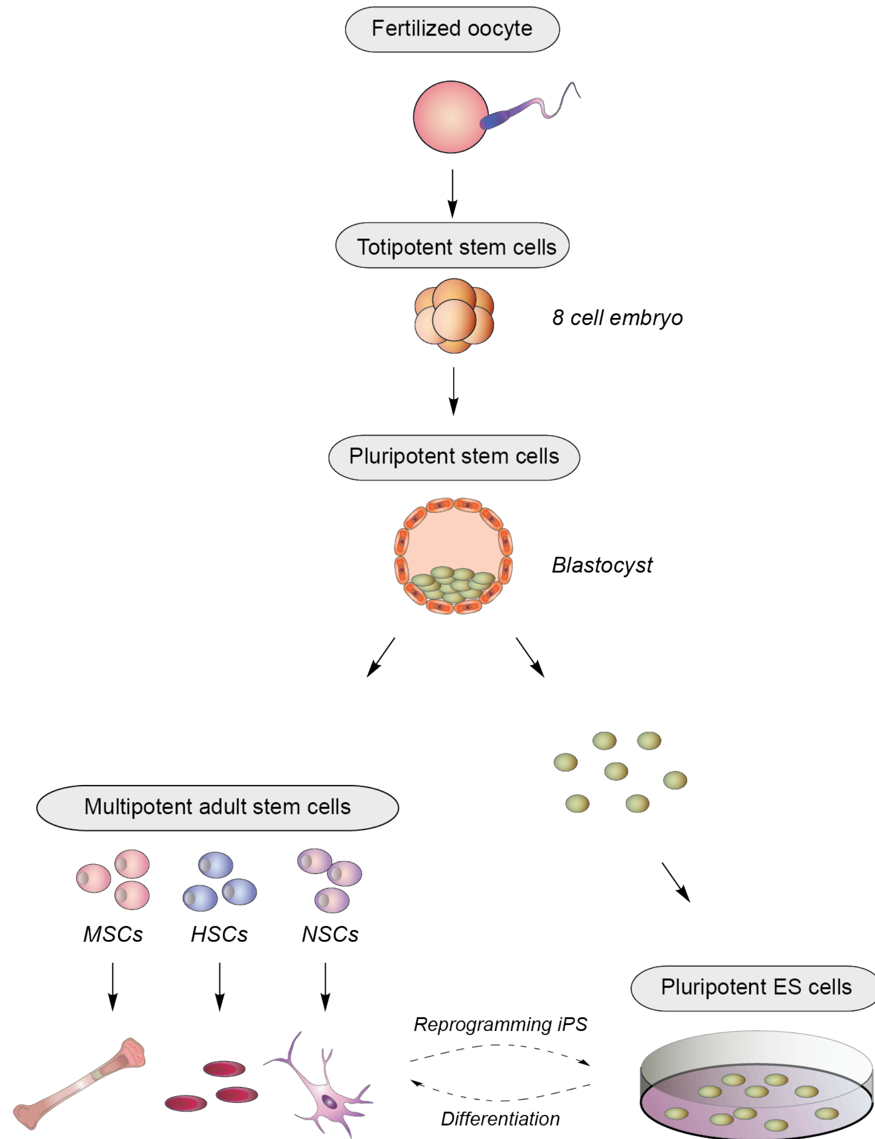

Figure 1. The stem cell potency ladder.

Multipotency - Adult stem cells

Further down the potency ladder are the stem cells that can give rise to several, but not all, cell types in an organism. The adult stem cell is sometimes also referred to as "*tissue stem cell*" or "*somatic stem cell*", from the greek word "***soma***" meaning "*of the body*". Adult stem cells are capable of self-renewal, same as stem cells of embryonic origin, but are more restricted in their capacity for producing specialized differentiated cells. They are usually limited to producing the cells of a particular tissue in the organism and are therefore referred to as multipotent from the Latin "***multus***" meaning "*many*" (Panchision, 2011).

Although adult stem cells all fulfill the basic criteria for stem cells they can differ a lot in terms of cellular behavior. Some are more or less regularly dividing to replenish cells in the tissue whilst others only divide under special conditions of cellular insult or tissue damage as a means of repair. For instance the epithelial stem cells located in the intestinal crypts and the epithelial stem cells in the skin are among the fastest dividing stem cells, continuously replacing cells in those tissues, since their barrier function necessitates a high cell turnover (Simons and Clevers, 2011; Tumbar, 2012). In contrast, stem cells in the liver and the pancreas are thought to only divide when an insult to the tissue triggers a repair response (Alison, 1998; Alison et al., 1998; Bonner-Weir and Sharma, 2002; Overturf et al., 1997).

Some dispute over the existence of true stem cells in different adult tissues still persists. For example the stem cell of the pancreas is hotly debated. Still, today stem cells or stem cell-like cells are thought to exist in the brain, bone, retina of the eye, hair follicle, muscles and several other tissues (Aubin, 1998; Coles et al., 2004; Cotsarelis et al., 1990; Dor et al., 2004; Gage, 2002; Goldring et al., 2002; Mauro, 1961; Pittenger et al., 1999; Reynolds and Weiss, 1992; Taupin and Gage, 2002; Tropepe et al., 2000; van Praag et al., 2002; Xu et al., 2008).

The disagreement on what cells are actually stem cells in adults is mainly due to limitations in the assays used to make sure the proposed cell meet the criteria for self-renewal and differentiation. In addition, the difficulty with assessing stem cell properties has been further complicated with the discovery that a certain degree of plasticity exists in some stem cells *in vivo*. Cells, previously described as differentiating into progressively more and more committed cells of a specific lineage have been revealed to, under special circumstances, cross tissue boundaries and differentiate to other lineages, so-called "*trans-differentiation*" (Gruh and Martin, 2009; Poulsom et al., 2002; Rovo and Gratwohl, 2008).

The controversy of potency and plasticity of stem cells was further augmented in 2006, when the Japanese scientist Yamanaka published his seminal paper on dedifferentiation of fibroblasts using a set of transcription factors to reprogram the cells into a cellular state that resembles that of the embryonic stem cell (Takahashi and Yamanaka, 2006). These cells, termed induced pluripotent stem (**iPS**) cells, have now been shown to have chimeric germline potential and today represent a massively expanding research field. iPS cells have been generated from differentiated cells from a wide spectrum of tissues and from several different species including humans(Loh et al., 2009; Lowry et al., 2008; Nakagawa et al., 2008; Okita et al., 2007; Ruiz et al., 2010; Tiscornia et al., 2011).

With regard to research and clinical applications, adult stem cells and iPS cells are a lot less controversial than their embryonic counterpart. Since adult stem cells and iPS cells in many cases can be collected from the adult source, without harm, less ethical objections are raised. However, this is not the case for all adult stem cell types; the neuronal stem cell for instance is not easily harvested without the use of invasive procedures (Haas et al., 2005; Liao et al., 2011; Prelle et al., 2002).

Finally, on the ladder of stem cell potency, there are also the oligo- and unipotent stem cells capable of giving rise to just a few or only one type of cells. The male germ stem cells giving rise to sperm is an example of a unipotent stem cell. The use of the word stem cell can vary quite a bit and

sometimes what would be described as a progenitor in one tissue is defined as a stem cell in another. In conclusion, although the theoretical definition of a stem cell has remained intact, the functional definition is more mottled today.

Cues governing stem cells – the niche

Stem cells generally represent only a very small portion of the cells of the tissue in which they reside (Morrison and Weissman, 1994; Tegelenbosch and de Rooij, 1993). This is linked to the need to protect these cells from genetic damage and also makes it easier to safeguard them from physical insult within the tissue. However, this low frequency is compensated by the enormous proliferation potential exhibited by the stem cells themselves and their direct progeny. In an obvious paradox, this ability is also what makes stem cells a potential danger. If stem cells were allowed to proliferate in an unrestricted manner they would cause disorganization of tissues and organs, reminiscent of the neoplastic invasive growth of tumors. It is therefore reasonable to assume that there exists an external, as well as an internal, way of regulating stem cell numbers and differentiation (Li and Xie, 2005; Lin, 2002).

The stem cell niche is a term that loosely signifies a microenvironment that stem cells reside in and that influences the stem cell fate choice. The theory of the stem cells niche has been around for decades but the first niche that was actually experimentally demonstrated was the one found in the *Drosophila* gonads. Stem cells in the gonads interact with the cap cells in the ovary and the hub cells in the testis. In fact these niche cells directly regulate stem cell numbers and behavior (Kiger et al., 2000; Spradling et al., 2001; Tran et al., 2000; Xie and Spradling, 2000).

Although the gonad niches are probably the most investigated, similar concepts are defined for a majority of stem cells, including those of the skin, intestine and the brain. Because the niche is usually located in a site that is relatively sheltered in those tissues, such as the bulge of the hair

follicle in the skin, it is also protected from external influences and tissue insults (Fuchs et al., 2001; Shen et al., 2004; Takeda et al., 2011).

Stem cell fitness and competition

The Darwinian concept of fitness and evolutionary competition has also been suggested to apply to cells in a multicellular organism. With the discovery of secreted growth factors it was hypothesized that cells compete for limiting space and nutrients in the niche (Purves, 1980; Raff, 1992; Ramón y Cajal, 1929).

In the *Drosophila* ovary, the cell fate of the stem cell daughter cells is determined by the relative position to the niche cells. If the progeny cell is adjacent to the cap cell it retains stem cell properties. But if the daughter cell has no direct contact with the niche cell, it instead becomes a differentiated cytoblast and moves away from the cap cells of the niche. Under normal circumstances this is however a passive, non-competitive process where intrinsic differences in cell fitness are never of consequence (Li and Xie, 2005). Another example of passive cell fate regulation is the self-sacrifice of cells in the *Caenorhabditis elegans* where cell autonomous programmed cell death occurs during development (Yuan and Horvitz, 1990).

Active competition for survival however, requires a measure of the fitness of a cell and a way of sensing the fitness of adjacent cells. In *Drosophila* mosaics it was shown that when cells of two different metabolic rates were mixed, cells with the reduced metabolic rate disappeared completely in the presence of cells with a normal metabolism. This was surprising since the cells with a reduced metabolic rate are viable on their own. These flies are the so-called *Minutes* mutants, with changes in different ribosomal genes leading to altered rate of protein synthesis. Homozygous *Drosophila Minutes* are lethal but heterozygotes are viable and normal, although with a longer developmental period. However, in wing mosaics with both wildtype and heterozygous cells, wildtype cells detect the decreased fitness of

Minute cells and actively kill the mutant cells by sending a signal to trigger apoptosis (Morata and Ripoll, 1975; Moreno et al., 2002; Simpson and Morata, 1981). A similar process with actively competing cells is believed to exist in mammalian development (Oliver et al., 2004).

Not only cells with reduced fitness can induce competition. Genetic mutations exist that can transform cells into super-competitors. When they are mixed with wildtype cells, super-competitors outcompete the normal cells. The *Myc* and *Hippo* family of genes can acquire mutations that can transform cells into super-competitors. Recently, the presence of active cell-cell competition between stem cells in their niche has been demonstrated in the *Drosophila* ovary. Intriguingly, the process of stem cell competition in the niche seems to trigger differentiation of the loosing cell rather than apoptosis (de la Cova et al., 2004; Jin et al., 2008; Johnston et al., 1999; Moreno and Basler, 2004; Rhiner et al., 2009; Tyler et al., 2007; Zhao and Xi, 2010). It is appealing to consider a possible relevance for active cell competition in adult mammalian stem cell niches, such as the one in the hematopoietic system (Domen, 2001; Gaudin et al., 2004).

HEMATOPOIETIC STEM CELLS

While a lot attention these days is given to the therapeutic potential of ES and iPS cells, the stem cell of the blood system, the hematopoietic stem cell (**HSC**), has already been used in treatment of disease for several decades. It is also by far the most explored and well defined of the adult stem cells.

Hematopoiesis

The process wherein the cells of the blood and immune system are continuously replenished is termed hematopoiesis. The word derives from the Greek word "***haima***" meaning "*blood*" and "**poieō**" meaning "*to make*". Under homeostatic conditions 10^{11}-10^{12} new cells are formed each day in an adult human and under situations of stress the output is even more numerous (Ogawa, 1993).

The function of the blood and immune system

Hematopoiesis generates several distinct cell types that are responsible for the function of the blood and immune system. The most abundant cell is the *red cell*, or erythrocyte, which is responsible for transporting oxygen from the lungs to all tissues in the body. Erythrocyte production responds to low levels of oxygen, e.g. due to loss of blood, chronic anemia or other situations of stress (Hattangadi et al., 2011). *Platelets*, or thrombocytes, are produced from megakaryocytes and respond to hemorrhage or inflammation by helping with clotting. All other cells produced in hematopoiesis are said to be *white blood cells* and are part of the immune system (Widmaier et al., 2004).

The immune system is generally divided into one innate and one acquired portion. Innate immunity, in the broadest sense, consists of all the elements that we are born with and that can be used for fighting against challenges

from foreign invaders. This includes the skin and mucous membranes as well as for example the cough reflex. The hematopoietic contribution to innate immunity is phagocytic cells including the *granulocytes*, *macrophages*, and *dendritic* cells. Phagocytic cells recognize foreign particles, such as bacteria, and ingest and digest them. *Natural-killer cells* instead use cell-cell contact to distinguish non-self cells and kill them by releasing various cytotoxic molecules (Aderem, 2003; Allen and Aderem, 1996).

Acquired immunity is more specialized than innate immunity and as such acts as a second line of defense. From an evolutionary perspective it is a rather late development, present only in vertebrates. *Lymphocytes* are the essential cells in this specific immune defense. The adaptive response of the *B* and *T-lymphocytes* is slower than that of the innate response and it can take up to a week for the clonal expansion necessary to occur, before *effector cells* can start eliminating an infection. On the other hand the *memory cells* can, after that first contact with a foreign substance, persist for the whole lifetime and prevent reinfection (Coico et al., 2003).

Discovery of the hematopoietic stem cell

Most people are familiar with the culmination of the World War II on the eastern front in Asia – the nuclear bomb. After the ending of the war the threat of a nuclear war spurred interest in radiation protection research and it was noticed early on that the bone marrow was particularly sensitive to irradiation. Early attempts in the 50's to cure victims of radiation injury with bone marrow transplantation failed because the cells were recognized as foreign and rejected. When researchers learned to tackle the problem of the allogeneic nature of the transplants faster progress was made and in 1968 the first successful bone marrow transplant was performed (Congdon, 1962; Ford et al., 1956; Gatti et al., 1968; Main and Prehn, 1955).

This strong relevance of radiation research also fueled an interest in the understanding of blood formation and progressed the idea that one cell was

the origin of all blood cells, the blood stem cell. In their seminal papers James Till and Ernest McCulloch first demonstrated in mice the multi-lineage potential of injected bone marrow and then later the self-renewal capacity of secondary transplants. In the first paper published in 1961 (Till and Mc, 1961) they showed that the spleens of irradiated mice transplanted with bone marrow contained colonies consisting of cells from multiple blood lineages (colony forming units-spleen, (**CFU-S**). In the research published two years later (Becker et al., 1963; Siminovitch et al., 1963) they demonstrated that upon re-injection, such colony forming spleen cells resulted in new spleen colonies and each colony was formed from one cell in a clonal manner. So, somewhat ironically, one of the greatest fears of the 20th century was also the fuel for a field of research that may in the future solve some of the toughest medical challenges of cancer, tissue repair and aging.

The hematopoietic hierarchy

The cell that Till and McCulloch discovered was of course not what we today consider a proper HSC but rather something approaching a multipotent progenitor cell (Baines et al., 1982; Jones et al., 1990; Magli et al., 1982). The true HSC sits at the top of the hematopoietic hierarchy and has the potential to sustain hematopoiesis throughout a lifetime. Functionally this is defined by the ability to reconstitute an ablated hematopoietic system in several sequential transplantations (Harrison et al., 1978). Using retroviral integration analysis to trace the clonality of transplanted bone marrow cells, later work has delivered more robust support for HSC self-renewal and single-cell transplants generating long-term multi-lineage reconstitution provided the definitive evidence (Dick et al., 1985; Jordan and Lemischka, 1990; Keller and Snodgrass, 1990; Osawa et al., 1996).

