Chapter Number

Adult Stem Cells in Tissue Homeostasis and Disease

Elena Lazzeri, Anna Peired, Lara Ballerini and Laura Lasagni
University of Florence,
Italy

1. Introduction

Stem cells (SCs) are a rare population of cells characterized by the ability to self-renew in order to preserve the SC pool and to differentiate in different lineage to produce progeny needed for the physiological functions of tissues and organs. SC can be classified as embryonic SC (ESC) and adult or somatic SC (ASC): ESC have been isolated from the inner cell mass of the blastocyst and are pluripotent cells, that is cells able to differentiate into all the cell types required to form an entire organism (Smith, 2001); ASC are tissue-resident SC that, based on their differentiation potency, can be classified as multipotent, oligopotent or even unipotent. It is still controversial whether every mammalian tissue and organ possesses an ASC, but many tissue-specific ASC have been successfully identified and isolated e.g., hematopoietic SCs (HSCs), mammary SCs, muscle SCs (satellite cells), intestinal SCs, and mesenchymal SCs. All these tissues need to constantly replace damaged or dead cells throughout the life of the animal. This process of continual cell replacement critical for the maintenance of adult tissues, is called tissue homeostasis, and is maintained through the presence of ASC (Fig. 1). The homeostatic replacement of cells varies substantially among different tissues. The epithelium of the intestine is one of the most rapidly self-renewing tissue in adult mammals and it completely self-renews in around 5 days (van der Flier & Clevers, 2009). By contrast, interfollicular epidermis takes 4 weeks to renew (Blanpain & Fuchs, 2009), whereas the lung epithelium can take as long as 6 months to be replaced (Rawlins & Hogan, 2006). Moreover, apart from the maintenance of tissue homeostasis, ASC are devoted to the regeneration and repair of highly specialized tissues. Regeneration refers to the proliferation of cells to replace lost structures, such as the growth of an amputated limb in amphibians. In mammals, whole organs and complex tissues rarely regenerate after injury, but tissues with high proliferative capacity, such as the hematopoietic system and the epithelia of the skin and gastrointestinal tract, renew themselves continuously and can regenerate after injury, as long as the SC of these tissues are not destroyed (Fig. 1). Repair most often consist of a combination of regeneration and scar formation by the deposition of collagen which relative contribution depends on the ability of the tissue to regenerate and the extent of the injury. For instance, in superficial injury of the skin, wound can heal through the regeneration of the surface epithelium. However, scar formation is the predominant healing process that occurs when the extracellular matrix framework is damaged by severe injury (Fig. 1). This last mechanism results in restoration of tissue continuity but with or without function (Gurtner et al., 2008).

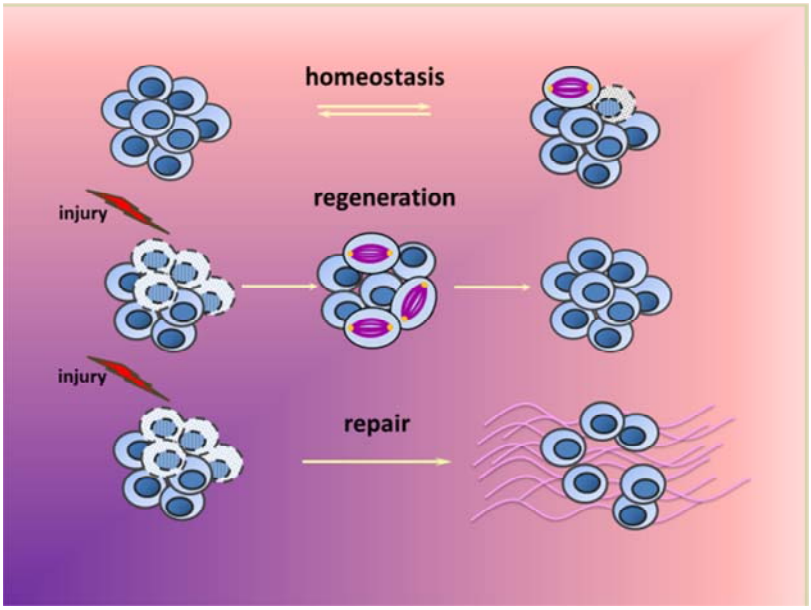

Fig. 1. Normal homeostasis and healing responses. In normal homeostasis a balance between proliferation and cell death maintains the tissue structure and function. Healing after acute injury can occur by regeneration, that restores normal tissue structure, or repair with deposition of collagen fibers and scar formation.

2. SCs and their niches

Self-renewal and differentiation of ASC are supported by two types of cell division known as symmetric and asymmetric (Morrison & Kimble, 2006). With symmetric division both the daughter cells acquire similar fates, while the asymmetric division, a fundamental and nearly universal mechanism for the generation of cellular diversity and pattern, gives rise to daughter cells with dissimilar fates. Divergent fates in daughter cells may be recognized by various characteristics: (i) morphological, such as cell size and shape; (ii) molecular, such as the segregation of proteins into only one daughter cell; or (iii) behavioural, such as the subsequent descendant types produced by either of the daughter cells. One mechanism for fate determination of daughter cells following symmetric and asymmetric cell divisions is the partitioning of fate-determining molecules during mitosis of the mother cell (Tajbakhsh et al., 2009). The idea that specific molecules can be partitioned unequally to daughter cells and behave as fate determinants had been hypothesized over a century earlier, following observations of cell divisions in simple organisms. When an intrinsic mechanism is used, cells establish an axis of polarity, orient the mitotic spindle along this axis and localize cell fate determinants to one side of the cell. During cytokinesis, determinants are then segregated into one of the two daughter cells where they direct cell fate (Betschinger & Knoblich, 2004). However, this hypothesis was only experimentally validated a little under two decades ago, with the identification of the first asymmetrically segregated cell fate determinant – Numb (Rhyu et al., 1994).

Alternatively, the SC depends on the contact with the surrounding microenvironment (the SC "niche") for maintaining the potential to self-renew (Li & Xie, 2005). By orienting its mitotic spindle perpendicularly to the niche surface, the SC will placed the two daughters in distinct cellular environments either inside or outside the SC niche, leading to asymmetric fate choice. However, when SC divides parallel to the niche it may also generate two identical SC in order to increase SC number or to compensate for occasional SC loss (Yamashita et al, 2010). The concept of the "niche" was proposed first by Schofield (Schofield, 1978) who hypothesized that proliferative, hematopoietic cells derived from the spleen displayed decreased proliferative potential when compared to HSC obtained from the bone marrow because they were no longer in association with a complement of cells, the "niche", which supports long term SC activity. This concept subsequently has proven relevant to many different SC systems, and the definition of the niche has been expanded further to include functional regulation of SC by both cellular and acellular (extracellular matrix) component of the niche. Thus the niche comprise all the microenvironment surrounding SCs, which provides diverse external cues to instruct SCs activities, preserve their proliferative potential and block maturation (Jones & Wagers, 2008).

3. Signaling pathways regulating SC function

Despite morphological and functional differences among different ASC, common signaling pathways appear to control SC self-renewal, activation, and differentiation, including Notch and Wingless-type (Wnt).

3.1 Notch signaling pathway

The Notch signaling pathway was discovered in flies more than 90 years ago (Morgan, 1917), and it is among the most well-conserved signaling pathways in animals. It arose with the evolution of multicellular organisms and the concomitant need for juxtacrine cell-to-cell communication to coordinate development. In mammals, four Notch transmembrane receptors (Notch1-4) have been described. Notch ligands are also transmembrane proteins comprising two different subtypes (Delta, Jagged), each containing several members (Jagged1-2, Delta-like1, 3, and 4) (Kopan & Ilagan, 2009). In Notch signaling, a 'signal-sending cell' presents the Notch ligand to the 'signal-receiving cell', which expresses the Notch receptor. Triggering of Notch receptor by ligand binding promotes two proteolytic cleavage events at the Notch receptor (Fig. 2) (Kopan & Ilagan, 2009). The first cleavage is catalyzed by the ADAM-family of metalloproteases, whereas the second cleavage is mediated by γ-secretase, an enzyme complex that contains presenilin, nicastrin, PEN2 and APH1. The second cleavage releases the Notch intracellular domain (NICD), which is free to translocate to the nucleus where it engages CSL, converting it from a transcriptional repressor to an activator and activates transcription of genes containing CSL binding sites (Kopan & Ilagan, 2009). In the absence of a Notch signal, CSL represses transcription of Notch target genes by interacting with the basal transcription machinery and recruiting ubiquitous corepressor proteins to form multiprotein transcriptional repressor complexes (Lai, 2002). In the presence of a Notch signal, NICD binding to CSL displaces corepressors from CSL. The best characterized Notch target genes belong to the hairy enhancer of split (Hes) complex and consist of the b-HLH transcription factors Hes (1-7) and Hey (1-3) (Bray & Bernard, 2010).