Between the HSC and the mature blood cells are a series of more restricted progenitors. Early on there is a division between a common myeloid progenitor (**CMP**) and a common lymphoid progenitor (**CLP**) and later

also a separation of the CMP into a bipotent megakaryocyte erythrocyte progenitior (**MEP**) and a granulocyte macrophage progenitor (**GMP**) (Orkin, 2000).

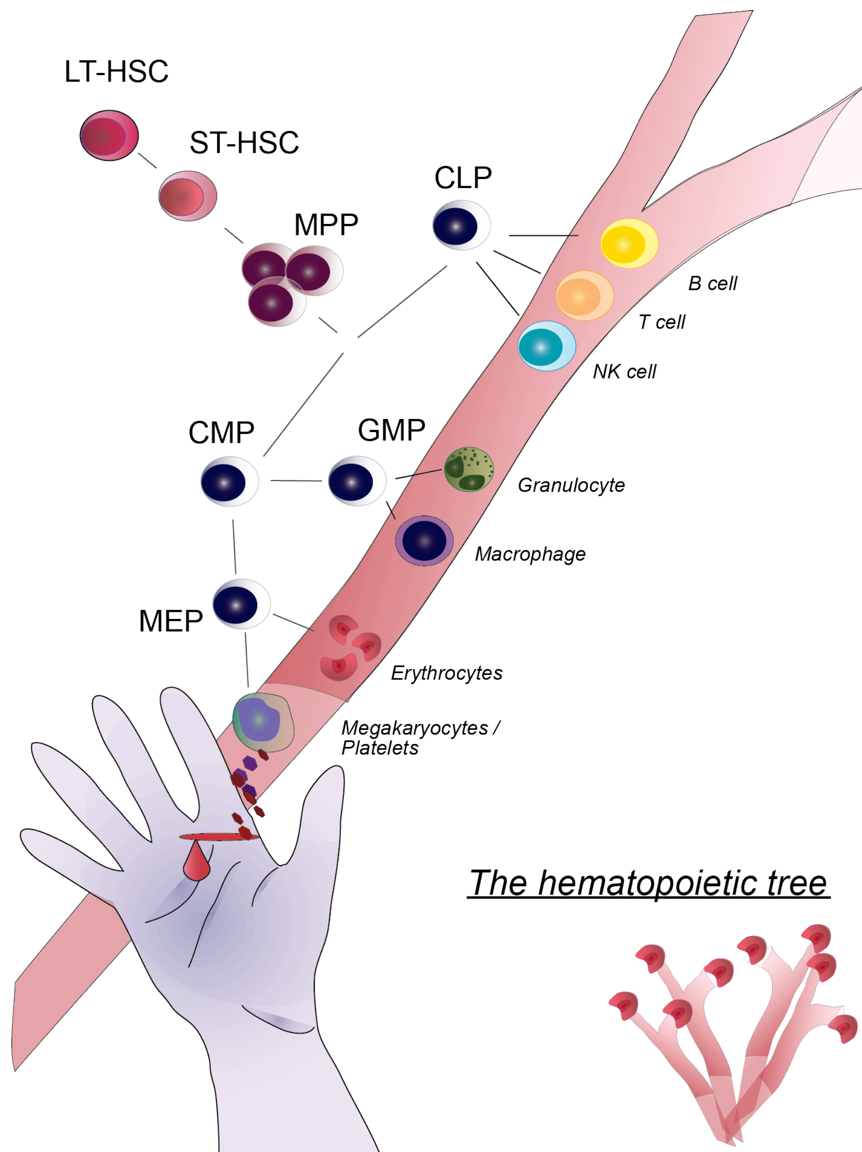

Figure 2. The hematopoietic hierarchy and function of the blood system.

Hematopoiesis during development

In mammalian development, hematopoiesis begins in the blood islands of the yolk sac. In the mouse this transpires at embryonic day 7.5 (**E7.5**). Extra-embryonic mesoderm crosses the posterior primitive streak and then establishes the islands (Cumano and Godin, 2007; Silver and Palis, 1997). Moore and Metcalf demonstrated more than 40 years ago the existence of hematopoietic myeloid progenitor cells, capable of forming clonal hematopoietic colonies *in vitro* and also some cells resembling adult HSCs and cells capable of forming CFU-S(Moore and Metcalf, 1970). Later, more stringent studies have challenged the existence of true HSC and CFU-S in the yolk sac (Medvinsky and Dzierzak, 1996; Medvinsky et al., 1993; Muller et al., 1994). Furthermore, because of the nucleated nature the of erythroblasts produced in the yolk sac, we now refer to this immature form of hematopoiesis as *primitive hematopoiesis* to distinguish it from the *definitive hematopoiesis* that can generate a fully formed blood system (Palis et al., 1999). Instead, *definitive hematopoiesis* is first detected in the embryo proper at E9.5-10.5, in the aorta-gonad-mesonephros (**AGM**) region, and includes the presence of both lymphoid progenitors and definitive HCS (**dHSC**) activity (Ivanovs et al., 2011; Medvinsky and Dzierzak, 1996; Medvinsky et al., 1996; Medvinsky et al., 1993). This is in agreement with our knowledge of hematopoietic development in other species, such as the well-studied chick embryo (Dieterlen-Lievre, 1975). However, alternative descriptions of the origin of the dHSC, including a placental, umbilical cord or later stage (E10.5-11) yolk sac model, continue to be debated and are reviewed by Medvinsky et al (Medvinsky et al., 2011).

HSCs from the AGM region enter circulation and proceed to seed the liver of the embryo where a massive expansion of the HSC pool ensues between E12.5-E15.5 (Ema and Nakauchi, 2000; Morrison et al., 1995). The fetal liver (**FL**) is considered the major hematopoietic organ during development. Following the expansion in the FL, starting at E16.5 HSCs then proceed to seed the bone marrow where they then reside until

parturition and into adulthood. Lastly, three to four weeks after birth HSCs go through a switch where the rapid cycling behavior, begun in the FL, culminate, and they become primarily quiescent cells (Bowie et al., 2006). This is also how the HSCs will remain in the niche for most of their remaining life.

The hematopoietic stem cell niche

When Schofield in 1968 proposed the existence of a specific microenvironment, a niche, which the hematopoietic stem cell resides in it was a pioneering idea (Schofield, 1978). Although studies in invertebrates would be first to experimentally prove the existence of a stem cell niche, it was studies of the hematopoietic system that generated the idea. Even before Schofield proposed his theory Lord and his colleagues showed that hematopoietic progenitor cells were located closer to the endosteal surface of the bone marrow than were differentiated cells (Lord and Hendry, 1972; Lord et al., 1975). During the years since, a series of studies have defined many of the components that constitute a niche that influence HSC behavior.

Osteoblasts have the potential to produce many of the cytokines that are necessary for myeloid progenitor expansion and were an early contender for a niche constituent (Taichman and Emerson, 1996; Taichman et al., 1996). Several studies have provided strong evidence in support of that idea. Dye-labeling experiments have followed the homing of injected hematopoietic stem and progenitor (**HSPC**) cells to their endosteal location were they tend to accumulate (Nilsson et al., 2001). In the beginning of the millennia, it was demonstrated in two different mouse models that either changing the volume of bone, and consequently the number and total surface of available osteoblasts, or increasing the number of osteoblasts by their over-activation, both lead to a corresponding increase in HSC number (Calvi et al., 2003; Zhang et al., 2003). More specifically, it seems to be the osteoprogentior rather than the mature osteoblast that is important for normal HCS function (Raaijmakers et al., 2010). The publication of these

two mouse models is considered the first definitive proof of the existence of a HCS niche, 25 years after it was first proposed (Calvi et al., 2003; Zhang et al., 2003).

Another proposed location for the niche is the perivascular area in the bone marrow cavity. The endothelial cells surrounding the sinusoids have been shown to be indispensable for engraftment in irradiated recipients (Hooper et al., 2009). Furthermore, over-activated endothelial cells result in increased number of HSCs, similar to over-activated osteoblasts do (Butler et al., 2010; Kobayashi et al., 2010). Finally, recent work with cytokines and chemokines necessary for HSC maintenance indicate that vascular niche cells secrete these factors and that HSCs reside in close proximity to a vascular niche (Ding et al., 2012; Sugiyama et al., 2006). However, there is also conflicting evidence indicating that the vascular areas have an active role in differentiation and that HSPC are located on a gradient away from the vessels (Avecilla et al., 2004; Winkler et al., 2010). Live imaging has tried to merge the opposing theories by indicating that the niches might not be physically separated but rather two parts of one niche (Lo Celso et al., 2009; Xie et al., 2009). Other important components of the niche, or niches, include osteoclasts, mesenchymal cells, adipocytes, and monocytes (Park et al., 2012; Purton and Scadden, 2008).

Finally it has been postulated that the HSC niche is hypoxic in its nature and the area close to the endosteum is indeed low in oxygen (Dello Sbarba et al., 1987; Harrison et al., 2002; Levesque et al., 2007). There is also strong evidence that HSC reside in a low oxygen environment; in vitro culture of bone marrow cells in hypoxia result in increased reconstitution (Cipolleschi et al., 1993) and HCS enriched cell fractions are positive for hypoxia markers (Parmar et al., 2007) and contain lower levels of reactive oxygen species (**ROS**) (Jang and Sharkis, 2007). Additionally, there exist evidence that instead implies that LT-HSCs, with a low oxygen content, are located close to the sinusoids (Kubota et al., 2008).

Regulation of hematopoietic stem cells

One of the mouse models of the osteoblastic HSC niche described previously also, identified the Notch signaling pathway as a major regulator of stem cell maintenance (Calvi et al., 2003). Other ways the osteoblast has been suggested to influence the HSC includes, cell-cell interactions via N-cadherins (Nakamura et al., 2010); secretion of the chemotactic agent CXCL12 (Schajnovitz et al., 2011); Thrombopoietin (**TPO**) expression (Qian et al., 2007); Angiopoietin-1 secretion (Arai et al., 2004; Hirao et al., 2004) and through production of the extra-cellular matrix protein osteopontin (OPN) (Nilsson et al., 2005).

HSCs express the tyrosine kinase receptor **c-kit** on their cell surface and its ligand, stem cell factor (**SCF**), is thought to be present in the extra-cellular matrix of the endosteal microenvironment and is secreted from vascular niche cells. The presence of SCF is necessary for the survival and retention of HSCs in the bone marrow (Ding et al., 2012; Driessen et al., 2003; Heissig et al., 2002). SCF is also vital for HSC survival and function in vitro and is one of a few that have the ability to support HCS maintenance in cultures (Keller et al., 1995). There exist a plethora of naturally occurring kit deficient mouse models with varying phenotypes (Miller et al., 1996; Nocka et al., 1990). Depending upon the severity of the mutations hematopoiesis is more or less effected. If partial receptor activity remains the HSC compartment seems to be functional unless rigorously challenged (Thoren et al., 2008). The W^{41}/W^{41} mouse strain has a point mutation in the c-kit receptor, which negatively affects the numbers and function of HSCs (Thoren et al., 2008).

Other important extrinsic regulators of HSCs include the transforming growth factor β (**TGFβ**)(Karlsson et al., 2007; Langer et al., 2004) and fibroblast growth factor (**FGF**)(de Haan et al., 2003) signaling pathways.

Signals imposed on HCS by the microenvironment will naturally impact on intrinsic signaling pathways, determining cell fate options such as cell cycling, differentiation, apoptosis, cytoskeleton organization and migration.

There are many such intrinsic factors shown to be indispensable for HCS activity including but not limited to cell cycle regulators such as p21 (Cheng et al., 2000), Gf1 (van der Meer et al., 2010) and Pbx1 (Shimabe et al., 2009) and transcription factors such as Bmi-1 (Park et al., 2003) and Prdm16 (Aguilo et al., 2011; Chuikov et al., 2010).

Functional assays for HSCs

The CFU-S assay described by Till and McCulloch was the first in vivo assay used in the study of HSCs (Till and Mc, 1961). The CFU-S is based on the ability of injected cells to form visible colonies in the spleen 7 to 12 days after transplantation. The number of colonies is easily determined and correlates to the number of progenitors injected (Jones et al., 1990). Many more assays have been developed since then and the gold standard today is long-term serial bone marrow transplantations. Recipients are conditioned, usually by irradiation, to remove endogenous cells and then the hematopoietic to be evaluated are injected intra-venously. HSPCs have the ability to home to the bone marrow and engraft the recipient. Progenitor and stem cells are then read out in CFU-S assays or by their contribution to hematopoiesis in peripheral blood and bone marrow at different time points. LT-HSC are generally considered to be present if multi-lineage contribution is detected in the bone marrow after 12 weeks or more and can be further challenged by harvesting bone marrow from the first recipient and injecting into a second host. To compare the quality of two different cell populations competitive transplantations are performed. Two cell populations are competed against each other to discern possible differences in reconstitution capacity. To complement the in vivo assays, and dissect progenitor function, colony formation in vitro is usually studied. Cells are seeded in a semi-solid medium supplemented with growth factors supporting differentiation to distinctive lineages. Each progenitor with the proper lineage potential will form a colony and the number of colonies can be counted. The size and cell composition of the colonies give further

information on proliferation capacity, and possible multi-lineage potential of the originating cell (Purton and Scadden, 2007).

THE HIPPO PATHWAY

Discovery of the Hippo pathway

In the middle of the 90's a set of mosaic studies in *Drosophila* unveiled a tumor suppressor gene that resulted in an irregular, warts-like, surface phenotype with clonal cell outgrowths. Loss of only one allele led to massive over proliferation of the fly's imaginal discs. The gene was aptly named *warts* (***wts***) (Justice et al., 1995; Xu et al., 1995). Later a similar phenotype was observed in other mosaics when the genes *salvador* (***sav***) and *hippo* (***hpo***) were mutated and the new pathway was pieced together and titled the Hippo pathway (Harvey et al., 2003; Jia et al., 2003; Kango-Singh et al., 2002; Tapon et al., 2002; Udan et al., 2003; Wu et al., 2003).

During the course of the *Drosophila* mosaics studies, it was established that the pathway suppresses overgrowth, at least in part, by the transcriptional regulation of *cyclin E* and *diap1* and it seemed natural to assume the existence of a downstream transcriptional regulator (Lai et al., 2005). Using Wts as bait in a yeast hybrid screen the Hippo pathway effector Yorkie (**Yki**) was discovered. Yorkie was shown to be negatively regulated by Wts. Wts is in turn activated by Hpo and Sav, linking the pathway together in a cascade that ultimately inactivates Yki (Huang et al., 2005) (Figure 3).

A large body of work in the past years have extended the pathway and uncovered several elements that may be acting upstream of Hpo.

The Hippo signaling cascade

Directly upstream of Hpo two cytoskeletal-binding proteins, Merlin (**Mer**) and Expanded (**Ex**) and their associated protein Kibra (**Kbr**), activates the Hippo pathway (Baumgartner et al., 2010; Genevet et al., 2010; Hamaratoglu et al., 2006; Yu et al., 2010). Further upstream the protocadherin Fat in conjunction with Dachsous (**Ds**), Discs overgrown

(**Dco**) Four-jointed (**Fj**) and Lowfat (**Lft**) act at the cell surface to regulate the pathway in an as yet unknown way (Bennett and Harvey, 2006; Cho et al., 2006; Mao et al., 2009; Silva et al., 2006; Simon et al., 2010; Sopko et al., 2009; Tyler and Baker, 2007; Willecke et al., 2006). The activity of Fat is also influenced by gradients of the morphogens Wingless (**Wg**) and Decapentaplegic (**Dpp**) (Rogulja et al., 2008).