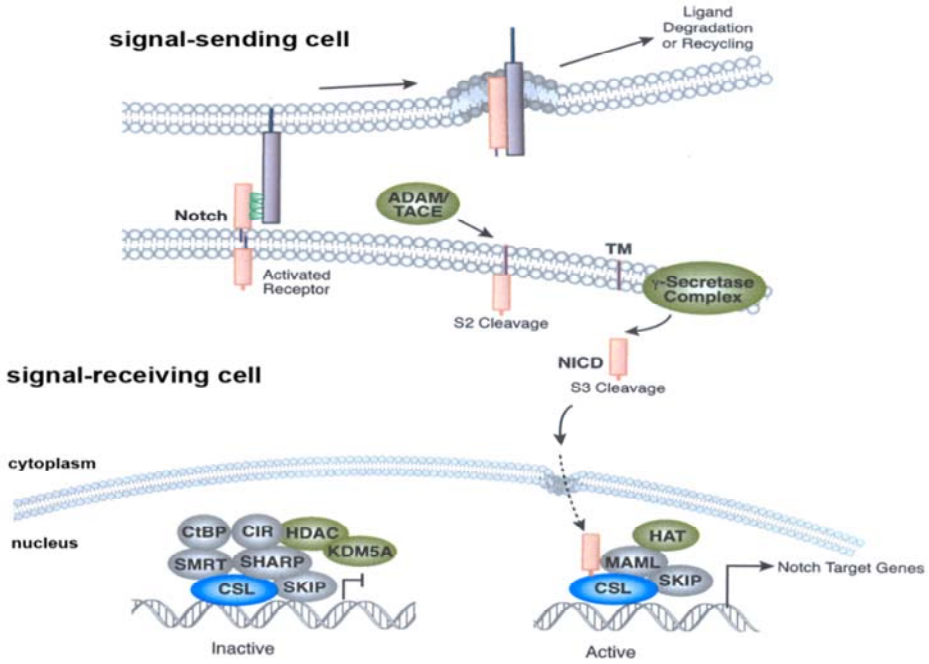

Fig. 2. Model of Notch signaling pathway. See the text for detail.

3.2 Wnt signaling pathway

The Wnt signaling pathway is a highly conserved developmental pathway, and orchestrates development and morphogenesis in many different tissues. Wnt proteins are secreted proteins, that bind to receptors of the Frizzled family (FZD) (Wodarz & Nusse, 1998), of which 10 members were found, and several coreceptors such as lipoprotein receptor-related protein (LRP)-5/6, (Pinson et al., 2000) Ryk, or Ror2 (Logan & Nusse, 2004). Wnt signals can be transduced to the canonical, or Wnt/β-catenin, pathway and to the noncanonical, or β-catenin independent, pathway.

3.2.1 Canonical Wnt signaling pathway

The canonical Wnt pathway involves the multifunctional protein β-catenin (MacDonald et al., 2009). In the absence of Wnt, β-catenin is targeted to a multimeric destruction complex with adenomatous polyposis coli (APC) and Axin and is phosphorylated by casein kinase 1α, followed by phosphorylation by glycogen synthase kinase (GSK)3β (Fig.34) (Ikeda et al., 1998). This phosphorylation targets β-catenin for ubiquitination and degradation by the proteasome. The binding of Wnt ligands to the FZD receptors results in the disassembly of the destruction complex and the stabilization of β-catenin. This process also involves the protein dishevelled (DVL). Cytoplasmic β-catenin accumulates and is eventually imported into the nucleus, where it serves as a transcriptional coactivator of transcription factors of the TCF/LEF family (Arce et al., 2006). TCF/LEF target genes are then involved in regulating cell proliferation, SC maintenance, or differentiation.

3.2.2 Noncanonical Wnt signaling pathway

Different noncanonical Wnt signals are transduced through FZD receptors and coreceptors. Depending on the major intracellular mediators used, those are called the Wnt/JNK (Veeman et al., 2003) or Wnt/calcium pathway (Fig. 3). The core element of the Wnt/JNK pathway (or planar cell polarity –PCP- pathway) includes the activation of small GTPases of the rho family, such as rac, cdc42, and rhoA. The GTPases can activate more downstream mediators like JNK or rho kinase (ROK). In this branch, Dvl is also recruited by a FZD receptor and promotes the asymmetrical localization of the PCP core proteins within the cell (Montcouquiol, et al. 2006). The asymmetrical subcellular localization of these elements in an epithelial sheet directs cytoskeletal reorganization. The same mechanism is used in mesenchymal cells to direct cell movement and migration during gastrulation (convergent and extension movements) (Roszko, et al., 2009).

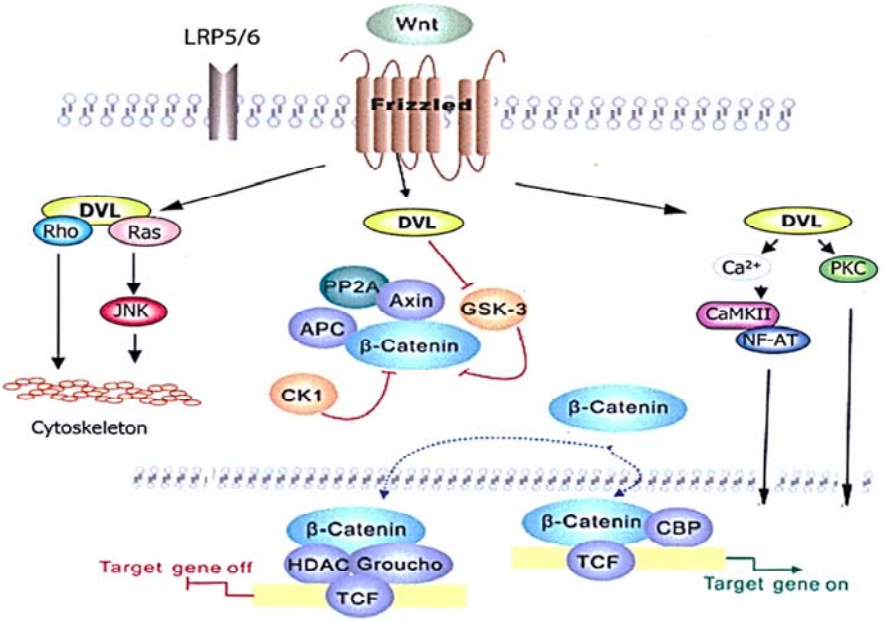

Fig. 3. Model of canonical and noncanonical Wnt signaling pathway. See the text for detail.

The existence of the Wnt/calcium pathway was hypothesized because injection of RNA coding for certain Wnts or FZD into early zebrafish embryos triggered intracellular calcium release (Slusarski et al., 1997) and loss of Wnt-11 or Wnt-5A function resulted in reduced intracellular calcium signaling (Eisenberg & Eisenberg, 1999; Westfall et al., 2003). This finding was subsequently expanded by the observation that the Wnt-induced release of intracellular calcium is sufficient to activate different intracellular calcium-sensitive enzymes such as protein kinase C, PKC (Sheldahl et al., 1999), calcium–calmodulin-dependent kinase II, CamKII (Kuhl et al., 2000) and the calcium-sensitive phosphatase calcineurin (Saneyoshi et al., 2002). Through calcineurin the Wnt/calcium pathway connects to NFAT (nuclear factor of activated T cells) transcription factor and gene expression.