Much less is known about the constituents and biochemical interactions of the mammalian Hippo pathway. There is however great evolutionary conservation of the signaling cascade and homologs of the core proteins and their interactions are known (Callus et al., 2006; Chan et al., 2005; Graves et al., 1998; Hao et al., 2008; Oka et al., 2008). Expression of the human Yes associated protein (**YAP**, a Yki homolog), large tumor suppressor (**LATS1**, a Wts homolog) and mammalian sterile twenty kinase 2 (**MST2**, a Hpo homolog) have all been shown to rescue their corresponding *Drosophila* mutants (Huang et al., 2005; Lai et al., 2005; Wu et al., 2003).

In mammals, Yap is subjected to the same inhibitory signaling cascade as in *Drosophila* and its phosphorylation by Lats results in increased retention of Yap in the cytoplasm and consequently less transcriptional regulatory activity in the nucleus (Zhao et al., 2007). As a transcriptional co-activator Yap has been coupled to numerous transcription factors in different tissues. However, complex formation between the TEA domain (**TEAD**) family of transcription factors and Yap is the only one preserved from the fly to humans, and is responsible for mediating a major part of the Hippo induced proliferation (Vassilev et al., 2001; Wu et al., 2008; Zhao et al., 2009; Zhao et al., 2008).

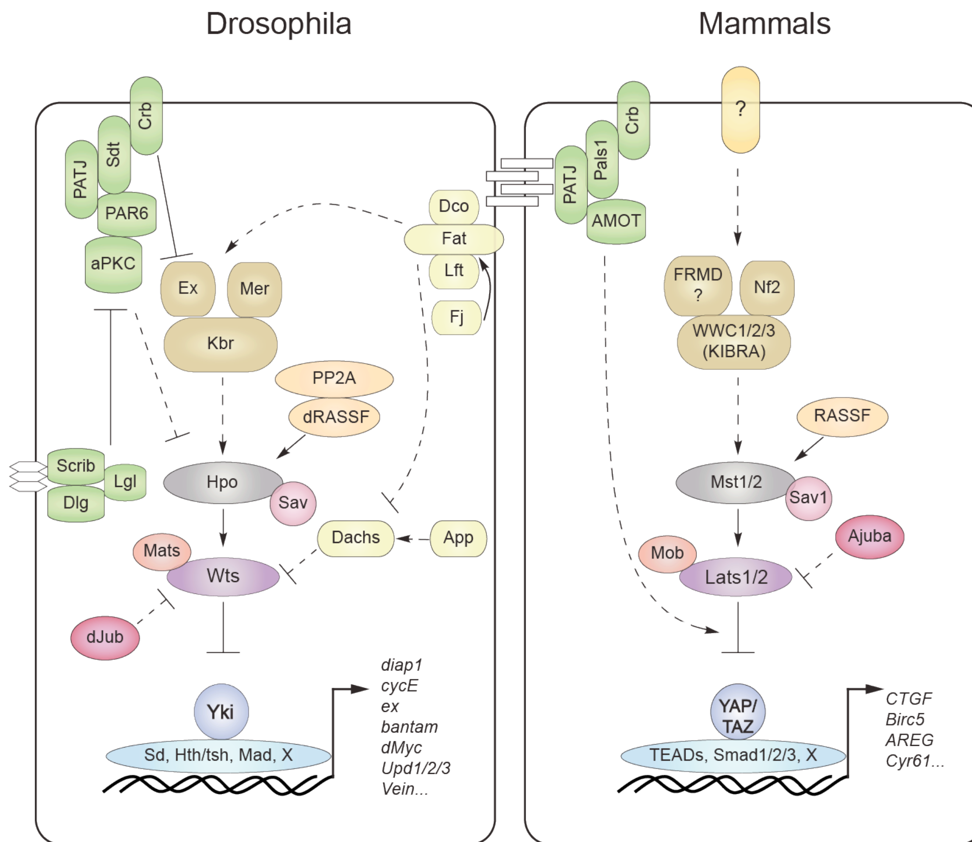

Figure 3. The Hippo signaling pathway in *Drosophila* and mammals. Boxes with the same color represent homologous proteins in *Drosophila* (left panel) and mammals (right panel). Several proteins involved in cell polarity, reviewed in (Grusche et al., 2010), such as dJub, Scrib, Dlg, Lgl, aPKC and Crb have all been implicated in upstream regulation of the *Drosophila* Hippo pathway. Adapted from Zhao et al (Zhao et al., 2011).

Hippo signaling redundancy - Wwtr1

In the mammalian Hippo pathway there exists a certain redundancy. Hpo and Wts both have two homologs, Mst 1/2 and Lats1/2 respectively. More important however, may be the existence of a Yap paralog, WW domain containing transcription regulator 1 (**Wwtr1**) also known as transcriptional co-activator with PDZ-binding motif (**Taz**) (Hong and Yaffe, 2006; Oh and Irvine, 2010; Wang et al., 2009). Taz and Yap have very similar structure, with 46% amino acid sequence identity, and they interact with many of the same proteins in the pathway. Both effectors, for example, can regulate transcription by binding to TEAD transcription factors (Li et al., 2010; Mahoney et al., 2005; Zhang et al., 2009a). However, there are also functional differences. Yap, but not Taz, can initiate pro-apoptotic transcriptional programs via interaction with p73. Taz on the other hand also has specific, distinct, binding partners, such as Pax3, which is involved in the embryo limb formation (Murakami et al., 2006). Additionally Taz has been shown to regulate osteoblastic differentiation of mesenchymal stem cells by binding Runx2, while simultaneously binding Pparγ to inhibit adipocyte differentiation (Hong et al., 2005). Thus, though mainly described as promoting proliferation, Yap and Taz can also promote cell cycle exit, cell death and differentiation. Moreover, they act in a highly cell and tissue dependent manner (Downward and Basu, 2008; Strano et al., 2005; Strano et al., 2001; Zhang et al., 2009b).

The role of Hippo signaling in disease and cancer

Most of the mammalian gene homologs in the Hippo pathway had been identified and studied before they were recognized as components of the pathway. The homolog of Merlin, **NF2**, has long been known as the gene that is responsible for neurofibromatosis 2, a genetically inherited disease that results in tumor-growth of nerve tissue (Rouleau et al., 1993; Trofatter et al., 1993). Mutations of NF2 are also found in some meningiomas and mesotheliomas (Hansson et al., 2007; Sekido, 2010).

LATS1 and LATS2 have been implicated in many different cancer forms. The promoters of both genes have been found to be hypermethylated in astrocytoma and breast cancer (Jiang et al., 2006; Takahashi et al., 2005). Low levels of LATS2 can even be an indication of a good prognosis in responding to treatment (Takahashi et al., 2007). Hypermethylated MST1 and MST2 promoters have similarly been found in soft tissue sarcomas (Seidel et al., 2007).

YAP levels are elevated in gastric adenocarcinoma (Lam-Himlin et al., 2006), medulloblastomas (Fernandez et al., 2009), as well as in cell lines from liver, colonic and lung cancers (Steinhardt et al., 2008). Additionally, YAP is located on chromosome 11q22, which is frequently amplified in various cancers (Overholtzer et al., 2006). In general, downregulation or reduced expression of upstreams components of the Hippo pathway, and conversely increased levels or increased nuclear localization of the downstream effector YAP, both correlate with a poor prognosis (Minoo et al., 2007; Xu et al., 2009). A summary of disease phenotypes in mouse models and patients for the different Hippo components can be found in Table 1.

Table 1. Summary of data on mouse models and disease pathology for different Hippo pathway components.

Hippo component	Mouse model phenotype	Human pathology	References
Fat4	Polycystic kidney disease	Potential pulmonary adenomas, breast cancer	(Saburi et al., 2008);(Berndt et al., 2011; Qi et al., 2009)
Sav1 (ww45)	Growth retardation, perinatal lethality	Renal cancer cell lines	(Lee et al., 2008);(Tapon et al., 2002)
Mst1	Viable, sarcomas, increased radiation and ROS sensitivity in T cells	Soft tissue sarcomas	(Choi et al., 2009; Zhou et al., 2009);(Seidel et al., 2007)
Mst2	Viable, mammary tumors	Soft tissue sarcomas	(Oh et al., 2009; Zhou et al., 2009);(Seidel et al., 2007)
Mst1/2	Embryonic lethal E 8.5. Growth retardation, impaired hematopoiesis		(Oh et al., 2009);
Lats1	Soft tissue sarcomas, ovarian tumors; predisposition T cell lymphomas	Astrocytoma, Breast cancer	(Cornils et al., 2010; St John et al., 1999);(Jiang et al., 2006; Takahashi et al., 2005)
Lats2	Embryonic lethal E12.5	13q12 deletion, Astrocytoma, breast cancer	(McPherson et al., 2004; Yabuta et al., 2007);(Jiang et al., 2006; Takahashi et al., 2005)
Yap1	Embryonic lethal E9.5	Liver, colonic, lung, ovarian tumors, 11q22, medulloblastomas	(Morin-Kensicki et al., 2006);(Fernandez et al., 2009; Overholtzer et al., 2006; Steinhardt et al., 2008)
Taz	Partial embryonic lethality, polycystic kidney disease	Breast and lung cancer	(Hossain et al., 2007; Makita et al., 2008);(Chan et al., 2008; Zhao et al., 2012; Zhou et al., 2011)

Hippo signaling in stem cells

Yap1 and Taz are both involved in the maintenance of *stemness*, usually in opposite manners. Yap1 tends to inhibit, while Taz induces differentiation. Whereas Taz was shown to induce differentiation in mesenchymal stem cells (Hong et al., 2005; Hong and Yaffe, 2006) Yap has been shown to instead impair differentiation in the intestinal crypt (Camargo et al., 2007), the sub ventricular zone in the developing brain (Cao et al., 2008) and in mouse ES cells (Lian et al., 2010). However, this may be an oversimplification; in human ES cells, TAZ is necessary for nuclear localization of the TGFβ signaling components Smad2/3/4, which are needed for self-renewal (Varelas et al., 2008).

Overexpression of Yap, specifically in the liver, neural tube and intestine, results in massive proliferation and an increased self-renewal of stem and progenitor cells (Camargo et al., 2007; Cao et al., 2008; Dong et al., 2007). Furthermore, Yap is highly expressed in dermal progenitors and controls hair follicle morphogenesis by expanding progenitors and inhibiting terminal differentiation, all in a TEAD dependent manner (Zhang et al., 2011).

A Hippo in hematopoiesis?

Although the Hippo pathway is well studied in Drosophila and mammalian tissues such as the liver and intestine, very little is known about is function in the hematopoietic system. A few studies have suggested that the pathway may be implicated in regulation of hematopoiesis. The promoter of the potential upstream Hippo pathway regulator Ras association domain family member 6 (**RASSF6**) is frequently hypermethylated in childhood B cell acute lymphocytic leukemia (**ALL**) and in almost half of T cell ALL (Hesson et al., 2009). Furthermore, the Mst1 knockout mouse display defective T cell homing and increased apoptosis. Finally, using a Nf2 knockout mouse, Hippo signaling has been implicated in HSC regulation through the niche (Larsson et al., 2008).

GENETIC MODELING

With the advent of the sequencing of the human genome came a whole new set of challenges in biomedical research; to translate all that sequence data into knowledge of gene function (Olivier et al., 2001; Venter et al., 2001; Venter et al., 1998). Fortunately, a tool was already established in the laboratory mouse. Sir Martin Evans, Mario Cappechi and Oliver Smithies consecutively discovered the potential of ES cells, isolated them from the mouse blastocyst, learned how they could be propagated in culture and finally developed techniques for homologous genetic recombination.

Gain of function models

A basic constitutive transgenic strategy involves injecting a DNA construct into a fertilized oocyte, also termed pronuclear injection (Gordon et al., 1980). A promoter and enhancer element is used to direct the expression, sometimes to a specific tissue or developmental stage. In theory, once the sequence has randomly integrated in the genome it directly produces the desired transcript and does so forever. In practice however, complications such as positional effects, gene silencing and insertional mutagenesis often occur (Rijkers et al., 1994; Wilson et al., 1990).

Another commonly used gain of function strategy is to introduce a gene of interest via a virus vector. This can be done in oocytes or ES cells in order to create a transgenic mouse or directly into cultured cell lines. Hematopoietic cells can be isolated from the bone marrow, targeted with retrovirus, and then injected into irradiated recipients (Haviernik et al., 2008).

Loss of function models

Building on the Nobel Prize winning work of Evans, Capecchi and Smithies the first traditional knockout (**KO**) mouse was generated in 1985 (Doetschman et al., 1987; Thomas and Capecchi, 1987). The chief advantage of gene targeting approaches such as homologous recombination over standard transgenic methods is that the integration locus can be clearly defined. Given that homologous recombination is a relatively rare event in ES cells some sort of selection strategy, negative and/or positive, is usually employed. More complex KO mice can have inducible deletion of the gene in a specific tissue or during a certain developmental stage. Combinations of approaches including mice with multiple targeted genes can also be generated through breeding strategies.

The careful studies done with gene targeting have revealed the influence of genetic background on phenotype. Mice with the same mutation on different genetic backgrounds have displayed quite varying symptoms (Phillips et al., 1999; Sanford et al., 2001).

With the discovery of RNA interference (**RNAi**) a new way of reducing gene expression became available. Using sequence specific post-transcriptional targeting of messenger RNA with so called small interfering RNAs (**siRNAs**), gene expression is down regulated instead of completely abolished (Fire et al., 1998). This approach has been successfully used both with virus delivery and in targeted mouse models (Jaako et al., 2011; Shi, 2003).

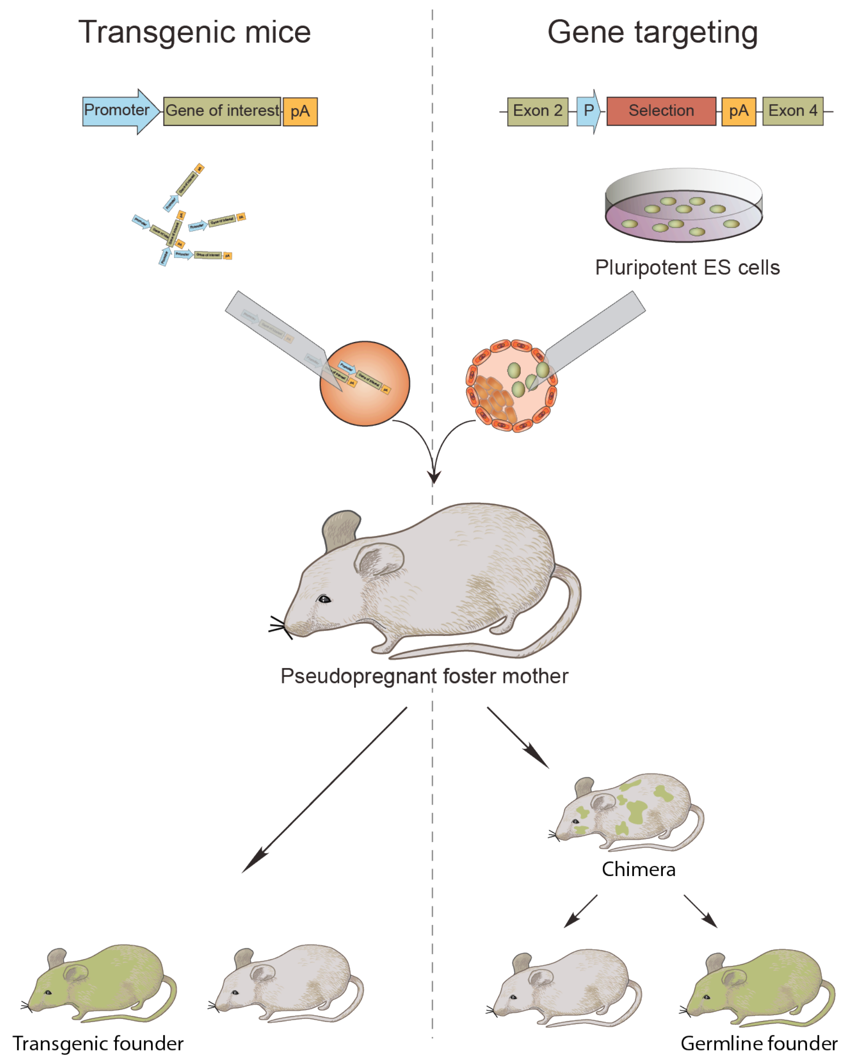

Figure 4. Basic transgenic versus gene targeting approaches for generating mouse models.