Presently, a series of recent findings clearly indicate that different Wnt signaling pathways are simultaneously active within the same cell type, supporting the idea that Wnt pathways are highly connected to form a Wnt signaling network. This network seems to be activated by either one or more ligands acting on a certain cell type (Kestler & Kuhl, 2008).

3.3 Wnt signaling inhibitors

Secreted frizzled-related proteins (SFRP1, 2, 3, 4, 5), WIF1, DKK1, -2, -3, and -4 are secreted-type Wnt signaling inhibitors. WIFs and SFRPs can directly bind to Wnt proteins in the extracellular space, thereby affecting receptor occupancy and, ultimately, the cellular response (Bovolenta et al., 2008). DKK1 is among the best-characterized inhibitors of the canonical Wnt pathway. DKK1 itself is a target gene of Wnt/β-catenin signaling, thereby establishing a negative-feedback loop (Niida et al., 2004). There are two possible mechanisms by which DKK1 inhibits β-catenin signaling. One possible mechanism is that DKK1 prevents the formation of Wnt–FZD–LRP6 complexes on the cell surface by binding to LRP6 (Seto et al., 2006). Another possibility, which is related to the internalization of LRP6, is that DKK1 binds to another class of receptor, Kremen (Krm). In this model, the binding of DKK1 to LRP6 and Krm results in the formation of a ternary structure and induces rapid endocytosis and the removal of LRP6 from the plasma membrane, and thereby attenuates β-catenin signaling (Mao et al., 2002).

4. Hematopoietic SCs

In adult mammals, HSCs form a rare population of multipotent SCs that reside primarily in the bone marrow (BM). They have the capability to both self-renew and constantly give rise to lineage-specific progenitor cells and effector blood cells that perform the physiological functions of the hematopoietic system. Blood cells can be classified into various cell types, from the myeloid (monocytes and macrophages, neutrophils, basophils, eosinophils, erythrocytes, megakaryocytes/platelets, dendritic cells), and lymphoid lineages (T-cells, B-cells, NK-cells) (Liu et al., 2010).

HSCs are functionally defined by their capacity to reconstitute the hematopoietic system of immunodeficient animals such as NOD/SCID mice or contribute to functional reconstitution in human transplant settings. HSCs can be identified and isolated by a combination of presence and absence of cell surface markers. The most commonly used combination is characterized by the positive expression of the tyrosine kinase receptor c-Kit (CD117) and the membrane glycoprotein Sca-1 (Okada et al., 1992), together with the lack of markers of terminal differentiation (Ter119, Gr-1, Mac-1, B220, CD4 and CD8), collectively known as Lineage markers. The resulting c-Kit+ Sca-1+ Lin- population, is commonly referred to as KSL cells. More recently, an alternative method was described, using a signature of SLAM (Signaling lymphocyte activation molecule) family of cell surface molecules, CD150+ CD244- CD48- (Kiel et al, 2005). This is the first family of receptors whose combinatorial expression precisely distinguishes HSCs from hematopoietic progenitor cells (HPC).

The BM microenvironment –also called niche- plays an important role in the regulation of self-renewal and differentiation of HSCs. It is composed of different types of cells and structures surrounding the bone, which regulates the fate of hematopoietic cells through

direct or indirect means, facilitating a stable generation of all the blood cells needed in a steady state situation. But the niche also adapts in times of hematopoietic stress. A failure to maintain a strict regulation of the hematopoietic cells can lead to a variety of malignancies such as leukemia, the most common form of cancer in humans (Renstrom et al., 2010).

4.1 Notch pathway as a regulator of HSC behavior

All Notch receptors and ligands are expressed on HSCs (Singh et al., 2000) and it is now well established that Notch signaling is essential for the production of HSCs during embryogenesis. However, its role in subsequent stages of mammalian HSC development is still controversial (Liu et al, 2010; Radtke et al., 2010).

In adult hematopoiesis, activation of Notch signaling has been reported to promote HSCs self-renewal, proliferation and differentiation *in vitro* and *in vivo*, and in both mice and humans. Constitutive expression of NICD by HSCs, leading to the constitutive activation of the Notch pathway, enhances proliferation and consequently delays hematopoiesis. Conversely, it inhibits differentiation in response to various cytokines, mostly under myeloid promoting conditions (Carlesso et al, 1999). Several reports show that HSCs stimulated with soluble or membrane-bound Notch ligand Delta 1 (Karanu et al, 2001) or Jagged1 (Karanu et al. 2000) increase in expansion potential *in vitro* and in reconstitution capacity *in vivo*. Although these gain-of-function studies show an important role for Notch in expanding the HSC pool, they do not prove that Notch is essential for post-natal hematopoiesis. The controversy arises from several loss-of-function studies in mice that did not fully support the previous conclusions. In particular, inactivation of Notch receptors (Notch1, Notch2), ligands (Jagged1) or downstream effectors (CSL/RBPJ, Mastermind-like1) does not impair HSC function (Cerdan & Bhatia, 2010). Additional studies failed to identify a protective role for Notch when HSCs were exposed to oxidative stress. Taken together, these results show that Notch signaling is not a major regulator of adult HSC maintenance *in vivo*. Downstream of HSCs, Notch signaling plays a critical role in cell fate decision of a variety of oligopotent progenitor cells in the hematopoietic system, such as in T-cell development. Inactivation of Notch signaling in HPCs results in early blockade of T-cell lymphopoiesis, due to a failure in commitment to the T-cell lineage. Transgenic mice with a conditional deletion of Notch1 do not develop T-cells but develop ectopic B-cells in the thymus, while immunodeficient mice expressing a constitutively active form of Notch1 develop ectopic T-cells in the bone marrow (BM) but no B-cell (Tanigaki & Honjo, 2007). Additionally, Notch1 signaling is necessary at various stages of T-cell development, such as progression through thymocyte maturation, regulation of T-cell Receptor β (TCR-β) gene rearrangement, regulation of lineage decisions between αβ and γδ lineages (Tanigaki & Honjo, 2007).

4.2 Role of Notch in T-cell leukemia

The pathological role for a deregulated Notch signaling was first described in a rare human T-cell acute lymphoblastic leukaemia/lymphoma (T-ALL), in which a t(7;9) chromosomal translocation results in the generation of a constitutively active, but truncated form of the Notch1 receptor named TAN1 (Translocation Associated Notch homolog) (Ellisen et al., 1991). Evidence that constitutively active Notch1 is responsible for disease development was provided by murine BM reconstitution experiments. Irradiated mice transplanted with BM

progenitors expressing activated forms of Notch1 developed clonal hematopoietic tumors characterized as T-ALL. Experiments performed using other truncated Notch isoforms, including Notch2 and Notch3, showed similar results. However, mice having a defect in T-cell development failed to produce tumors. These results reveal that Notch1 has a special oncotropism for T-cell progenitors (Radtke et al., 2010). These findings became extremely relevant when a study of a large number of T-ALL patients revealed in more than 50% of them the presence of at least one gain-of-function mutation in the Notch1 receptor, emphasizing the oncogenic role of Notch (Weng et al., 2004). Notch1 mutations found in T-ALL affect critical domains responsible for preventing the spontaneous activation of the receptor in the absence of ligand or for terminating Notch1 signaling in the nucleus.