AIM OF THE THESIS

It is not known whether the Hippo signaling pathway plays a role in hematopoiesis and hematopoietic stem cells. Many studies have underlined the importance of the pathway in organ development, cell proliferation and stem cell renewal. My main focus for this thesis was to through genetic modeling investigate if and how Hippo signaling effects mouse HSCs.

1. In the first part of the work I focused on improving the genetic models available for studying overexpression in HSCs.
2. In the second part of the work I employed the model generated in (1) to study effects of overexpressing the Hippo effector YAP1 in mouse HSCs.
3. In the final part of the work I am complementing the YAP1 studies with an investigation of the other downstream effector Taz using a traditional knockout mouse.

SUMMARY OF THE RESULTS

ARTICLE I

W41/W41 blastocyst complementation: a system for genetic modeling of hematopoiesis

In this work we wanted to improve upon available methods to study genetic changes in HSCs. We decided to create a new system for overexpression in the hematopoietic system to complement the use of transgenic mice and viral vectors. We injected GFP-labeled ES cells into blastocysts from the c-kit-deficient W^{41}/W^{41} mouse strain to give the ES cells an advantage in founding the hematopoietic system. Investigating the fetal liver (FL) hematopoietic cells from W^{41}/W^{41} blastocyst complementation embryos we showed that we could generate embryos with hematopoietic cells with an almost complete ES cell origin. We further demonstrated that the FL cells can be transplanted to establish large cohorts of bone marrow chimeras with hematopoiesis of pure ES-cell origin. Furthermore, using ES cells with tet-inducible GFP expression, we could show that expression, both *in vitro* and *in vivo* is doxycycline dose-dependent with low background levels and that expression in HSPCs is feasible.

ARTICLE II

Normal hematopoietic stem cell function in mice with enforced expression of the Hippo signaling effector YAP1

To study the role of Hippo signaling in HSCs, we used or W^{41}/W^{41} system to create a transgenic model with inducible YAP1 expression exclusively within the hematopoietic system. After transplanting engineered FL cells

into irradiated recipients we waited 6 weeks and induced YAP expression. Mice were then analyzed 12 weeks post-induction. In our examination of blood and bone marrow no changes were detected in the lineage distribution compared to control mice. Furthermore, colony formation was normal and using flow cytometry we determined that the number of HSPCs was not affected. To address whether YAP1 affects the quantity and function of HSCs we also performed competitive transplantation experiments. No difference in the reconstitution ability of YAP1 expressing cells was detected after transplantations. Taken together this indicates that YAP overexpression does not affect HSC function during steady state or during regeneration.

ARTICLE III

The role of Taz in fetal and adult hematopoiesis

In the last project we are studying the effect of knocking out the other Hippo effector Taz on the hematopoietic system in mice. Taz KO mice are born well below the expected mendelian ratios and we have found no changes in steady state hematopoiesis in adult homozygous and heterozygous mice. At E14.5-15.5 the number of homozygous embryos are still below the expected mendelian ratio but are more frequent than post partum. Out of two homozygous embryos recovered, one had a reduced fetal liver cellularity while the other was normal. The first embryonic FL performed normal in colony assays but gave no short-term reconstitution when transplanted. The second embryo FL reconstituted irradiated mice comparable to wildtype cells. Although this is very preliminary data and more embryos have to be investigated, the divergent behavior of the homozygous FL cells correlates with the reduced penetrance observed and raises the question of why some homozygous embryos are viable while others succumb before birth. In summary, Taz seems dispensable for adult hematopoiesis but Taz deficiency leads to partial embryonic lethality; possibly by mechanisms that affect HSC function.

GENERAL DISCUSSION

GENETIC MODELING OF HEMATOPOIESIS

In **Article I** we generated a new model for studying genetic alterations in HSCs to complement already existing approaches. The benefit of using ES cells to generate FL cells for the production of bone marrow chimeras is twofold. First, it is considerably faster than generating a transgenic mouse model. Second, via available ES cell recombination techniques, it lets us control integration site, copy number and take advantage of an inducible system. Compared to using viral vectors this results in fewer confounding factors affecting the results and a more consistent level of expression. Pros and cons of the different models are summarized in Figure 5.

To achieve full ES cell chimerism in the fetal livers used for transplantations, we employed blastocysts from the W^{41}/W^{41} mouse. The idea is that hematopoietic cells from the W^{41}/W^{41} mouse are generally of normal viability until challenged, for example in a competitive transplantation assay. In such a situation they are outcompeted by wildtype cells (Thoren et al., 2008). This prompted us to assume that W^{41}/W^{41} blastocyst would not only be viable but will contribute to both embryonic and extra-embryonic tissues when ES cells are injected, but not be able to partake in establishing HSCs during development. Indeed other parts of the E14.5 embryo, excluding the fetal liver, displayed on average about 50% ES to W^{41}/W^{41} chimerism (data not shown). However, when it came to founding the hematopoietic system, W^{41}/W^{41} cells were outcompeted by the

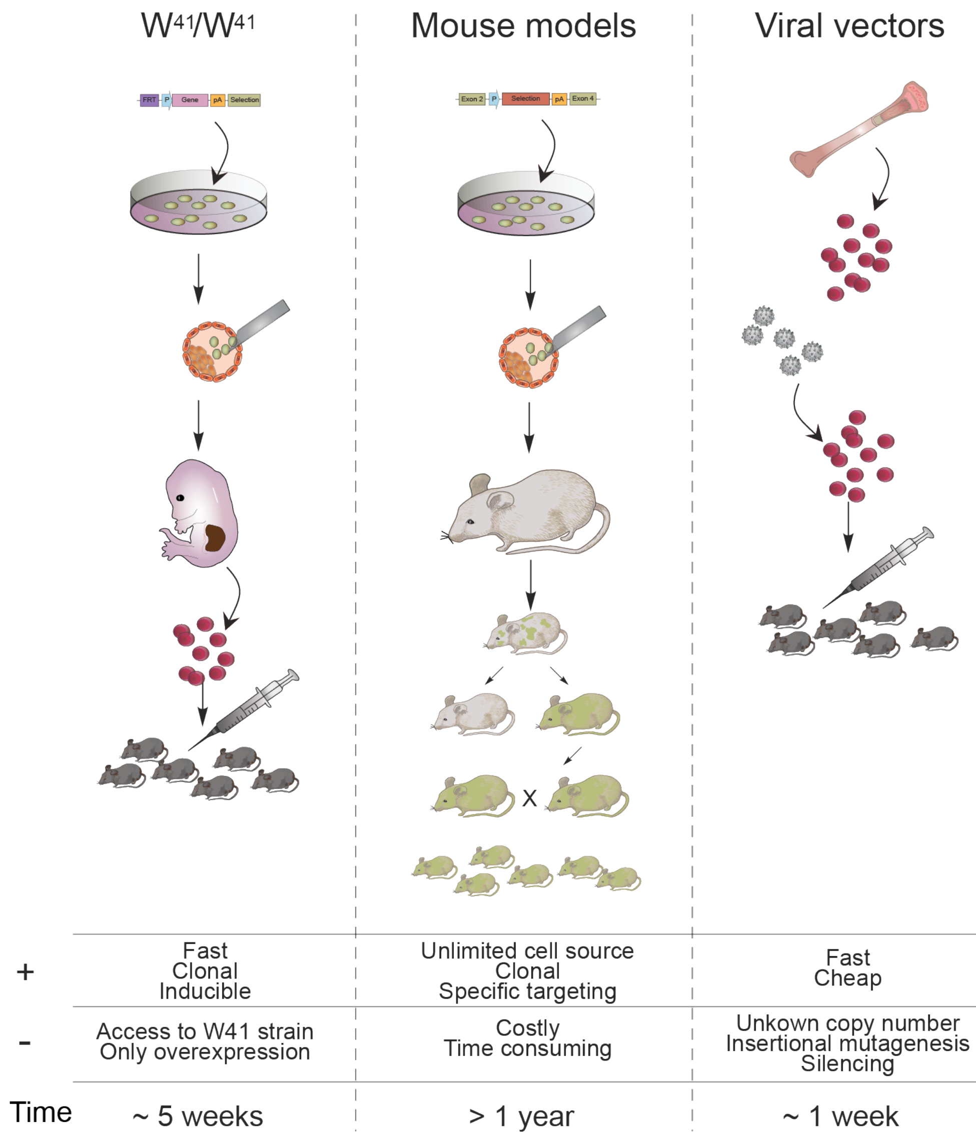

Figure 5. A comparison between different approaches for studying genetic changes in the hematopoietic system.

ES cells. The nature and timing of this out-competition is unknown, as we did not investigate embryos at an earlier stage than E14.5. It is known that c-kit signaling is required in fetal liver HSCs and it is believed that dHSCs in the AGM are c-kit^{+} but the functional relevance of c-kit signaling for HSC formation is still unclear (Bowie et al., 2006; Miller et al., 1997; Nishikawa et al., 2001; Nobuhisa et al., 2012; Sanchez et al., 1996; Sasaki et al., 2010).

THE HIPPO PATHWAY IN HEMATOPOIESIS

In **Article II** we used the new system to investigate the effects of YAP overexpression in HSCs. Because of the site-specific and clonal nature of the genetic modification introduced using the W^{41}/W^{41} approach, there is less variability compared to the use of viral vectors. We therefore feel comfortable in concluding that no positive effect is seen from ectopic YAP expression in HSCs. Indeed if anything, there might be a small negative effect seen with the mutated version of YAP when enumerating HSCs by flow cytometry (Article II, figure 4D). However, this effect was not significant and did not read out in transplantation assays. We did notice a decrease in p21 levels that hints at another possible scenario, wherein active YAP signaling in HSCs might lead to a loss of stem cell potential through exhaustion. Serial transplantation assays could be done to examine this possibility.

The knockout model for Yap1 is embryonic lethal, and thus to continue investigating the Hippo pathway in hematopoiesis in **Article III** we chose to study the Taz KO mouse. There are two different models, targeting the same exon, but on different strain backgrounds. The model in our possession is on a pure 129 genetic background while the other is on a 129/B6 mixed background (Hossain et al., 2007; Makita et al., 2008). There are differences in the penetrance of the genotypes between the strains, with the first model having lower numbers of homozygotes born. The second model also reports homozygotes to succumb perinatally. Our findings

indicate that homozygotes at E14.5-15.5 are still not found at mendelian ratios. Therefore the exact stage where homozygotes die is yet to be determined. Out of the two null embryos examined the first was smaller, with a pale hypo-cellular liver. The other liver was normal. However, the first null liver performed on par with wildtype cells in colony assays but not when competitively transplanted. A possible explanation is that this is the result of a HSC specific defect but then the question arises why this only happens in some of the homozygous embryos. It is most likely not due to variations in the genetic background as the mice are on a pure inbred background. Rather, this points towards a multifactorial situation where small fluctuations in external environmental parameters at a certain stage of development in combination with the genetic lesion, sometimes tips the scale. Adult homozygotes had normal blood parameters. It therefore looks like Taz is dispensable for adult steady state hematopoiesis but it cannot be ruled out that it plays a role during embryonic hematopoietic development.

Solid versus liquid tissues

Hippo signaling has been demonstrated to be regulated in a highly cell and tissue context dependent manner. We can therefore not rule out that the pathway as a whole has no endogenous function in HSCs. Recent publications indicates a strong link between cell components coupled to cell polarity, such as Crumbs, and Hippo signaling in other tissues (Varelas et al., 2010). This then raises the question of how that would be related to our knowledge of the HSC niche. There is strong evidence for some sort of cell-cell interactions being involved in HSC regulation in the niche. On the other hand the hematopoietic system is a liquid tissue where cells are not in fixed position relative each other. HSCs do migrate to other parts of the body through the circulation upon tissue insult. It is also believed that a certain percentage of HCS at all times can be found in the circulation and that there is a continuous cycle of migration to and from the bone marrow. Evidence for the involvement of other mechanisms besides cell-cell contact in regulating cell behavior exists. For example gradients of growth factors

and chemokines are known to influence motility and differentiation. Furthermore, HSCs are thought to reside in a hypoxic area where an oxygen gradient may dictate stem cell potential.

Taken together this indicates that the anti-proliferative Hippo signaling cascade, believed to be influenced mainly by cell-cell contact, may be obsolete in the bone marrow, which is a liquid tissue. Moreover, differences in how cells react to cell-cell contacts As an example, epithelial cell lines grown as a monolayer will stop proliferating when becoming confluent in contact with neighboring cells though contact-mediated inhibition. Hematopoietic cells on the other hand, when grown in suspension, are not growth inhibited by cell contact. If similar mechanism apply in vivo, this could explain the differences seen when Yap is overexpressed in solid organs compared to hematopoietic cells.

It would further be interesting to investigate Hippo signaling in the niche to address any niche-mediated Hippo effect on HSCs.

Yap and Wwtr1 specificity

Since Yap and Taz have also been demonstrated to have partly redundant, yet divergent tissue or species specific function, one can of course not rule out Hippo involvement in HSCs without looking at both effectors simultaneously in gain of function as well as loss of function studies.

Characteristics of fetal liver HSCs

Since the FL is a place of enormous HSC cell activity, with a more than 50 fold expansion during a few days during development, it is easy to assume that a pathway such as Hippo, which regulates proliferation, might be involved. Moreover, as discussed above, hematopoiesis in a solid organ such as the liver may be regulated by different mechanisms compared to the liquid bone marrow. In depth studies of a potential FL niche is lacking and we don’t have a complete picture of how the massive HSC expansion is

regulated(Sugiyama et al., 2011). SCF signaling is necessary, but likely not solely responsible, as SCF/kit signaling has been demonstrated to have mainly survival- and maintenance functions in adult HSCs. Both Yap and Taz mRNA is expressed in FL cells, specifically in HSCs. Yap protein levels are also high in cell extracts from FLs. Since the Yap KO mouse is early embryonic lethal (E9.5) it would be interesting to study a conditional model where Yap/Taz is deleted later during development.

CONCLUSIONS

- W^{41}/W^{41} blastocyst complementation can be used as a method to model genetic modifications in HSCs. In this system we can use the sophisticated site-directed manipulations that are possible in ES cells to generate inducible genetic changes.
- The W^{41}/W^{41} approach can be used to avoid the costly and time consuming methods of standard transgenic mouse strains
- YAP overexpression does not influence *in vivo* HSC function during steady state or regeneration. This is contrast to effects seen in other tissue stem cells and suggests tissue specific functions of YAP1 in regulation of stem cells
- Taz is dispensable for adult hematopoiesis but may effect embryonic development.

ACKNOWLEDGEMENTS

First and foremost I want to thank my supervisor Jonas for taking a chance on hiring me when he started his group and then being an incredible supportive boss throughout my whole PhD studies.

I would also like to give a hand for my co-supervisor Stefan Karlsson who has managed to create a lab with such an exceptional positive atmosphere that I have never encountered before. It makes it a real pleasure to do science in this environment. Also big shout-outs to Karin, Eva and all the other PIs for having a large part in that…

With that said it is natural to start thanking all the wonderful co-workers at the Department of Molecular Medicine and Gene Therapy to name but a few; Aurélie for being a great mentor when I started my PhD; Pekka and Evan for endless hours of chattering; Justyna and Lyndon for wonderful times trekking across California (including bear scares!); Ida and Matilda for the most fascinating discussions on life, work and…well everything; Sauna-buddies Marianne and Carolina for lots of shared discussions (and sweat); Gym-buddies Alex and Jens for putting the b in bench; Kristian for teaching me *proper* ping-pong technique; Sofie, Göran and Kenichi for helping with, well science!; All Larsson group members past and present, Christine, Praveen, Natsumi, Karolina, Ann-Margreth, Mehrnaz, Roman and Ineke for making our meetings hilarious; and all you other guys you are the best!