Studies of the genes and pathways controlled by Notch in T-ALL identified Notch1 as a central regulator, promoting leukemia cell growth by multiple direct and indirect mechanisms (Fig. 4) (Paganin & Ferrando, 2011). Analysis of Notch1 expression in T-ALL showed that it acts as a direct transcriptional activator of multiple genes. Notch1 also promotes the expression of the MYC oncogene, which in turn further enhances its direct effect on anabolic genes and facilitates cell growth. Indeed, many of the anabolic genes directly controlled by Notch1 are also direct targets of MYC, creating a feed-forward-loop transcriptional network that promotes leukemic cell growth (Palomero et al., 2006). Additionally, Notch1 facilitates the activation of the PI3K-AKT-mTOR signaling pathway, a critical regulator of cell growth and metabolism, via transcriptional downregulation of the PTEN tumor suppressor gene by Hes1, a transcriptional repressor directly downstream of Notch1 signaling (Palomero et al., 2007). The mTOR signaling was suppressed in T-ALL cells upon inhibition of Notch signaling, illustrating the importance of this indirect mechanism of regulation. The transcriptional program activated by oncogenic Notch1 also has a direct effect on cell cycle progression, promoting of G1/S cell cycle progression in T-ALL. This effect is mediated in part by transcriptional upregulation of CCND3, CDK4 and CDK6. Moreover, Notch1 induces the transcription of the S phase kinase-associated protein 2 (SKP2), which mediate the proteasomal degradation of CDKN1B (p27/Kip1) and CDKN1A (p21/Cip1), promoting premature entry of the cells into S phase (Sarmento et al, 2005). Notch1 can also modulate the survival of T-ALL cells by interacting with NF-κB, upregulating its activity by increasing expression of IκB kinase and upregulating both the expression and the nuclear localization of NF-κB. Inhibition of NF-κB in T-ALL can efficiently restrict tumor growth both *in vitro* and *in vivo* (Vilimas et al., 2007).

In addition, Notch1 modulates the NFAT cascade through the activation of calcineurin, which is a calcium-activated phosphatase that is important for the activation and translocation of NFAT factors to the nucleus. Calcineurin inhibition resulted in T-ALL cell death, as well as tumor regression and prolonged survival of leukemic mice (Medyouf et al., 2007). Finally, Notch1 regulates the activity of p53, lowering its expression through repression of the ARF-mdm2-p53 surveillance network. Attenuation of Notch signaling led to increase p53 expression and to tumor regression by inducing apoptosis (Beverly et al., 2005). A strong body of evidence supports a central role of Notch1 in promoting cell metabolism, growth and proliferation, as well as in enhancing the activity of signaling pathways that reinforce these functions and also promote cell survival. These results suggest that blocking Notch1 signaling may reduce the self-renewal capacity of T-ALL cells and/or selectively affect the leukemia initiating cell population.

Only few Notch mutations have been reported in myelogenous leukemias, but it is unclear whether Notch aberrant expression is responsible for the disease.

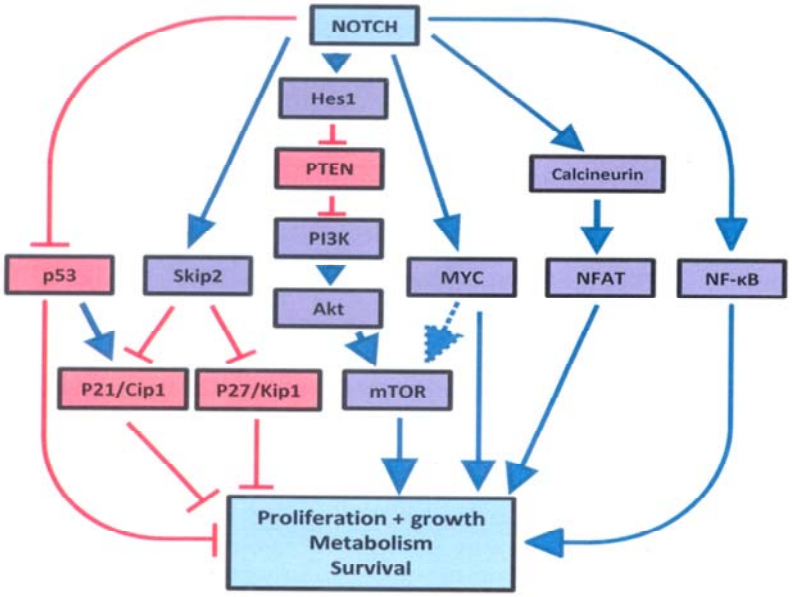

Fig. 4. Genes and pathways controlled by Notch in T-ALL

4.3 Wnt pathway and HSC

In hematopoiesis, Wnt pathway activity is required in the BM niche to regulate HSC proliferation and preserve self-renewal capacity (Malhotra & Kincade, 2009). Even though the role of canonical signaling on the regulation of adult hematopoiesis has been studied in great detail, controversy remains, possibly explained by differences in strength and duration of Wnt signaling or redundancy with other pathways. A role for Wnt signaling in hematopoiesis is supported by observations that Wnt ligands enhance proliferation of HSCs ex vivo (Van Den Berg et al, 1998) and that Wnt antagonists inhibit HSC proliferation and reconstitution. In particular, only short-term repopulation was reported using HSCs from normal mice cultured with Wnt3A (Reya et al., 2003; Willert et al., 2003). Subsequent studies reported that noncanonical Wnt5a inhibited canonical Wnt3a-mediated signaling to promote the maintenance of quiescent, functionally transplantable HSCs. In addition constitutively active nuclear β-catenin signaling reduces HSC quiescence and blocks HSC differentiation (Kirstetter et al., 2006). On the other hand, osteoblast-specific expression of Dkk1 results in increased HSC cycling and reduced regenerative capacity (Fleming et al, 2008). These findings suggest that Wnt pathway activation in the niche limits HSC proliferation and preserves self-renewal. These observations suggest that fine-tuning of Wnt/β-catenin activity in the microenvironment is crucial for maintaining SC quiescence.

The canonical Wnt pathway has also been shown to be necessary for appropriate HSC development (Zhao et al., 2008). In this model, Ctnnb1-/- bone marrow cells are deficient in long-term HSC maintenance and compete poorly against wild-type cells. However, experiments in adult HSC revealed that Ctnnb1 is dispensable for HSC maintenance in fully developed HSC (Koch et al., 2008). This indicates differential requirements for self-renewal pathways in development versus maintenance of HSC.

In the context of development, genetic studies have demonstrated the requirement for canonical signaling in the formation of mesoderm (Kelly et al., 2004; Liu et al., 1999). Recent advances have provided insights into the uniqueness of the biological functions of canonical and noncanonical pathways. It has been found that non-canonical and canonical Wnts affected different target populations and stages of hematopoietic development (Vijayaragavan et al., 2009). Consistent with its previously defined role in human adult cells (Van Den Berg et al., 1998), canonical signaling increased proliferation of blood committed progenitors when administered during the proper window of time during EB development. However, a short pulse of non-canonical signaling was necessary and sufficient to control exit of hESCs from the pluripotent state and subsequent entry into the mesendoderm/mesoderm lineages (Vijayaragavan et al., 2009). Taken together, these findings provide the first evidence of a unique role for non-canonical signaling in early specification of hematopoiesis from hESCs, whereas canonical signaling affects the proliferation of cells already fated to blood. These studies provide a valuable model system for examining the possibility of chronological activation and interaction between non-canonical and canonical signaling in the cellular progression from mesoderm to blood. The controversial function of canonical signaling on the reconstituting capacity of adult HSCs, combined with these present findings in hESCs, underscores the importance of fine tuning the strength and duration of Wnt signaling towards therapeutically exploiting the balance between self-renewal and lineage commitment of HSCs.

However, there are conflicting reports on the requirement for Wnt/β-catenin signaling in basal hematopoiesis: conditional disruption of β-catenin in adult HSCs does not affect their ability to self-renew and reconstitute hematopoietic lineages (Huang et al, 2009). In addition, although overexpression of stabilized β-catenin increases immunophenotypic HSCs, this is associated with a loss of repopulating activity and hematopoietic failure *in vivo* (Kirstetter et al., 2006), findings that appear incompatible with a positive role for β-catenin in hematopoiesis. A general conclusion from these apparently conflicting reports is that the role of Wnt signaling in hematopoiesis is complex and context dependent (Staal & Sen,. 2008). However, although the β-catenin loss-of-function studies suggest that canonical Wnt signaling is not essential for basal hematopoiesis in adults, they do not rule out a possible role for the Wnt/β-catenin pathway under nonbasal conditions and are still compatible with gain-of-function experiments in which the pathway is activated.