I could go on forever, but I'll skip to the essential part…of course this would not have been possible with out the fantastic support from my friends and family! You know I love you! I should say it more often…

POPULÄRVETENSKAPLIG SAMMANFATTNING

I vår kropp genereras konstant ett stort antal nya blodceller för att ersätta de som går förlorade. Olika sorters blodceller är ansvariga för att transportera syre till alla våra vävnader, hjälpa till med blodets koagulering vid skador och att slåss mot infektioner orsakade av bakterier och virus. All blodceller härstammar från en liten population med blodstamceller som finns i benmärgen. Dessa stamceller har kapaciteten att både dela sig och bilda nya dotterceller som även de är stamceller, samt att dela sig och bilda dotterceller som mognar till de specialiserade blodcellerna. Man vet idag inte riktigt hur blodstamcellerna instrueras att välja mellan att bli dessa två typer av dotterceller och inte heller vad för faktorer som gör att de är stamceller. I mitt projekt har jag tittat på en signalerings väg, kallad Hippo, som finns i celler och som ännu inte har undersökts i blodceller. För att göra det så började jag med att komma på ett system för hur man kan titta på vad som händer i blodstamceller när uttrycket av en gen ökar. I våra laboratorier använder vi mest möss för att göra våra studier eftersom de liknar oss människor ganska mycket. Så mitt system går ut på att skapa ett uttryck av en gen som kan slås av och på bara i blodceller genom att ge möss en antibiotika i deras dricksvatten. Efter att ha kontrollerat så att systemet fungerade så använde jag det för att titta på vad som händer med blodstamceller om en gen i min signalerings väg, YAP, har mycket högre uttryck än normalt. Jag testade funktionen hos stamcellerna genom att transplantera dem till möss som fått hela sitt blodsystem utslaget med strålning och kunde därmed visa att YAP-cellerna varken hade bättre eller sämre förmåga att ersätta det gamla blodsystemet. Eftersom det finns två gener i Hippo signaleringsvägen som kan täcka upp för varandra, YAP och

Taz, så studerade jag sen också vad som händer om man tar bort allt genuttryck från Taz i blod celler. Att ta bort Taz i vuxna möss påverkar inte deras blodceller utan dessa förblir normala vilket indikerar att Taz inte behövs för blodbildning i vuxna. Sammantaget är detta de första studier som gjorts på Hippo signalering i blodsystemet och även om varken överuttryck av YAP eller borttagande av Taz verkar ha någon inverkan så måste man göra många fler studier för att se om Hippo signaleringsvägen reglerar stamcells processer i blodet.

REFERENCES

Aderem, A. (2003). Phagocytosis and the inflammatory response. The Journal of infectious diseases *187 Suppl 2*, S340-345.

Aguilo, F., Avagyan, S., Labar, A.S., Sevilla, A., Lee, D.F., Kumar, P., Lemischka, I.R., Zhou, B.Y., and Snoeck, H.W. (2011). Prdm16 is a physiological regulator of hematopoietic stem cells. Blood.

Alison, M. (1998). Liver stem cells: a two compartment system. Current opinion in cell biology *10*, 710-715.

Alison, M., Golding, M., Lalani el, N., and Sarraf, C. (1998). Wound healing in the liver with particular reference to stem cells. Philosophical transactions of the Royal Society of London Series B, Biological sciences *353*, 877-894.

Allen, L.A., and Aderem, A. (1996). Mechanisms of phagocytosis. Current opinion in immunology *8*, 36-40.

Arai, F., Hirao, A., Ohmura, M., Sato, H., Matsuoka, S., Takubo, K., Ito, K., Koh, G.Y., and Suda, T. (2004). Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell *118*, 149-161.

Aubin, J.E. (1998). Bone stem cells. Journal of cellular biochemistry Supplement *30-31*, 73-82.

Avecilla, S.T., Hattori, K., Heissig, B., Tejada, R., Liao, F., Shido, K., Jin, D.K., Dias, S., Zhang, F., Hartman, T.E., *et al.* (2004). Chemokine-

mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. Nature medicine *10*, 64-71.

Baines, P., Bol, S.J., and Rosendaal, M. (1982). Physical and kinetic properties of haemopoietic progenitor cell populations from mouse marrow detected in five different assay systems. Leukemia research *6*, 81-88.

Baumgartner, R., Poernbacher, I., Buser, N., Hafen, E., and Stocker, H. (2010). The WW domain protein Kibra acts upstream of Hippo in Drosophila. Developmental cell *18*, 309-316.

Becker, A.J., Mc, C.E., and Till, J.E. (1963). Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature *197*, 452-454.

Bennett, F.C., and Harvey, K.F. (2006). Fat cadherin modulates organ size in Drosophila via the Salvador/Warts/Hippo signaling pathway. Curr Biol *16*, 2101-2110.

Berndt, A., Cario, C.L., Silva, K.A., Kennedy, V.E., Harrison, D.E., Paigen, B., and Sundberg, J.P. (2011). Identification of fat4 and tsc22d1 as novel candidate genes for spontaneous pulmonary adenomas. Cancer research *71*, 5779-5791.

Bockamp, E., Maringer, M., Spangenberg, C., Fees, S., Fraser, S., Eshkind, L., Oesch, F., and Zabel, B. (2002). Of mice and models: improved animal models for biomedical research. Physiological genomics *11*, 115-132.

Bonner-Weir, S., and Sharma, A. (2002). Pancreatic stem cells. The Journal of pathology *197*, 519-526.

Bowie, M.B., McKnight, K.D., Kent, D.G., McCaffrey, L., Hoodless, P.A., and Eaves, C.J. (2006). Hematopoietic stem cells proliferate until after birth and show a reversible phase-specific engraftment defect. The Journal of clinical investigation *116*, 2808-2816.

Brinster, R.L. (1974a). The effect of cells transferred into the mouse blastocyst on subsequent development. J Exp Med *140*, 1049-1056.

Brinster, R.L. (1974b). Embryo development. Journal of animal science *38*, 1003-1012.

Butler, J.M., Nolan, D.J., Vertes, E.L., Varnum-Finney, B., Kobayashi, H., Hooper, A.T., Seandel, M., Shido, K., White, I.A., Kobayashi, M.*, et al.* (2010). Endothelial cells are essential for the self-renewal and repopulation of Notch-dependent hematopoietic stem cells. Cell stem cell *6*, 251-264.

Callus, B.A., Verhagen, A.M., and Vaux, D.L. (2006). Association of mammalian sterile twenty kinases, Mst1 and Mst2, with hSalvador via C-terminal coiled-coil domains, leads to its stabilization and phosphorylation. The FEBS journal *273*, 4264-4276.

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*., et al.* (2003). Osteoblastic cells regulate the haematopoietic stem cell niche. Nature *425*, 841-846.

Camargo, F.D., Gokhale, S., Johnnidis, J.B., Fu, D., Bell, G.W., Jaenisch, R., and Brummelkamp, T.R. (2007). YAP1 increases organ size and expands undifferentiated progenitor cells. Curr Biol *17*, 2054-2060.

Campbell, K.H., McWhir, J., Ritchie, W.A., and Wilmut, I. (1996). Sheep cloned by nuclear transfer from a cultured cell line. Nature *380*, 64-66.

Cao, X., Pfaff, S.L., and Gage, F.H. (2008). YAP regulates neural progenitor cell number via the TEA domain transcription factor. Genes & development *22*, 3320-3334.

Cauffman, G., De Rycke, M., Sermon, K., Liebaers, I., and Van de Velde, H. (2009). Markers that define stemness in ESC are unable to identify the totipotent cells in human preimplantation embryos. Hum Reprod *24*, 63-70.

Chan, E.H., Nousiainen, M., Chalamalasetty, R.B., Schafer, A., Nigg, E.A., and Sillje, H.H. (2005). The Ste20-like kinase Mst2 activates the human large tumor suppressor kinase Lats1. Oncogene *24*, 2076-2086.

Chan, S.W., Lim, C.J., Guo, K., Ng, C.P., Lee, I., Hunziker, W., Zeng, Q., and Hong, W. (2008). A role for TAZ in migration, invasion, and tumorigenesis of breast cancer cells. Cancer research *68*, 2592-2598.

Chen, L., Wang, D., Wu, Z., Ma, L., and Daley, G.Q. (2010). Molecular basis of the first cell fate determination in mouse embryogenesis. Cell research *20*, 982-993.

Cheng, T., Rodrigues, N., Shen, H., Yang, Y., Dombkowski, D., Sykes, M., and Scadden, D.T. (2000). Hematopoietic stem cell quiescence maintained by p21cip1/waf1. Science *287*, 1804-1808.

Cho, E., Feng, Y., Rauskolb, C., Maitra, S., Fehon, R., and Irvine, K.D. (2006). Delineation of a Fat tumor suppressor pathway. Nature genetics *38*, 1142-1150.

Choi, J., Oh, S., Lee, D., Oh, H.J., Park, J.Y., Lee, S.B., and Lim, D.S. (2009). Mst1-FoxO signaling protects Naive T lymphocytes from cellular oxidative stress in mice. PLoS One *4*, e8011.

Chuikov, S., Levi, B.P., Smith, M.L., and Morrison, S.J. (2010). Prdm16 promotes stem cell maintenance in multiple tissues, partly by regulating oxidative stress. Nature cell biology *12*, 999-1006.

Cipolleschi, M.G., Dello Sbarba, P., and Olivotto, M. (1993). The role of hypoxia in the maintenance of hematopoietic stem cells. Blood *82*, 2031-2037.

Clarke, A.R. (2000). Manipulating the germline: its impact on the study of carcinogenesis. Carcinogenesis *21*, 435-441.

Coico, R., Sunshine, G., and Benjamini, E. (2003). Immunology : a short course, 5th edn (Hoboken, N.J., Wiley-Liss).

Coles, B.L., Angenieux, B., Inoue, T., Del Rio-Tsonis, K., Spence, J.R., McInnes, R.R., Arsenijevic, Y., and van der Kooy, D. (2004). Facile isolation and the characterization of human retinal stem cells. Proceedings of the National Academy of Sciences of the United States of America *101*, 15772-15777.

Congdon, C.C. (1962). Radiation injury:bone marrow transplantation. Annual review of medicine *13*, 203-212.

Cornils, H., Stegert, M.R., Hergovich, A., Hynx, D., Schmitz, D., Dirnhofer, S., and Hemmings, B.A. (2010). Ablation of the kinase NDR1 predisposes mice to the development of T cell lymphoma. Science signaling *3*, ra47.

Cotsarelis, G., Sun, T.T., and Lavker, R.M. (1990). Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell *61*, 1329-1337.

Cumano, A., and Godin, I. (2007). Ontogeny of the hematopoietic system. Annual review of immunology *25*, 745-785.

de Haan, G., Weersing, E., Dontje, B., van Os, R., Bystrykh, L.V., Vellenga, E., and Miller, G. (2003). In vitro generation of long-term repopulating hematopoietic stem cells by fibroblast growth factor-1. Developmental cell *4*, 241-251.

de la Cova, C., Abril, M., Bellosta, P., Gallant, P., and Johnston, L.A. (2004). Drosophila myc regulates organ size by inducing cell competition. Cell *117*, 107-116.

Dello Sbarba, P., Cipolleschi, M.G., and Olivotto, M. (1987). Hemopoietic progenitor cells are sensitive to the cytostatic effect of pyruvate. Experimental hematology *15*, 137-142.

Dick, J.E., Magli, M.C., Huszar, D., Phillips, R.A., and Bernstein, A. (1985). Introduction of a selectable gene into primitive stem cells capable of long-term reconstitution of the hemopoietic system of W/Wv mice. Cell *42*, 71-79.

Dieterlen-Lievre, F. (1975). On the origin of haemopoietic stem cells in the avian embryo: an experimental approach. Journal of embryology and experimental morphology *33*, 607-619.

Ding, L., Saunders, T.L., Enikolopov, G., and Morrison, S.J. (2012). Endothelial and perivascular cells maintain haematopoietic stem cells. Nature *481*, 457-462.

Doetschman, T., Gregg, R.G., Maeda, N., Hooper, M.L., Melton, D.W., Thompson, S., and Smithies, O. (1987). Targetted correction of a mutant HPRT gene in mouse embryonic stem cells. Nature *330*, 576-578.

Domen, J. (2001). The role of apoptosis in regulating hematopoietic stem cell numbers. Apoptosis : an international journal on programmed cell death *6*, 239-252.

Dong, J., Feldmann, G., Huang, J., Wu, S., Zhang, N., Comerford, S.A., Gayyed, M.F., Anders, R.A., Maitra, A., and Pan, D. (2007). Elucidation of a universal size-control mechanism in Drosophila and mammals. Cell *130*, 1120-1133.

Dor, Y., Brown, J., Martinez, O.I., and Melton, D.A. (2004). Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature *429*, 41-46.

Downward, J., and Basu, S. (2008). YAP and p73: a complex affair. Molecular cell *32*, 749-750.

Driessen, R.L., Johnston, H.M., and Nilsson, S.K. (2003). Membrane-bound stem cell factor is a key regulator in the initial lodgment of stem cells within the endosteal marrow region. Experimental hematology *31*, 1284-1291.

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

Estrov, Z. (2009). Stem cells and somatic cells: reprogramming and plasticity. Clinical lymphoma & myeloma *9 Suppl 3*, S319-328.

Evans, M.J. (1972). The isolation and properties of a clonal tissue culture strain of pluripotent mouse teratoma cells. Journal of embryology and experimental morphology *28*, 163-176.

Evans, M.J., and Kaufman, M.H. (1981). Establishment in culture of pluripotential cells from mouse embryos. Nature *292*, 154-156.

Fernandez, L.A., Northcott, P.A., Dalton, J., Fraga, C., Ellison, D., Angers, S., Taylor, M.D., and Kenney, A.M. (2009). YAP1 is amplified and up-regulated in hedgehog-associated medulloblastomas and mediates Sonic hedgehog-driven neural precursor proliferation. Genes & development *23*, 2729-2741.

Findikli, N., Candan, N.Z., and Kahraman, S. (2006). Human embryonic stem cell culture: current limitations and novel strategies. Reproductive biomedicine online *13*, 581-590.

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature *391*, 806-811.

Ford, C.E., Hamerton, J.L., Barnes, D.W., and Loutit, J.F. (1956). Cytological identification of radiation-chimaeras. Nature *177*, 452-454.

Fuchs, E., Merrill, B.J., Jamora, C., and DasGupta, R. (2001). At the roots of a never-ending cycle. Developmental cell *1*, 13-25.

Gage, F.H. (2002). Neurogenesis in the adult brain. The Journal of neuroscience : the official journal of the Society for Neuroscience *22*, 612-613.

Gatti, R.A., Meuwissen, H.J., Allen, H.D., Hong, R., and Good, R.A. (1968). Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet *2*, 1366-1369.

Gaudin, E., Rosado, M., Agenes, F., McLean, A., and Freitas, A.A. (2004). B-cell homeostasis, competition, resources, and positive selection by self-antigens. Immunol Rev *197*, 102-115.

Genevet, A., Wehr, M.C., Brain, R., Thompson, B.J., and Tapon, N. (2010). Kibra is a regulator of the Salvador/Warts/Hippo signaling network. Developmental cell *18*, 300-308.

Goldring, K., Partridge, T., and Watt, D. (2002). Muscle stem cells. The Journal of pathology *197*, 457-467.

Gordon, J.W., Scangos, G.A., Plotkin, D.J., Barbosa, J.A., and Ruddle, F.H. (1980). Genetic transformation of mouse embryos by microinjection of purified DNA. Proceedings of the National Academy of Sciences of the United States of America *77*, 7380-7384.