4.4 Wnt signaling and malignant HSC

Stem cell quiescence is closely associated with protection from myelotoxic insults (Cheshier et al, 1999). Similar to the role of tissue SCs in normal tissues, several cancers are also propagated by small populations of quiescent cancer stem cells (CSCs) that are resistant to both conventional chemotherapy and targeted therapies, and are retained and contribute to relapse following discontinuation of therapy (Dick, 2008).

When Ctnnb1 was deleted contemporaneously with activation of BCR-ABL using retroviral infection and transformation of HSC, chronic myeloid leukemia stem cell (CML-LSC) failed to engraft in secondary recipient mice (Hu Y et al., 2009). These experiments clearly indicate a pivotal role of Wnt signaling in CML-LSC development. More recently, Ctnnb1 has been investigated in the maintenance of already engrafted CML-LSC. In this clinically relevant setting, pharmacologic or genetic inactivation of Ctnnb1 after onset of the myeloproliferative disease acted synergistically with imatinib, reduced LSC numbers, and improved survival in a BM transplant model (Abrahamsson et al., 2009). Thus, despite its dispensability for adult HSC, CML-LSCs seem to retain dependency on canonical Ctnnb1 to maintain self-renewal capacity. In human disease, Ctnnb1 activation via the canonical Wnt pathway has been shown to occur in CML-blast crisis LSCs. Aberrant splicing of GSK3 appears to contribute to this hyperactivation in blast crisis samples (Abrahamsson et al., 2009). Thus, there is growing evidence that canonical Wnt signaling is an attractive target pathway in the treatment of CML-LSC. Moreover, cell extrinsic inhibition of Wnt signaling through ectopic DKK1 expression impairs leukemia cell proliferation in vitro (Zhu et al., 2009).

5. Intestinal SCs

Homeostasis of the intestinal epithelium is maintained by an intestinal SC (ISC) compartment that resides at the bottom of the crypt, safely far from the shear stresses and potentially toxic agents. These ISC are at the top of a cellular hierarchy and are crucial for the renewal of the differentiated progeny within the intestinal layer (Medema & Vermeulen, 2011). Indeed, as they migrate out of their niche, they cease to proliferate and initiate differentiation into the different cell lineages of the mature villi: absorptive enterocytes, mucin-secreting-goblet cells, peptide hormone-secreting neuroendocrine cells, and microbicide-secreting Paneth cells. Until relatively recently, ISCs were a rather elusive entity at the bottom of the intestinal crypt, and the discovery of ISC markers has only partly detailed the organization of the intestinal crypt and villi. Briefly, the marker LGR5 identifies crypt base columnar cells (CBCC) located in between the Paneth cells at the crypt bottom (Barker et al., 2007), whereas the markers BMI1 and TERT identify the +4 position in the crypt, just above the Paneth cells (Montgomery et al., 2011; Sangiorgi & Capecchi, 2008). Knock-in constructs that allow expression of GFP and Cre from the Lgr5 locus show that LGR5 expression is confined to CBCCs, and that these cells give rise to the variety of epithelial cells present in crypts, proving that CBCCs function as ISCs as well (Barker et al., 2007, Sato et al., 2009). The existence of these different types of ISC remains a matter of debate and notably, remains to be determined whether and how BMI1+ +4 cells ISCs and LGR5+ ISCs relate to each other. Interestingly, recent data indicate that TERT-expressing ISCs can generate LGR5+ISCs (Montgomery et al., 2011) suggesting that these different ISC types may act in a hierarchical fashion. Regardless of this dispute about ISC identity, there is a consensus that ISCs reside in a niche that provides the cells with essentials signals such as Wnt, Notch and Hedgehog. Under normal circumstances, the Paneth cell signals dictate the size of the SC pool to maintain the total number of SCs within the niche constant. SCs may divide asymmetrically, so that one SC remains within the niche, resulting in self-renewal, whilst the other daughter cell gives rise to progenitor cells that can migrate up the crypt and become more differentiated as they reach the top. Alternatively, two recent studies (Lopez-Garcia et al., 2010; Snippert et al., 2010) support that SCs may divide symmetrically either

forming two daughter SCs (leading to expansion) or two daughter non-stem progenitor cells (leading to extinction). Several pathways play a role in maintaining and regulating stem ISCs, including Wnt and Notch.

5.1 Notch signaling in intestinal epithelium

In the intestine, Notch activity determines lineage decisions between enterocyte and secretory cell differentiation. Several components of the Notch pathway are expressed in adult intestinal crypt cells, suggesting a role for Notch signaling in gene expression programs in immature proliferating compartment cells (Sander & Powell, 2004; Schroder & Gossler, 2002). The first evidence that Notch signaling plays a role in cell-type specification in the intestine was reported in Hes1 knockout mice (Jensen et al., 2000). The deletion of the Hes1 gene resulted in the generation of excessive numbers of goblet cells, enteroendocrine cells, and Paneth cells. Subsequently, it was shown that Math1 (mouse atonal homolog1), one of the genes repressed by Hes1, is required for the differentiation into the three secretory lineages, because the intestinal epithelium of Math1-mutant mice is populated only by absorptive cells (Yang et al., 2001). These data suggest that the choice between the absorptive or secretory fate might be the first decision made by each progenitor cells, and that Hes1 and Math1 activated by Notch signal play opposite roles in this decision making. Recently, using the villin promoter to drive the expression of a constitutively active form of mouse Notch1 receptor, it was noticed an expansion of proliferating intestinal progenitor cells (Fre et al., 2005). Moreover, Notch activation inhibited the differentiation of secretory cells in the mouse intestine, as there was a complete depletion of goblet cells, a marked reduction in enteroendocrine cells, and a low expression of early marker for Paneth cells. These results clearly suggest that Notch signaling is required for maintaining crypt cells in a proliferative state, at least in part, through its negative regulation of Math1. Conversely, conditional removal of the Notch pathway transcription factor CSL/RBP-J increases the proportion of goblet cells in the murine intestine, and a similar phenotype was observed using a γ-secretase inhibitor (van Es et al., 2005). These results suggest that Notch pathway is not only a gatekeeper for proliferating crypt progenitor cells, but is also involved in controlling the balance between secretory and absorptive cell types. Data suggest that the ISC microenvironment delivers Notch-activating signals to maintain stemness, which is consistent with the observation that Paneth cells express Notch ligands (Sato et al., 2011). In particular, recent papers identified Dll1 and Dll4 as the physiologically relevant Notch1 and Notch2 ligands within the small intestine of the mouse. These ligands cooperate and exhibit a partial functional redundancy to maintain the crypt progenitor compartment (Pellegrinet et al., 2011). However, Notch seems to have dual functions in the crypt, as it acts together with Wnt to affect significantly crypt homeostasis (Fre et al., 2005; van Es et al., 2005).

5.2 Canonical Wnt signaling in intestinal epithelium

The Wnt pathway proteins regulate cellular fate along the crypt-villus axis in normal gut epithelium and have been implicated in ISC self-renewal. The nuclear accumulation of β-catenin is preferentially observed in cells located at the base of crypts and decreases as cells move toward the top of the crypts (van der Wetering et al., 2002). Wnt target genes EphB2