Graves, J.D., Gotoh, Y., Draves, K.E., Ambrose, D., Han, D.K., Wright, M., Chernoff, J., Clark, E.A., and Krebs, E.G. (1998). Caspase-mediated activation and induction of apoptosis by the mammalian Ste20-like kinase Mst1. The EMBO journal *17*, 2224-2234.

Gruh, I., and Martin, U. (2009). Transdifferentiation of stem cells: a critical view. Advances in biochemical engineering/biotechnology *114*, 73-106.

Grusche, F.A., Richardson, H.E., and Harvey, K.F. (2010). Upstream regulation of the hippo size control pathway. Curr Biol *20*, R574-582.

Haas, S., Weidner, N., and Winkler, J. (2005). Adult stem cell therapy in stroke. Current opinion in neurology *18*, 59-64.

Hamaratoglu, F., Willecke, M., Kango-Singh, M., Nolo, R., Hyun, E., Tao, C., Jafar-Nejad, H., and Halder, G. (2006). The tumour-suppressor genes NF2/Merlin and Expanded act through Hippo signalling to regulate cell proliferation and apoptosis. Nature cell biology *8*, 27-36.

Hansson, C.M., Buckley, P.G., Grigelioniene, G., Piotrowski, A., Hellstrom, A.R., Mantripragada, K., Jarbo, C., Mathiesen, T., and Dumanski, J.P. (2007). Comprehensive genetic and epigenetic analysis of sporadic meningioma for macro-mutations on 22q and micro-mutations within the NF2 locus. BMC genomics *8*, 16.

Hao, Y., Chun, A., Cheung, K., Rashidi, B., and Yang, X. (2008). Tumor suppressor LATS1 is a negative regulator of oncogene YAP. The Journal of biological chemistry *283*, 5496-5509.

Harrison, D.E., Astle, C.M., and Delaittre, J.A. (1978). Loss of proliferative capacity in immunohemopoietic stem cells caused by serial transplantation rather than aging. J Exp Med *147*, 1526-1531.

Harrison, J.S., Rameshwar, P., Chang, V., and Bandari, P. (2002). Oxygen saturation in the bone marrow of healthy volunteers. Blood *99*, 394.

Harvey, K.F., Pfleger, C.M., and Hariharan, I.K. (2003). The Drosophila Mst ortholog, hippo, restricts growth and cell proliferation and promotes apoptosis. Cell *114*, 457-467.

Hattangadi, S.M., Wong, P., Zhang, L., Flygare, J., and Lodish, H.F. (2011). From stem cell to red cell: regulation of erythropoiesis at multiple levels by multiple proteins, RNAs, and chromatin modifications. Blood *118*, 6258-6268.

Haviernik, P., Zhang, Y., and Bunting, K.D. (2008). Retroviral transduction of murine hematopoietic stem cells. Methods Mol Biol *430*, 229-241.

Heissig, B., Hattori, K., Dias, S., Friedrich, M., Ferris, B., Hackett, N.R., Crystal, R.G., Besmer, P., Lyden, D., Moore, M.A.*, et al.* (2002). Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. Cell *109*, 625-637.

Heng, B.C., Liu, H., and Cao, T. (2005). Transplanted human embryonic stem cells as biological 'catalysts' for tissue repair and regeneration. Medical hypotheses *64*, 1085-1088.

Hesson, L.B., Dunwell, T.L., Cooper, W.N., Catchpoole, D., Brini, A.T., Chiaramonte, R., Griffiths, M., Chalmers, A.D., Maher, E.R., and Latif, F. (2009). The novel RASSF6 and RASSF10 candidate tumour suppressor genes are frequently epigenetically inactivated in childhood leukaemias. Molecular cancer *8*, 42.

Hirao, A., Arai, F., and Suda, T. (2004). Regulation of cell cycle in hematopoietic stem cells by the niche. Cell Cycle *3*, 1481-1483.

Hong, J.H., Hwang, E.S., McManus, M.T., Amsterdam, A., Tian, Y., Kalmukova, R., Mueller, E., Benjamin, T., Spiegelman, B.M., Sharp, P.A.*, et al.* (2005). TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. Science *309*, 1074-1078.

Hong, J.H., and Yaffe, M.B. (2006). TAZ: a beta-catenin-like molecule that regulates mesenchymal stem cell differentiation. Cell Cycle *5*, 176-179.

Hooper, A.T., Butler, J.M., Nolan, D.J., Kranz, A., Iida, K., Kobayashi, M., Kopp, H.G., Shido, K., Petit, I., Yanger, K.*, et al.* (2009). Engraftment and

reconstitution of hematopoiesis is dependent on VEGFR2-mediated regeneration of sinusoidal endothelial cells. Cell stem cell *4*, 263-274.

Hossain, Z., Ali, S.M., Ko, H.L., Xu, J., Ng, C.P., Guo, K., Qi, Z., Ponniah, S., Hong, W., and Hunziker, W. (2007). Glomerulocystic kidney disease in mice with a targeted inactivation of Wwtr1. Proceedings of the National Academy of Sciences of the United States of America *104*, 1631-1636.

Huang, J., Wu, S., Barrera, J., Matthews, K., and Pan, D. (2005). The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. Cell *122*, 421-434.

Hwang, Y.S., Randle, W.L., Bielby, R.C., Polak, J.M., and Mantalaris, A. (2006). Enhanced derivation of osteogenic cells from murine embryonic stem cells after treatment with HepG2-conditioned medium and modulation of the embryoid body formation period: application to skeletal tissue engineering. Tissue engineering *12*, 1381-1392.

Ivanovs, A., Rybtsov, S., Welch, L., Anderson, R.A., Turner, M.L., and Medvinsky, A. (2011). Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region. J Exp Med *208*, 2417-2427.

Jaako, P., Flygare, J., Olsson, K., Quere, R., Ehinger, M., Henson, A., Ellis, S., Schambach, A., Baum, C., Richter, J.*, et al.* (2011). Mice with ribosomal protein S19 deficiency develop bone marrow failure and symptoms like patients with Diamond-Blackfan anemia. Blood *118*, 6087-6096.

Jang, Y.Y., and Sharkis, S.J. (2007). A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. Blood *110*, 3056-3063.

Jia, J., Zhang, W., Wang, B., Trinko, R., and Jiang, J. (2003). The Drosophila Ste20 family kinase dMST functions as a tumor suppressor by restricting cell proliferation and promoting apoptosis. Genes & development *17*, 2514-2519.

Jiang, Z., Li, X., Hu, J., Zhou, W., Jiang, Y., Li, G., and Lu, D. (2006). Promoter hypermethylation-mediated down-regulation of LATS1 and LATS2 in human astrocytoma. Neuroscience research *56*, 450-458.

Jin, Z., Kirilly, D., Weng, C., Kawase, E., Song, X., Smith, S., Schwartz, J., and Xie, T. (2008). Differentiation-defective stem cells outcompete normal stem cells for niche occupancy in the Drosophila ovary. Cell stem cell *2*, 39-49.

Johnston, L.A., Prober, D.A., Edgar, B.A., Eisenman, R.N., and Gallant, P. (1999). Drosophila myc regulates cellular growth during development. Cell *98*, 779-790.

Jones, R.J., Wagner, J.E., Celano, P., Zicha, M.S., and Sharkis, S.J. (1990). Separation of pluripotent haematopoietic stem cells from spleen colony-forming cells. Nature *347*, 188-189.

Jordan, C.T., and Lemischka, I.R. (1990). Clonal and systemic analysis of long-term hematopoiesis in the mouse. Genes & development *4*, 220-232.

Justice, R.W., Zilian, O., Woods, D.F., Noll, M., and Bryant, P.J. (1995). The Drosophila tumor suppressor gene warts encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. Genes & development *9*, 534-546.

Kahan, B.W., and Ephrussi, B. (1970). Developmental potentialities of clonal in vitro cultures of mouse testicular teratoma. Journal of the National Cancer Institute *44*, 1015-1036.

Kango-Singh, M., Nolo, R., Tao, C., Verstreken, P., Hiesinger, P.R., Bellen, H.J., and Halder, G. (2002). Shar-pei mediates cell proliferation arrest during imaginal disc growth in Drosophila. Development (Cambridge, England) *129*, 5719-5730.

Karlsson, G., Blank, U., Moody, J.L., Ehinger, M., Singbrant, S., Deng, C.X., and Karlsson, S. (2007). Smad4 is critical for self-renewal of hematopoietic stem cells. J Exp Med *204*, 467-474.

Kaufman, D.S., Hanson, E.T., Lewis, R.L., Auerbach, R., and Thomson, J.A. (2001). Hematopoietic colony-forming cells derived from human

embryonic stem cells. Proceedings of the National Academy of Sciences of the United States of America *98*, 10716-10721.

Keller, G., and Snodgrass, R. (1990). Life span of multipotential hematopoietic stem cells in vivo. J Exp Med *171*, 1407-1418.

Keller, J.R., Ortiz, M., and Ruscetti, F.W. (1995). Steel factor (c-kit ligand) promotes the survival of hematopoietic stem/progenitor cells in the absence of cell division. Blood *86*, 1757-1764.

Kiger, A.A., White-Cooper, H., and Fuller, M.T. (2000). Somatic support cells restrict germline stem cell self-renewal and promote differentiation. Nature *407*, 750-754.

Kleinsmith, L.J., and Pierce, G.B., Jr. (1964). Multipotentiality of Single Embryonal Carcinoma Cells. Cancer research *24*, 1544-1551.

Kobayashi, H., Butler, J.M., O'Donnell, R., Kobayashi, M., Ding, B.S., Bonner, B., Chiu, V.K., Nolan, D.J., Shido, K., Benjamin, L., *et al.* (2010). Angiocrine factors from Akt-activated endothelial cells balance self-renewal and differentiation of haematopoietic stem cells. Nature cell biology *12*, 1046-1056.

Kubota, Y., Takubo, K., and Suda, T. (2008). Bone marrow long label-retaining cells reside in the sinusoidal hypoxic niche. Biochemical and biophysical research communications *366*, 335-339.

Lai, Z.C., Wei, X., Shimizu, T., Ramos, E., Rohrbaugh, M., Nikolaidis, N., Ho, L.L., and Li, Y. (2005). Control of cell proliferation and apoptosis by mob as tumor suppressor, mats. Cell *120*, 675-685.

Lam-Himlin, D.M., Daniels, J.A., Gayyed, M.F., Dong, J., Maitra, A., Pan, D., Montgomery, E.A., and Anders, R.A. (2006). The hippo pathway in human upper gastrointestinal dysplasia and carcinoma: a novel oncogenic pathway. International journal of gastrointestinal cancer *37*, 103-109.

Langer, J.C., Henckaerts, E., Orenstein, J., and Snoeck, H.W. (2004). Quantitative trait analysis reveals transforming growth factor-beta2 as a positive regulator of early hematopoietic progenitor and stem cell function. J Exp Med *199*, 5-14.

Larsson, J., Ohishi, M., Garrison, B., Aspling, M., Janzen, V., Adams, G.B., Curto, M., McClatchey, A.I., Schipani, E., and Scadden, D.T. (2008). Nf2/merlin regulates hematopoietic stem cell behavior by altering microenvironmental architecture. Cell stem cell *3*, 221-227.

Lee, J.H., Kim, T.S., Yang, T.H., Koo, B.K., Oh, S.P., Lee, K.P., Oh, H.J., Lee, S.H., Kong, Y.Y., Kim, J.M.*, et al.* (2008). A crucial role of WW45 in developing epithelial tissues in the mouse. The EMBO journal *27*, 1231-1242.

Levesque, J.P., Winkler, I.G., Hendy, J., Williams, B., Helwani, F., Barbier, V., Nowlan, B., and Nilsson, S.K. (2007). Hematopoietic progenitor cell mobilization results in hypoxia with increased hypoxia-inducible transcription factor-1 alpha and vascular endothelial growth factor A in bone marrow. Stem Cells *25*, 1954-1965.

Li, L., and Xie, T. (2005). Stem cell niche: structure and function. Annual review of cell and developmental biology *21*, 605-631.

Li, Z., Zhao, B., Wang, P., Chen, F., Dong, Z., Yang, H., Guan, K.L., and Xu, Y. (2010). Structural insights into the YAP and TEAD complex. Genes & development *24*, 235-240.

Lian, I., Kim, J., Okazawa, H., Zhao, J., Zhao, B., Yu, J., Chinnaiyan, A., Israel, M.A., Goldstein, L.S., Abujarour, R.*, et al.* (2010). The role of YAP transcription coactivator in regulating stem cell self-renewal and differentiation. Genes & development *24*, 1106-1118.

Liao, Y., Geyer, M.B., Yang, A.J., and Cairo, M.S. (2011). Cord blood transplantation and stem cell regenerative potential. Experimental hematology *39*, 393-412.

Lin, H. (2002). The stem-cell niche theory: lessons from flies. Nature reviews Genetics *3*, 931-940.

Lo Celso, C., Fleming, H.E., Wu, J.W., Zhao, C.X., Miake-Lye, S., Fujisaki, J., Cote, D., Rowe, D.W., Lin, C.P., and Scadden, D.T. (2009). Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. Nature *457*, 92-96.

Loh, Y.H., Agarwal, S., Park, I.H., Urbach, A., Huo, H., Heffner, G.C., Kim, K., Miller, J.D., Ng, K., and Daley, G.Q. (2009). Generation of induced pluripotent stem cells from human blood. Blood *113*, 5476-5479.

Lord, B.I., and Hendry, J.H. (1972). The distribution of haemopoietic colony-forming units in the mouse femur, and its modification by x rays. Br J Radiol *45*, 110-115.

Lord, B.I., Testa, N.G., and Hendry, J.H. (1975). The relative spatial distributions of CFUs and CFUc in the normal mouse femur. Blood *46*, 65-72.

Lowry, W.E., Richter, L., Yachechko, R., Pyle, A.D., Tchieu, J., Sridharan, R., Clark, A.T., and Plath, K. (2008). Generation of human induced pluripotent stem cells from dermal fibroblasts. Proceedings of the National Academy of Sciences of the United States of America *105*, 2883-2888.

Magli, M.C., Iscove, N.N., and Odartchenko, N. (1982). Transient nature of early haematopoietic spleen colonies. Nature *295*, 527-529.

Mahoney, W.M., Jr., Hong, J.H., Yaffe, M.B., and Farrance, I.K. (2005). The transcriptional co-activator TAZ interacts differentially with transcriptional enhancer factor-1 (TEF-1) family members. Biochem J *388*, 217-225.

Main, J.M., and Prehn, R.T. (1955). Successful skin homografts after the administration of high dosage X radiation and homologous bone marrow. Journal of the National Cancer Institute *15*, 1023-1029.

Makita, R., Uchijima, Y., Nishiyama, K., Amano, T., Chen, Q., Takeuchi, T., Mitani, A., Nagase, T., Yatomi, Y., Aburatani, H.*, et al.* (2008). Multiple renal cysts, urinary concentration defects, and pulmonary emphysematous changes in mice lacking TAZ. American journal of physiology Renal physiology *294*, F542-553.

Mao, Y., Kucuk, B., and Irvine, K.D. (2009). Drosophila lowfat, a novel modulator of Fat signaling. Development (Cambridge, England) *136*, 3223-3233.

Martin, G.R. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proceedings of the National Academy of Sciences of the United States of America *78*, 7634-7638.

Mauro, A. (1961). Satellite cell of skeletal muscle fibers. The Journal of biophysical and biochemical cytology *9*, 493-495.

McPherson, J.P., Tamblyn, L., Elia, A., Migon, E., Shehabeldin, A., Matysiak-Zablocki, E., Lemmers, B., Salmena, L., Hakem, A., Fish, J.*, et al.* (2004). Lats2/Kpm is required for embryonic development, proliferation control and genomic integrity. The EMBO journal *23*, 3677-3688.

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

Medvinsky, A., Rybtsov, S., and Taoudi, S. (2011). Embryonic origin of the adult hematopoietic system: advances and questions. Development (Cambridge, England) *138*, 1017-1031.

Medvinsky, A.L., Gan, O.I., Semenova, M.L., and 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.