and EphB3 control crypt cellular segregation (Batlle et al., 2002), Sox9 regulates Paneth cell differentiation (Mori-Akiyama et al., 2007), and Lgr5 (Barker et al., 2007). TCF4 null mice died shortly after birth and showed an embryonic epithelium made entirely of differentiated cells without proliferative compartments in the crypts (Korinek et al., 1998) suggesting that TCF4 maintains the proliferation of SCs in the murine small intestine. Notably, deletion of the Wnt/TCF4 target gene c-Myc led to a loss of intestinal crypts in a murine model (Muncan et al., 2006). The importance of the Wnt signaling pathway in maintaining the architecture and homeostasis of the adult intestinal epithelium was also shown in a murine model through adenoviral expression of Dkk1. This induced Wnt inhibition in fully adult mice, resulted in inhibition of proliferation in the small intestine and colon, with progressive loss of crypts, villi and glandular structure (Kuhnert et al., 2004). By contrast, when the Wnt pathway is overactivated by mutations in APC or β-catenin, many of the epithelial cells enter into the proliferative state and display a failure of the differentiation programs (Andreu et al., 2005; Sansom et al., 2004). According with these data, recent papers demonstrated that injection of R-spondin1 (R-Spo1), a potent activator of the Wnt signaling pathways, induced rapid onset of crypt cell proliferation displaying epithelial hyperplasia in the intestine of normal mice through β-catenin stabilization and subsequent transcriptional activation of target genes such as murine Axin2, Ascl2, and Lgr5 (Kim et al., 2005; Takashima et al., 2011). The effects of R-Spo1 administration determine protection against radiation-induced colitis by stimulating proliferation of intestinal SCs and protect them against a damage after allogeneic bone-marrow transplantation, suppressing inflammatory cytokine cascades and donor T cell activation (Takashima et al., 2011). These, *in vivo,* data suggest that Wnt signaling is directly linked to the promotion of cellular proliferation and, more specifically, the regulation of progression through cell cycle. In this regard, previous papers pointed to the downregulation of $p21^{cip1waf1}$, a cyclin-dependent kinase inhibitor (CKI), as an important mechanism that might mediate Wnt-dependent growth promotion. A microarray analysis showed that $p21^{cip1waf1}$ was one of the genes whose expression was increased by inhibition of Wnt signaling in human colorectal cancer-derived LS174T cells (van der Wetering et al., 2002). Furthermore, the TCF4 target gene c-Myc has been shown to play a central role in Wnt-mediated repression of $p21^{cip1waf1}$ expression at the transcriptional level through its direct binding to the $p21^{cip1waf1}$ gene promoter (van der Wetering et al., 2002). These data suggest that the repression of $p21^{cip1waf1}$ by c-Myc might be the intracellular mechanism by which Wnt signaling regulates the G1/S transition and cell cycle progression. This signaling cascade has been shown to be functional *in vivo*, because abnormal features of proliferation/differentiation in the adult murine intestine, which occur with the single deletion of APC, are mostly rescued when c-Myc gene is simultaneously deleted (Sansom et al., 2007). Furthermore, this restoration of the morphologically normal phenotype in double mutant mice for APC and c-Myc is accompanied by restoration of p21 expression within the crypts, suggesting the involvement of p21 in the Wnt-c-Myc pathway-mediated growth control of progenitor cells. Indeed, raises the possibility that p21 is an intracellular molecular switch between proliferation and differentiation. Moreover, it has been shown that conditional expression of $p21^{cip1waf1}$ alone allow cells to differentiate (van der Wetering et al., 2002) suggesting that the cell fate choice between proliferation and differentiation is regulated by modulation of the expression of $p21^{cip1waf1}$ via the direct induction of c-Myc by Wnt signaling.

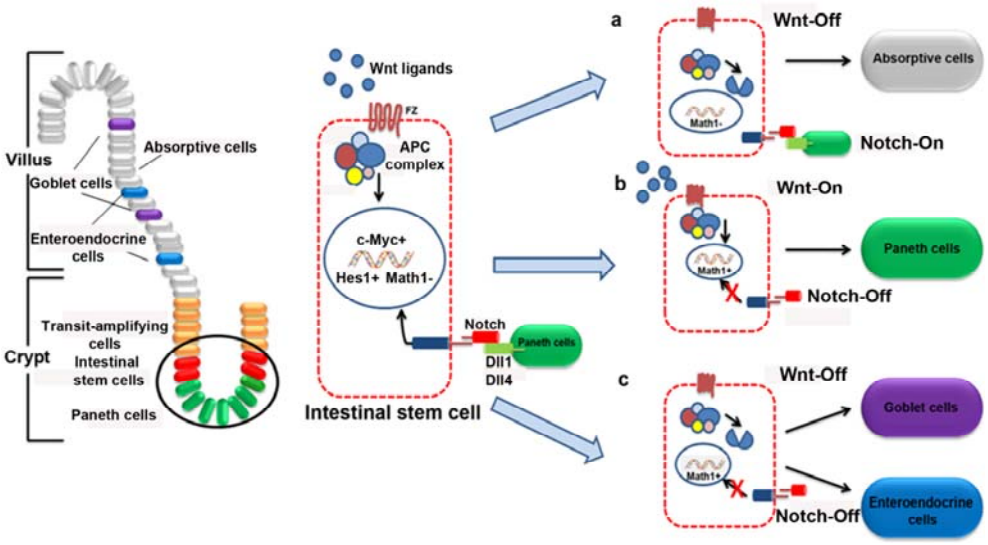

Fig. 5. The role for Notch and Wnt pathways in intestinal epithelial proliferation and differentiation. The ISC can give rise to four lineages of terminally differentiated cells: a is absorptive cells, b and c (Paneth, goblet and enteroendocrine cells) have secretory phenotypes. See the text for detail.

In general, the data strongly support a model in which Notch directs proliferation when Wnt signal activity is high, and directs enterocyte differentiation when Wnt activity levels drop towards the top of the crypt. The multipotent progenitors require both Wnt and Notch signals to be activated for fulfilling continuous proliferation without differentiation. Once some cells in this Wnt and Notch-activated population escape from the Notch signal, they stop proliferating and acquire the Math1 function. These cells raise the terminally differentiation in secretory cells in areas where the Wnt signal is not active (Pinto et al., 2003), whereas they differentiate in Paneth cells if they remain at the bottom of the crypt where Wnt ligands are abundant. By contrast, if cells in this Wnt and Notch-active population lose the Wnt signal, for example, because of their positional changes along the vertical axis, they differentiate as absorptive cells (Fig. 5).

5.3 SCs and the origin of intestinal cancer

Despite stringent homeostatic maintenance in the intestine, the high number of patients with colorectal cancer (CRC) indicates that these regulatory mechanisms often fall short in protecting against malignant transformation. Both environmental and genetic risk factors have been defined for CRC, and deregulation of morphogenetic pathways plays a key part in cancer development. Notably, the vast majority of sporadic CRC cases carry Wnt pathway mutations, highlighting the importance of this pathway in CRC. The hit that induces transition from normal to polypoid tissue is accompanied by several changes in crypt appearance and behavior, cells show a more immature phenotype and a higher proliferative index which results in expansion of the pre-malignant clone. Although

mutation of APC or β-catenin is an early event in the transformation of colonic epithelial cells, studies have revealed that colon carcinomas do not contain nuclear β-catenin homogeneously (Fodde & Brabletz, 2007). This so-called β-catenin paradox indicate that Wnt signaling has a preponderant role only for a subset of tumour cells, cancer SCs (CSCs), which are endowed with tumorigenic capacity (Vermeulen et al., 2008). Indeed, the past decade has seen a shift in the way tumours are perceived, and the now widely accepted model is that tumours contain a small population of self-renewing CSCs, as well as a large compartment of more differentiated tumour cells (Vermeulen et al., 2008). Cellular hierarchy within CRC is maintained, at least in part, by microenvironmental factors regulating stemness and differentiation. In agreement, tumour cells located next to myofibroblast-rich regions, have a much higher incidence of nuclear-localized β-catenin, suggesting for microenvironment-modulated Wnt signaling (Fodde & Brabletz, 2007). A recent paper point to hepatocyte growth factor (HGF) as the myofibroblast-derived signal that, at least in part, orchestrates this intimate relationship and enhances Wnt activity in more differentiated tumour cells, thereby reinstalling CSCs features (dedifferentiation) (Vermeulen et al., 2010). Indeed, using a TCF/LEF reporter that directs the expression of enhanced green fluorescent protein, authors provided evidence that Wnt signaling activity is a marker for colon CSCs and is regulated by the microenvironment. Moreover, they show that differentiated cancer cells can be reprogrammed to express CSC markers and regain their tumorigenic capacity when stimulated with myofibroblast-derived factors (Vermeulen et al., 2010). Although, these data clearly ascertain a role for the Wnt pathway in CRC stemness, Notch inhibition with an antibody against the Notch ligand Dll4 results in human colon CSCs differentiation, reduction of CRC growth in a xenotransplantation model and chemosensitization (Hoey et al., 2009).

6. Identification of Renal SCs.

The mammalian kidney shares with the majority of organs the ability to repopulate and at least partially repair structures that have sustained some degree of injury. Indeed, tubular integrity can be rescued after acute damage, and even severe glomerular disorders sometimes may undergo regression and remission, suggesting that glomerular injury is also reparable (Imai & Iwatani, 2007; Remuzzi, et al., 2006). However, the existence of renal SC (RSC) has been a matter of long debate. Recently, converging data definitively demonstrated the existence of a population of stem/progenitor cells in the parietal epithelium of the Bowman's capsule of adult human kidney (Sagrinati, et al., 2006) (Fig.6). These SC coexpress both CD24, a surface molecule that has been used to identify different types of human SC, and CD133, a marker of several types of adult tissue SC, lack lineage-specific markers, express transcription factors that are characteristic of multipotent SC, and exhibit self-renewal, high clonogenic efficiency and multidifferentiation potential. When injected intravenously in SCID mice that had acute kidney injury, RSC regenerated tubular structures from different portions of the nephron and also reduced the morphological and functional kidney damage (Sagrinati, et al., 2006).

In addition, it was demonstrated that RSC are arranged in a precise sequence within Bowman's capsule of adult human kidneys (Ronconi, et al., 2009) (Fig. 6).

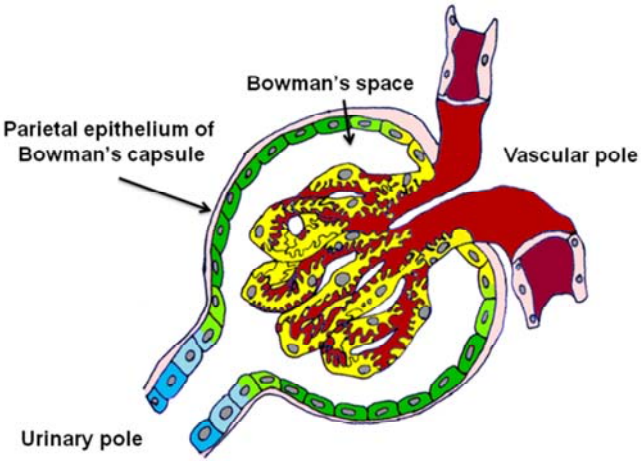

Fig. 6. Localization of RPC in the glomerulus. RPC (green) are localized in the Bowman's capsule epithelium. A transitional cell population (podocyte progenitors, green/yellow) displays features of either RPC or podocyte (yellow) and localize between the urinary pole and the vascular stalk. Cells that express only podocyte markers and the phenotypic features of differentiated podocytes (yellow) localize at the vascular stalk of the glomerulus.

These findings obtained in human kidneys were confirmed in a parallel study performed in murine kidney by Appel (Appel, et al., 2009), who also demonstrated the existence of transitional cells with morphological and immunohistochemical features of both parietal epithelial cells in proximity of the glomerular vascular stalk and that podocytes are recruited from parietal epithelial cells, which proliferate and differentiate from the urinary to the vascular stalk, then generating novel podocytes (Fig. 6). This occurs as the kidney grows, during childhood and adolescence, and may also take place following an injury which allows a slow, regulated generation of novel podocytes, such as uninephrectomy. Recently, a rare subpopulation of CD133+CD24+ cells has also been describe in renal tubules (Lindgren, et al., 2011). These cells are able to proliferate and differentiate after tubular injury. Accordingly, tubular epithelium regenerating on acute tubular necrosis displayed long stretches of CD133+CD24+ cells, further substantiating that the cells that are repairing tubular epithelium may simply represent the result of proliferation and differentiation of CD133+CD24+ tubular progenitors.

6.1 Involvement of RSC in glomerular disorders and cancer.

It has been widely recognized that a disruption in the strictly regulated balance of SC self-renewal and differentiation not only impairs regenerative mechanisms but can even generate disorders. In the glomerulus, the response to podocyte injury may cause aberrant epithelial cell proliferation, hypercellular lesions formation and Bowman's space obliteration, as seen in collapsing glomerulopathy and in crescentic glomerulonephritis (Albaqumi & Barisoni, 2008; Thorner, et al., 2008). Until now, theories explaining the origin of aberrant epithelial cells in collapsing glomerulopathy and crescentic glomerulonephritis have been controversial. One possibility is that these cells are exclusively of parietal epithelial origin (Thorner et al., 2008), while another is that some dedifferentiated

podocytes acquire markers of parietal epithelial cells (Moeller et al., 2004). It was recently demonstrated that the majority of cells present in the hyperplastic lesions in collapsing glomerulopathy or crescentic glomerulonephritis exhibits the RSC markers CD133 and CD24, with or without coexpression of podocyte markers (Smeets et al., 2009). Therefore, it is suggested that the glomerular hyperplastic lesions are generated by RSC of Bowman's capsule at different stages of their differentiation towards mature podocytes. Support for this hypothesis came from lineage tracing experiments performed in transgenic mice with genetically labeled parietal epithelial cells in a model of inflammatory crescentic glomerulonephritis, and of collapsing glomerulopathy (Smeets et al., 2009).

Finally, a close relationship between the transcriptome of CD133+ tubular progenitors and the one derived by papillary renal cell carcinomas was demonstrated (Lindgren et al. 2011). Moreover, a strong CD133 expression was observed in the papillary renal cell carcinomas analysed. Thus, these observations raise the provocative hypothesis that papillary renal cell carcinomas may directly derive from $CD133^{+}CD24^{+}$ renal tubular progenitors, whereas clear renal cell carcinomas may derive from other more differentiated proximal tubular cells.

6.2 Signaling pathway regulating the RSC niche.

The molecular mechanisms regulating the proliferation of RSC, as well as the cell fate determination in the podocyte lineage are unknown. We recently demonstrate the role of the Notch signaling pathway in both these processes (Lasagni et al., 2010). Notch activation triggers the expansion of renal progenitors by promoting their entry into the S-phase of the cell cycle and mitotic division. Moreover, Notch downregulation is required for differentiation toward the podocyte lineage. However, Notch downregulation was neither sufficient nor necessary for the acquisition of a podocyte phenotype, but an impaired downregulation of the Notch pathway led to podocyte death. Indeed, renal progenitor differentiation into podocytes was associated with cell cycle checkpoint activation and G_2/M arrest, reflecting an intrinsic barrier to replication of mature podocytes. Persistent activation of the Notch pathway induced podocytes to cross the G_2/M checkpoint, resulting in cytoskeleton disruption and cell death (Lasagni et al., 2010). Notch expression was virtually absent in the glomeruli of healthy adult kidneys, while a strong upregulation was observed in renal progenitors and podocytes in patients affected by glomerular disorders. Accordingly, inhibition of the Notch pathway in mouse models of focal segmental glomerulosclerosis ameliorated proteinuria and reduced podocyte loss during the initial phases of glomerular injury, while inducing reduction of progenitor proliferation during the regenerative phases of glomerular injury with worsening of proteinuria and glomerulosclerosis. Taken altogether, these results suggest that the severity of glomerular disorders depends on the Notch-regulated balance between podocyte death and regeneration provided by renal progenitors (Lasagni et al., 2010).

7. References

Abrahamsson AE, Geron, I., Gotlib, J., Dao, KH., Barroga, CF., Newton, IG., Giles, FJ., Durocher, J., Creusot, RS., Karimi, M., Jones, C., Zehnder, JL., Keating, A., Negrin, RS., Weissman, IL.& Jamieson, CH. (2009). Glycogen synthase kinase 3beta missplicing contributes to leukemia stem cell generation. *Proc Natl Acad Sci USA*, Vol.106, No.10, (March 2009), pp.3925–9, ISSN1091-6490.