Medvinsky, A.L., Samoylina, N.L., Muller, A.M., and Dzierzak, E.A. (1993). An early pre-liver intraembryonic source of CFU-S in the developing mouse. Nature *364*, 64-67.

Miller, C.L., Rebel, V.I., Helgason, C.D., Lansdorp, P.M., and Eaves, C.J. (1997). Impaired steel factor responsiveness differentially affects the detection and long-term maintenance of fetal liver hematopoietic stem cells in vivo. Blood *89*, 1214-1223.

Miller, C.L., Rebel, V.I., Lemieux, M.E., Helgason, C.D., Lansdorp, P.M., and Eaves, C.J. (1996). Studies of W mutant mice provide evidence for alternate mechanisms capable of activating hematopoietic stem cells. Experimental hematology *24*, 185-194.

Minoo, P., Zlobec, I., Baker, K., Tornillo, L., Terracciano, L., Jass, J.R., and Lugli, A. (2007). Prognostic significance of mammalian sterile20-like kinase 1 in colorectal cancer. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc *20*, 331-338.

Mintz, B., and Illmensee, K. (1975). Normal genetically mosaic mice produced from malignant teratocarcinoma cells. Proceedings of the National Academy of Sciences of the United States of America *72*, 3585-3589.

Moore, M.A., and Metcalf, D. (1970). Ontogeny of the haemopoietic system: yolk sac origin of in vivo and in vitro colony forming cells in the developing mouse embryo. British journal of haematology *18*, 279-296.

Morata, G., and Ripoll, P. (1975). Minutes: mutants of drosophila autonomously affecting cell division rate. Developmental biology *42*, 211-221.

Moreno, E., and Basler, K. (2004). dMyc transforms cells into super-competitors. Cell *117*, 117-129.

Moreno, E., Basler, K., and Morata, G. (2002). Cells compete for decapentaplegic survival factor to prevent apoptosis in Drosophila wing development. Nature *416*, 755-759.

Morin-Kensicki, E.M., Boone, B.N., Howell, M., Stonebraker, J.R., Teed, J., Alb, J.G., Magnuson, T.R., O'Neal, W., and Milgram, S.L. (2006). Defects in yolk sac vasculogenesis, chorioallantoic fusion, and embryonic axis elongation in mice with targeted disruption of Yap65. Mol Cell Biol *26*, 77-87.

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.

Morrison, S.J., and Weissman, I.L. (1994). The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. Immunity *1*, 661-673.

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

Murakami, M., Tominaga, J., Makita, R., Uchijima, Y., Kurihara, Y., Nakagawa, O., Asano, T., and Kurihara, H. (2006). Transcriptional activity of Pax3 is co-activated by TAZ. Biochemical and biophysical research communications *339*, 533-539.

Nakagawa, M., Koyanagi, M., Tanabe, K., Takahashi, K., Ichisaka, T., Aoi, T., Okita, K., Mochiduki, Y., Takizawa, N., and Yamanaka, S. (2008). Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nature biotechnology *26*, 101-106.

Nakamura, Y., Arai, F., Iwasaki, H., Hosokawa, K., Kobayashi, I., Gomei, Y., Matsumoto, Y., Yoshihara, H., and Suda, T. (2010). Isolation and characterization of endosteal niche cell populations that regulate hematopoietic stem cells. Blood *116*, 1422-1432.

Nilsson, S.K., Johnston, H.M., and Coverdale, J.A. (2001). Spatial localization of transplanted hemopoietic stem cells: inferences for the localization of stem cell niches. Blood *97*, 2293-2299.

Nilsson, S.K., Johnston, H.M., Whitty, G.A., Williams, B., Webb, R.J., Denhardt, D.T., Bertoncello, I., Bendall, L.J., Simmons, P.J., and Haylock, D.N. (2005). Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. Blood *106*, 1232-1239.

Nishikawa, M., Tahara, T., Hinohara, A., Miyajima, A., Nakahata, T., and Shimosaka, A. (2001). Role of the microenvironment of the embryonic aorta-gonad-mesonephros region in hematopoiesis. Annals of the New York Academy of Sciences *938*, 109-116.

Nobuhisa, I., Yamasaki, S., Ramadan, A., and Taga, T. (2012). CD45(low)c-Kit(high) cells have hematopoietic properties in the mouse aorta-gonad-mesonephros region. Experimental cell research.

Nocka, K., Tan, J.C., Chiu, E., Chu, T.Y., Ray, P., Traktman, P., and Besmer, P. (1990). Molecular bases of dominant negative and loss of

function mutations at the murine c-kit/white spotting locus: W37, Wv, W41 and W. The EMBO journal *9*, 1805-1813.

Odorico, J.S., Kaufman, D.S., and Thomson, J.A. (2001). Multilineage differentiation from human embryonic stem cell lines. Stem Cells *19*, 193-204.

Ogawa, M. (1993). Differentiation and proliferation of hematopoietic stem cells. Blood *81*, 2844-2853.

Oh, H., and Irvine, K.D. (2010). Yorkie: the final destination of Hippo signaling. Trends in cell biology *20*, 410-417.

Oh, S., Lee, D., Kim, T., Kim, T.S., Oh, H.J., Hwang, C.Y., Kong, Y.Y., Kwon, K.S., and Lim, D.S. (2009). Crucial role for Mst1 and Mst2 kinases in early embryonic development of the mouse. Mol Cell Biol *29*, 6309-6320.

Oka, T., Mazack, V., and Sudol, M. (2008). Mst2 and Lats kinases regulate apoptotic function of Yes kinase-associated protein (YAP). The Journal of biological chemistry *283*, 27534-27546.

Okita, K., Ichisaka, T., and Yamanaka, S. (2007). Generation of germline-competent induced pluripotent stem cells. Nature *448*, 313-317.

Oliver, E.R., Saunders, T.L., Tarle, S.A., and Glaser, T. (2004). Ribosomal protein L24 defect in belly spot and tail (Bst), a mouse Minute. Development (Cambridge, England) *131*, 3907-3920.

Olivier, M., Aggarwal, A., Allen, J., Almendras, A.A., Bajorek, E.S., Beasley, E.M., Brady, S.D., Bushard, J.M., Bustos, V.I., Chu, A.*, et al.* (2001). A high-resolution radiation hybrid map of the human genome draft sequence. Science *291*, 1298-1302.

Orkin, S.H. (2000). Diversification of haematopoietic stem cells to specific lineages. Nature reviews Genetics *1*, 57-64.

Osawa, M., Hanada, K., Hamada, H., and Nakauchi, H. (1996). Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. Science *273*, 242-245.

Overholtzer, M., Zhang, J., Smolen, G.A., Muir, B., Li, W., Sgroi, D.C., Deng, C.X., Brugge, J.S., and Haber, D.A. (2006). Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. Proceedings of the National Academy of Sciences of the United States of America *103*, 12405-12410.

Overturf, K., al-Dhalimy, M., Ou, C.N., Finegold, M., and Grompe, M. (1997). Serial transplantation reveals the stem-cell-like regenerative potential of adult mouse hepatocytes. Am J Pathol *151*, 1273-1280.

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

Pan, G.J., Chang, Z.Y., Scholer, H.R., and Pei, D. (2002). Stem cell pluripotency and transcription factor Oct4. Cell research *12*, 321-329.

Panchision, D.M. (2011). Molecular Mechanisms Regulating Adult Stem Cell Self-Renewal. In Adult Stem Cells: Biology and Methods of Analysis, D.G. Phinney, ed. (Springer Verlag).

Papaioannou, V.E., McBurney, M.W., Gardner, R.L., and Evans, M.J. (1975). Fate of teratocarcinoma cells injected into early mouse embryos. Nature *258*, 70-73.

Park, D., Sykes, D.B., and Scadden, D.T. (2012). The hematopoietic stem cell niche. Frontiers in bioscience : a journal and virtual library *17*, 30-39.

Park, I.K., Qian, D., Kiel, M., Becker, M.W., Pihalja, M., Weissman, I.L., Morrison, S.J., and Clarke, M.F. (2003). Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. Nature *423*, 302-305.

Parmar, K., Mauch, P., Vergilio, J.A., Sackstein, R., and Down, J.D. (2007). Distribution of hematopoietic stem cells in the bone marrow

according to regional hypoxia. Proceedings of the National Academy of Sciences of the United States of America *104*, 5431-5436.

Phillips, T.J., Hen, R., and Crabbe, J.C. (1999). Complications associated with genetic background effects in research using knockout mice. Psychopharmacology *147*, 5-7.

Pierce, G.B., and Dixon, F.J., Jr. (1959). Testicular teratomas. II. Teratocarcinoma as an ascitic tumor. Cancer *12*, 584-589.

Pittenger, M.F., Mackay, A.M., Beck, S.C., Jaiswal, R.K., Douglas, R., Mosca, J.D., Moorman, M.A., Simonetti, D.W., Craig, S., and Marshak, D.R. (1999). Multilineage potential of adult human mesenchymal stem cells. Science *284*, 143-147.

Polak, J.M., and Bishop, A.E. (2006). Stem cells and tissue engineering: past, present, and future. Annals of the New York Academy of Sciences *1068*, 352-366.

Poulsom, R., Alison, M.R., Forbes, S.J., and Wright, N.A. (2002). Adult stem cell plasticity. The Journal of pathology *197*, 441-456.

Prelle, K., Zink, N., and Wolf, E. (2002). Pluripotent stem cells--model of embryonic development, tool for gene targeting, and basis of cell therapy. Anatomia, histologia, embryologia *31*, 169-186.

Purton, L.E., and Scadden, D.T. (2007). Limiting factors in murine hematopoietic stem cell assays. Cell stem cell *1*, 263-270.

Purton, L.E., and Scadden, D.T. (2008). The hematopoietic stem cell niche. In StemBook (Cambridge (MA)).

Purves, D. (1980). Neuronal competition. Nature *287*, 585-586.

Qi, C., Zhu, Y.T., Hu, L., and Zhu, Y.J. (2009). Identification of Fat4 as a candidate tumor suppressor gene in breast cancers. Int J Cancer *124*, 793-798.

Qian, H., Buza-Vidas, N., Hyland, C.D., Jensen, C.T., Antonchuk, J., Mansson, R., Thoren, L.A., Ekblom, M., Alexander, W.S., and Jacobsen, S.E. (2007). Critical role of thrombopoietin in maintaining adult quiescent hematopoietic stem cells. Cell stem cell *1*, 671-684.

Raaijmakers, M.H., Mukherjee, S., Guo, S., Zhang, S., Kobayashi, T., Schoonmaker, J.A., Ebert, B.L., Al-Shahrour, F., Hasserjian, R.P., Scadden, E.O.*, et al.* (2010). Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia. Nature *464*, 852-857.

Raff, M.C. (1992). Social controls on cell survival and cell death. Nature *356*, 397-400.

Ramón y Cajal, S. (1929). Studies on vertebrate neurogenesis (Thomas, 1960).

Reynolds, B.A., and Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science *255*, 1707-1710.

Rhiner, C., Diaz, B., Portela, M., Poyatos, J.F., Fernandez-Ruiz, I., Lopez-Gay, J.M., Gerlitz, O., and Moreno, E. (2009). Persistent competition among stem cells and their daughters in the Drosophila ovary germline niche. Development (Cambridge, England) *136*, 995-1006.

Rijkers, T., Peetz, A., and Ruther, U. (1994). Insertional mutagenesis in transgenic mice. Transgenic research *3*, 203-215.

Rogulja, D., Rauskolb, C., and Irvine, K.D. (2008). Morphogen control of wing growth through the Fat signaling pathway. Developmental cell *15*, 309-321.

Rosenthal, M.D., Wishnow, R.M., and Sato, G.H. (1970). In vitro growth and differetiation of clonal populations of multipotential mouse clls derived from a transplantable testicular teratocarcinoma. Journal of the National Cancer Institute *44*, 1001-1014.

Rossant, J. (1975). Investigation of the determinative state of the mouse inner cell mass. II. The fate of isolated inner cell masses transferred to the

oviduct. Journal of embryology and experimental morphology *33*, 991-1001.

Rossant, J. (1976). Postimplantation development of blastomeres isolated from 4- and 8-cell mouse eggs. Journal of embryology and experimental morphology *36*, 283-290.

Rouleau, G.A., Merel, P., Lutchman, M., Sanson, M., Zucman, J., Marineau, C., Hoang-Xuan, K., Demczuk, S., Desmaze, C., Plougastel, B., *et al.* (1993). Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2. Nature *363*, 515-521.

Rovo, A., and Gratwohl, A. (2008). Plasticity after allogeneic hematopoietic stem cell transplantation. Biological chemistry *389*, 825-836.

Ruiz, S., Brennand, K., Panopoulos, A.D., Herrerias, A., Gage, F.H., and Izpisua-Belmonte, J.C. (2010). High-efficient generation of induced pluripotent stem cells from human astrocytes. PLoS One *5*, e15526.

Saburi, S., Hester, I., Fischer, E., Pontoglio, M., Eremina, V., Gessler, M., Quaggin, S.E., Harrison, R., Mount, R., and McNeill, H. (2008). Loss of Fat4 disrupts PCP signaling and oriented cell division and leads to cystic kidney disease. Nature genetics *40*, 1010-1015.

Sanchez, M.J., Holmes, A., Miles, C., and Dzierzak, E. (1996). Characterization of the first definitive hematopoietic stem cells in the AGM and liver of the mouse embryo. Immunity *5*, 513-525.

Sanford, L.P., Kallapur, S., Ormsby, I., and Doetschman, T. (2001). Influence of genetic background on knockout mouse phenotypes. Methods Mol Biol *158*, 217-225.

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

Schajnovitz, A., Itkin, T., D'Uva, G., Kalinkovich, A., Golan, K., Ludin, A., Cohen, D., Shulman, Z., Avigdor, A., Nagler, A., *et al.* (2011). CXCL12 secretion by bone marrow stromal cells is dependent on cell contact and

mediated by connexin-43 and connexin-45 gap junctions. Nature immunology *12*, 391-398.

Schofield, R. (1978). The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood cells *4*, 7-25.

Seidel, C., Schagdarsurengin, U., Blumke, K., Wurl, P., Pfeifer, G.P., Hauptmann, S., Taubert, H., and Dammann, R. (2007). Frequent hypermethylation of MST1 and MST2 in soft tissue sarcoma. Molecular carcinogenesis *46*, 865-871.

Sekido, Y. (2010). Genomic abnormalities and signal transduction dysregulation in malignant mesothelioma cells. Cancer science *101*, 1-6.

Senner, C.E., and Hemberger, M. (2010). Regulation of early trophoblast differentiation - lessons from the mouse. Placenta *31*, 944-950.

Shen, Q., Goderie, S.K., Jin, L., Karanth, N., Sun, Y., Abramova, N., Vincent, P., Pumiglia, K., and Temple, S. (2004). Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. Science *304*, 1338-1340.

Shi, Y. (2003). Mammalian RNAi for the masses. Trends in genetics : TIG *19*, 9-12.

Shimabe, M., Goyama, S., Watanabe-Okochi, N., Yoshimi, A., Ichikawa, M., Imai, Y., and Kurokawa, M. (2009). Pbx1 is a downstream target of Evi-1 in hematopoietic stem/progenitors and leukemic cells. Oncogene *28*, 4364-4374.

Silva, E., Tsatskis, Y., Gardano, L., Tapon, N., and McNeill, H. (2006). The tumor-suppressor gene fat controls tissue growth upstream of expanded in the hippo signaling pathway. Curr Biol *16*, 2081-2089.

Silver, L., and Palis, J. (1997). Initiation of murine embryonic erythropoiesis: a spatial analysis. Blood *89*, 1154-1164.

Siminovitch, L., McCulloch, E.A., and Till, J.E. (1963). The Distribution of Colony-Forming Cells among Spleen Colonies. Journal of cellular physiology *62*, 327-336.

Simon, M.A., Xu, A., Ishikawa, H.O., and Irvine, K.D. (2010). Modulation of fat:dachsous binding by the cadherin domain kinase four-jointed. Curr Biol *20*, 811-817.

Simons, B.D., and Clevers, H. (2011). Stem cell self-renewal in intestinal crypt. Experimental cell research *317*, 2719-2724.

Simpson, P., and Morata, G. (1981). Differential mitotic rates and patterns of growth in compartments in the Drosophila wing. Developmental biology *85*, 299-308.

Sopko, R., Silva, E., Clayton, L., Gardano, L., Barrios-Rodiles, M., Wrana, J., Varelas, X., Arbouzova, N.I., Shaw, S., Saburi, S.*, et al.* (2009). Phosphorylation of the tumor suppressor fat is regulated by its ligand Dachsous and the kinase discs overgrown. Curr Biol *19*, 1112-1117.

Spradling, A., Drummond-Barbosa, D., and Kai, T. (2001). Stem cells find their niche. Nature *414*, 98-104.

St John, M.A., Tao, W., Fei, X., Fukumoto, R., Carcangiu, M.L., Brownstein, D.G., Parlow, A.F., McGrath, J., and Xu, T. (1999). Mice deficient of Lats1 develop soft-tissue sarcomas, ovarian tumours and pituitary dysfunction. Nature genetics *21*, 182-186.

Steinhardt, A.A., Gayyed, M.F., Klein, A.P., Dong, J., Maitra, A., Pan, D., Montgomery, E.A., and Anders, R.A. (2008). Expression of Yes-associated protein in common solid tumors. Human pathology *39*, 1582-1589.

Stevens, L.C., and Little, C.C. (1954). Spontaneous Testicular Teratomas in an Inbred Strain of Mice. Proceedings of the National Academy of Sciences of the United States of America *40*, 1080-1087.

Strano, S., Monti, O., Pediconi, N., Baccarini, A., Fontemaggi, G., Lapi, E., Mantovani, F., Damalas, A., Citro, G., Sacchi, A.*, et al.* (2005). The transcriptional coactivator Yes-associated protein drives p73 gene-target specificity in response to DNA Damage. Molecular cell *18*, 447-459.

Strano, S., Munarriz, E., Rossi, M., Castagnoli, L., Shaul, Y., Sacchi, A., Oren, M., Sudol, M., Cesareni, G., and Blandino, G. (2001). Physical interaction with Yes-associated protein enhances p73 transcriptional activity. The Journal of biological chemistry *276*, 15164-15173.

Sugiyama, D., Kulkeaw, K., Mizuochi, C., Horio, Y., and Okayama, S. (2011). Hepatoblasts comprise a niche for fetal liver erythropoiesis through cytokine production. Biochemical and biophysical research communications *410*, 301-306.

Sugiyama, T., Kohara, H., Noda, M., and Nagasawa, T. (2006). Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity *25*, 977-988.

Suwinska, A., Czolowska, R., Ozdzenski, W., and Tarkowski, A.K. (2008). Blastomeres of the mouse embryo lose totipotency after the fifth cleavage division: expression of Cdx2 and Oct4 and developmental potential of inner and outer blastomeres of 16- and 32-cell embryos. Developmental biology *322*, 133-144.

Taichman, R.S., and Emerson, S.G. (1996). Human osteosarcoma cell lines MG-63 and SaOS-2 produce G-CSF and GM-CSF: identification and partial characterization of cell-associated isoforms. Experimental hematology *24*, 509-517.

Taichman, R.S., Reilly, M.J., and Emerson, S.G. (1996). Human osteoblasts support human hematopoietic progenitor cells in vitro bone marrow cultures. Blood *87*, 518-524.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *126*, 663-676.

Takahashi, Y., Miyoshi, Y., Morimoto, K., Taguchi, T., Tamaki, Y., and Noguchi, S. (2007). Low LATS2 mRNA level can predict favorable response to epirubicin plus cyclophosphamide, but not to docetaxel, in breast cancers. Journal of cancer research and clinical oncology *133*, 501-509.

Takahashi, Y., Miyoshi, Y., Takahata, C., Irahara, N., Taguchi, T., Tamaki, Y., and Noguchi, S. (2005). Down-regulation of LATS1 and LATS2 mRNA expression by promoter hypermethylation and its association with biologically aggressive phenotype in human breast cancers. Clin Cancer Res *11*, 1380-1385.

Takeda, N., Jain, R., LeBoeuf, M.R., Wang, Q., Lu, M.M., and Epstein, J.A. (2011). Interconversion between intestinal stem cell populations in distinct niches. Science *334*, 1420-1424.

Tapon, N., Harvey, K.F., Bell, D.W., Wahrer, D.C., Schiripo, T.A., Haber, D.A., and Hariharan, I.K. (2002). salvador Promotes both cell cycle exit and apoptosis in Drosophila and is mutated in human cancer cell lines. Cell *110*, 467-478.

Tarkowski, A.K., and Rossant, J. (1976). Haploid mouse blastocysts developed from bisected zygotes. Nature *259*, 663-665.

Tarkowski, A.K., Suwinska, A., Czolowska, R., and Ozdzenski, W. (2010). Individual blastomeres of 16- and 32-cell mouse embryos are able to develop into foetuses and mice. Developmental biology *348*, 190-198.

Tarkowski, A.K., and Wroblewska, J. (1967). Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. Journal of embryology and experimental morphology *18*, 155-180.

Taupin, P., and Gage, F.H. (2002). Adult neurogenesis and neural stem cells of the central nervous system in mammals. Journal of neuroscience research *69*, 745-749.

Tegelenbosch, R.A., and de Rooij, D.G. (1993). A quantitative study of spermatogonial multiplication and stem cell renewal in the C3H/101 F1 hybrid mouse. Mutation research *290*, 193-200.

Thomas, K.R., and Capecchi, M.R. (1987). Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. Cell *51*, 503-512.

Thoren, L.A., Liuba, K., Bryder, D., Nygren, J.M., Jensen, C.T., Qian, H., Antonchuk, J., and Jacobsen, S.E. (2008). Kit regulates maintenance of quiescent hematopoietic stem cells. J Immunol *180*, 2045-2053.

Till, J.E., and Mc, C.E. (1961). A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. Radiat Res *14*, 213-222.

Tiscornia, G., Vivas, E.L., and Belmonte, J.C. (2011). Diseases in a dish: modeling human genetic disorders using induced pluripotent cells. Nature medicine *17*, 1570-1576.

Tran, J., Brenner, T.J., and DiNardo, S. (2000). Somatic control over the germline stem cell lineage during Drosophila spermatogenesis. Nature *407*, 754-757.

Trofatter, J.A., MacCollin, M.M., Rutter, J.L., Murrell, J.R., Duyao, M.P., Parry, D.M., Eldridge, R., Kley, N., Menon, A.G., Pulaski, K*., et al.* (1993). A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. Cell *75*, 826.

Tropepe, V., Coles, B.L., Chiasson, B.J., Horsford, D.J., Elia, A.J., McInnes, R.R., and van der Kooy, D. (2000). Retinal stem cells in the adult mammalian eye. Science *287*, 2032-2036.

Tumbar, T. (2012). Ontogeny and Homeostasis of Adult Epithelial Skin Stem Cells. Stem cell reviews.

Tyler, D.M., and Baker, N.E. (2007). Expanded and fat regulate growth and differentiation in the Drosophila eye through multiple signaling pathways. Developmental biology *305*, 187-201.

Tyler, D.M., Li, W., Zhuo, N., Pellock, B., and Baker, N.E. (2007). Genes affecting cell competition in Drosophila. Genetics *175*, 643-657.

Udan, R.S., Kango-Singh, M., Nolo, R., Tao, C., and Halder, G. (2003). Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway. Nature cell biology *5*, 914-920.

van der Meer, L.T., Jansen, J.H., and van der Reijden, B.A. (2010). Gfi1 and Gfi1b: key regulators of hematopoiesis. Leukemia *24*, 1834-1843.

van Praag, H., Schinder, A.F., Christie, B.R., Toni, N., Palmer, T.D., and Gage, F.H. (2002). Functional neurogenesis in the adult hippocampus. Nature *415*, 1030-1034.

Varelas, X., Sakuma, R., Samavarchi-Tehrani, P., Peerani, R., Rao, B.M., Dembowy, J., Yaffe, M.B., Zandstra, P.W., and Wrana, J.L. (2008). TAZ controls Smad nucleocytoplasmic shuttling and regulates human embryonic stem-cell self-renewal. Nature cell biology *10*, 837-848.

Varelas, X., Samavarchi-Tehrani, P., Narimatsu, M., Weiss, A., Cockburn, K., Larsen, B.G., Rossant, J., and Wrana, J.L. (2010). The Crumbs complex couples cell density sensing to Hippo-dependent control of the TGF-beta-SMAD pathway. Developmental cell *19*, 831-844.

Vassilev, A., Kaneko, K.J., Shu, H., Zhao, Y., and DePamphilis, M.L. (2001). TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm. Genes & development *15*, 1229-1241.

Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., *et al.* (2001). The sequence of the human genome. Science *291*, 1304-1351.

Venter, J.C., Adams, M.D., Sutton, G.G., Kerlavage, A.R., Smith, H.O., and Hunkapiller, M. (1998). Shotgun sequencing of the human genome. Science *280*, 1540-1542.

Wang, K., Degerny, C., Xu, M., and Yang, X.J. (2009). YAP, TAZ, and Yorkie: a conserved family of signal-responsive transcriptional coregulators in animal development and human disease. Biochem Cell Biol *87*, 77-91.

Widmaier, E.P., Raff, H., Strang, K.T., and Vander, A.J. (2004). Vander, Sherman, & Luciano's human physiology : the mechanisms of body function, 9th edn (Boston, McGraw-Hill Higher Education).

Willecke, M., Hamaratoglu, F., Kango-Singh, M., Udan, R., Chen, C.L., Tao, C., Zhang, X., and Halder, G. (2006). The fat cadherin acts through the hippo tumor-suppressor pathway to regulate tissue size. Curr Biol *16*, 2090-2100.

Wilson, C., Bellen, H.J., and Gehring, W.J. (1990). Position effects on eukaryotic gene expression. Annual review of cell biology *6*, 679-714.

Winkler, I.G., Barbier, V., Wadley, R., Zannettino, A.C., Williams, S., and Levesque, J.P. (2010). Positioning of bone marrow hematopoietic and stromal cells relative to blood flow in vivo: serially reconstituting hematopoietic stem cells reside in distinct nonperfused niches. Blood *116*, 375-385.

Wu, S., Huang, J., Dong, J., and Pan, D. (2003). hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with salvador and warts. Cell *114*, 445-456.

Wu, S., Liu, Y., Zheng, Y., Dong, J., and Pan, D. (2008). The TEAD/TEF family protein Scalloped mediates transcriptional output of the Hippo growth-regulatory pathway. Developmental cell *14*, 388-398.

Xie, T., and Spradling, A.C. (2000). A niche maintaining germ line stem cells in the Drosophila ovary. Science *290*, 328-330.

Xie, Y., Yin, T., Wiegraebe, W., He, X.C., Miller, D., Stark, D., Perko, K., Alexander, R., Schwartz, J., Grindley, J.C.*, et al.* (2009). Detection of functional haematopoietic stem cell niche using real-time imaging. Nature *457*, 97-101.

Xu, M.Z., Yao, T.J., Lee, N.P., Ng, I.O., Chan, Y.T., Zender, L., Lowe, S.W., Poon, R.T., and Luk, J.M. (2009). Yes-associated protein is an independent prognostic marker in hepatocellular carcinoma. Cancer *115*, 4576-4585.

Xu, T., Wang, W., Zhang, S., Stewart, R.A., and Yu, W. (1995). Identifying tumor suppressors in genetic mosaics: the Drosophila lats gene encodes a putative protein kinase. Development (Cambridge, England) *121*, 1053-1063.

Xu, X., D'Hoker, J., Stange, G., Bonne, S., De Leu, N., Xiao, X., Van de Casteele, M., Mellitzer, G., Ling, Z., Pipeleers, D.*, et al.* (2008). Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell *132*, 197-207.

Yabuta, N., Okada, N., Ito, A., Hosomi, T., Nishihara, S., Sasayama, Y., Fujimori, A., Okuzaki, D., Zhao, H., Ikawa, M., *et al.* (2007). Lats2 is an essential mitotic regulator required for the coordination of cell division. The Journal of biological chemistry *282*, 19259-19271.

Yu, J., Zheng, Y., Dong, J., Klusza, S., Deng, W.M., and Pan, D. (2010). Kibra functions as a tumor suppressor protein that regulates Hippo signaling in conjunction with Merlin and Expanded. Developmental cell *18*, 288-299.

Yuan, J.Y., and Horvitz, H.R. (1990). The Caenorhabditis elegans genes ced-3 and ced-4 act cell autonomously to cause programmed cell death. Developmental biology *138*, 33-41.

Zhang, H., Liu, C.Y., Zha, Z.Y., Zhao, B., Yao, J., Zhao, S., Xiong, Y., Lei, Q.Y., and Guan, K.L. (2009a). TEAD transcription factors mediate the function of TAZ in cell growth and epithelial-mesenchymal transition. The Journal of biological chemistry *284*, 13355-13362.

Zhang, H., Pasolli, H.A., and Fuchs, E. (2011). Yes-associated protein (YAP) transcriptional coactivator functions in balancing growth and differentiation in skin. Proceedings of the National Academy of Sciences of the United States of America *108*, 2270-2275.

Zhang, J., Niu, C., Ye, L., Huang, H., He, X., Tong, W.G., Ross, J., Haug, J., Johnson, T., Feng, J.Q., *et al.* (2003). Identification of the haematopoietic stem cell niche and control of the niche size. Nature *425*, 836-841.

Zhang, S.C., Wernig, M., Duncan, I.D., Brustle, O., and Thomson, J.A. (2001). In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nature biotechnology *19*, 1129-1133.

Zhang, X., Milton, C.C., Humbert, P.O., and Harvey, K.F. (2009b). Transcriptional output of the Salvador/warts/hippo pathway is controlled in distinct fashions in Drosophila melanogaster and mammalian cell lines. Cancer research *69*, 6033-6041.

Zhao, B., Kim, J., Ye, X., Lai, Z.C., and Guan, K.L. (2009). Both TEAD-binding and WW domains are required for the growth stimulation and

oncogenic transformation activity of yes-associated protein. Cancer research *69*, 1089-1098.

Zhao, B., Tumaneng, K., and Guan, K.L. (2011). The Hippo pathway in organ size control, tissue regeneration and stem cell self-renewal. Nature cell biology *13*, 877-883.

Zhao, B., Wei, X., Li, W., Udan, R.S., Yang, Q., Kim, J., Xie, J., Ikenoue, T., Yu, J., Li, L., *et al.* (2007). Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. Genes & development *21*, 2747-2761.

Zhao, B., Ye, X., Yu, J., Li, L., Li, W., Li, S., Lin, J.D., Wang, C.Y., Chinnaiyan, A.M., Lai, Z.C., *et al.* (2008). TEAD mediates YAP-dependent gene induction and growth control. Genes & development *22*, 1962-1971.

Zhao, D., Zhi, X., Zhou, Z., and Chen, C. (2012). TAZ antagonizes the WWP1-mediated KLF5 degradation and promotes breast cell proliferation and tumorigenesis. Carcinogenesis *33*, 59-67.

Zhao, R., and Xi, R. (2010). Stem cell competition for niche occupancy: emerging themes and mechanisms. Stem cell reviews *6*, 345-350.

Zhou, D., Conrad, C., Xia, F., Park, J.S., Payer, B., Yin, Y., Lauwers, G.Y., Thasler, W., Lee, J.T., Avruch, J., *et al.* (2009). Mst1 and Mst2 maintain hepatocyte quiescence and suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. Cancer cell *16*, 425-438.

Zhou, Z., Hao, Y., Liu, N., Raptis, L., Tsao, M.S., and Yang, X. (2011). TAZ is a novel oncogene in non-small cell lung cancer. Oncogene *30*, 2181-2186.