Albaqumi, M. & Barisoni, L. (2008) Current views on collapsing glomerulopath,. *J Am Soc Nephrol*., Vol.19, No.7, (July 2008), pp. 1276-1281, ISSN 1046-6673

Andreu, P.; Colnot, S.; Godard, C.; Gad, S.; Chafey, P.; Niwa-Kawakita, M.; Laurent-Puig, P.; Kahn, A.; Robine, S.; Perret, C. & Romagnolo, B. (2005). Crypt-restricted proliferation and commitment to the Paneth cell lineage following Apc loss in the mouse intestine. *Development*, Vol.132, No.6, (March 2005), pp. 1443-1451, ISSN 1011-6370

Appel, D.; Kershaw, D.B.; Smeets, B.; Yuan, G.; Fuss, A.; Frye, B.; Elger, M.; Kriz, W.; Floege, J. & Moeller, M.J. (2009) Recruitment of podocytes from glomerular parietal epithelial cells, *J Am Soc Nephrol*, Vol.20, No.2, (February 2009), pp. 333-343, ISSN 1046-6673

Arce, L.; Yokoyama, N.N. & Waterman, M.L. (2006) Diversity of LEF/TCF action in development and disease. *Oncogene*. Vol.25, No.57, (December 2006), pp. 7492-504, ISSN 0950-9232

Barker, N.; van Es, JH.; Kuipers, J.; Kujala, P.; van den Born, M.; Cozijnsen, M.; Haegebarth, A.; Korving, J.; Begthel, H.; Peters, PJ. & Clevers, H. (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature*, Vol.449, No.7165, (October 2007), pp. 1003-7, ISSN 00280836

Batlle, E.; Henderson, J.T.; Beghtel, H.; van den Born, M.M.; Sancho, E.; Huls, G.; Meeldijk, J.; Robertson, J.; van de Wetering,; M. Pawson, T. & Clevers, H. (2002). Beta-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. *Cell*, Vol.111, No.2, (October 2002), pp. 251-263, ISSN 0092-8674

Betschinger, J. & Knoblich, J.A. (2004) Dare to be different: asymmetric cell division in Drosophila, C. elegans and vertebrates, *Curr Biol*, Vol.14, No.16, (August 2004), pp. R674-685, ISSN 0960-9822

Beverly, L.J.; Felsher, D.W. & Capobianco, A.J. (2005). Suppression of p53 by Notch in lymphomagenesis: Implications for initiation and regression. *Cancer Research*, Vol.65, No.16, (August 2005), pp. 7159-7168, ISSN 1538-7445.

Blanpain C, Fuchs E. (2009) Epidermal homeostasis: a balancing act of stem cells in the skin, *Nat Rev Mol Cell Biol*, Vol.10, No.3, (March 2009), pp. 207-217, ISSN 1471-0072

Bovolenta, P.; Esteve, P.; Ruiz, J.M.; Cisneros, E. & Lopez-Rios, J.(2008) Beyond Wnt inhibition: new functions of secreted Frizzled-related proteins in development and disease. *J Cell Sci*, Vol.121, No.6, (March 2008), pp. 737–746, ISSN 0021-9533

Bray, S. & Bernard, F. (2010) Notch targets and their regulation. *Curr. Top. Dev. Biol.,* Vol.92, 253–275 ISSN: 0070-2153

Carlesso, N.; Aster, J.C.; Sklar, J. & Scadden, D.T., (1999). Notch1-induced delay of human hematopoietic progenitor cell differentiation is associated with altered cell cycle kinetics. *Blood*, Vol.93, No.3, (February 1999), pp. 838-848, ISSN 0006-4971.

Cedarn, C. & Bhatia, M. (2010) Novel roles for Notch, Wnt and Hh in hematopoiesis derived from human pluripotent stem cells. *Int J Dev Biol*, Vol.54, No.6-7, (2010), pp. 955-963, ISSN 02146282

Cheshier, SH., Morrison, SJ., Liao, X. & Weissman IL. (1999). In vivo proliferation and cell cycle kinetics of long-term self-renewing hematopoietic stem cells. *Proc Natl Acad Sci USA* , Vol.96, No.6, (March 1999), pp.3120–5, ISSN 1091-6490.

Dick, JE. (2008). Stem cell concepts renew cancer research. *Blood*, Vol.112, No.13, (December 2008), pp.4793–807, ISSN 0006-4971.

Eisenberg, C.A. & Eisenberg, L.M. (1999) WNT11 promotes cardiac tissue formation of early mesoderm, *Dev. Dyn.*, Vol.216, No.1, (September 1999), pp. 45–58, ISSN 1058-8388

Ellisen, L.W.; Bird, J.; West, D.C.; Soreng, A.L.; Reynolds, T.C.; Smith, S.D. & Sklar, J. (1991). TAN-1, the human homolog of the Drosophila Notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell*, Vol.66, No.4, (August 1991), pp. 649-661, ISSN 0092-8674.

Fleming, HE., Janzen, V., Lo Celso, C., Guo, J., Leahy, KM., Kronenberg, HM. & Scadden DT. (2008). Wnt signaling in the niche enforces hematopoietic stem cell quiescence and is necessary to preserve self-renewal in vivo. *Cell Stem Cell,* Vol.2, No.3, (March 2008), pp.274–83, ISSN 19345909

Fodde, R. & Brabletz, T. (2007). Wnt/beta-catenin signaling in cancer stemness and malignant behavior. *Curr Opin Cell Biol*, Vol.19, No. , (April 2007), pp. 150-158, ISSN 09550674

Fre, S.; Huyghe, M.; Mourikis, P.; Robine, S.; Louvard, D. & Artavanis-Tsakonas, S. (2005). Notch signals control the fate of immature progenitor cells in the intestine. *Nature*, Vol. 435, No. 7044, (June 2005), pp. 964-968, ISSN 0028-0836

Gurtner, G.C.; Werner, S.; Barrandon, Y. & Longaker, M.T. (2008) Wound repair and regeneration, *Nature*, Vol.453, No.7193, (May 2008), pp. 314-321, ISSN 0028-0836

Hoey, T.; Yen, W.C.; Axelrod, F.; Basi, J.; Donigian, L.; Dylla, S.; Fitch-Bruhns, M.; Lazetic, S.; Park, I.K.; Sato, A.; Satyal, S.; Wang, X.; Clarke, M.F.; Lewicki, J. & Gurney, A. (2009). DLL4 blockade inhibits tumor growth and reduces tumor-initiating cell frequency. *Cell Stem Cell*, Vol.5, No.2, (August 2009), pp. 168-177, ISSN 19345909

Hu Y, Chen, Y., Douglas, L. & Li, S. (2009). beta-Catenin is essential for survival of leukemic stem cells insensitive to kinase inhibition in mice with BCR-ABLinduced chronic myeloid leukemia. *Leukemia*, Vol.23, No.1, (January 2009), pp.109–16, ISSN 08876924.

Huang, J., Zhang, Y., Bersenev, A., O'Brien, WT., Wei Tong, W., Emerson, SG. & Klein, PS. (2009). Pivotal role for glycogen synthase kinase–3 in hematopoietic stem cell homeostasis in mice *J. Clin. Invest,* Vol.119, No.12, (December 2009), pp.3519–3529, ISSN 00219738.

Ikeda, S.; Kishida, S.; Yamamoto, H.; Murai, H.; Koyama, S. & Kikuchi, A. (1998) Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin, *EMBO J.*, Vol.17, No.5, (March 1998), pp. 1371-84, ISSN 0261-4189

Imai, E. & Iwatani, H. (2007) The continuing story of renal repair with stem cells. *J Am Soc Nephrol.*, Vol.18, No.9, (September 2007), pp. 2423-2424, ISSN 1046-6673

Jensen, J.; Pedersen, E.E.; Galante, P.; Hald, J.; Heller, R.S.; Ishibashi, M.; Kageyama, R.; Guillemot, F.; Serup, P. & Madsen, O.D. (2000). Control of endodermal endocrine development by Hes-1. *Nat Genet*, Vol.24, No.1, (January 2000), pp. 36-44, ISSN 1061-4036

Jones, D.L. & Wagers, A.J. No place like home: anatomy and function of the stem cell niche, *Nature Review Molecular Cell Biology*, Vol.9, No.1, (January 2008), pp. 11-21, ISSN: 1471-0072

Karanu, F.N.; Murdoch, B.; Gallacher, L.; Wu, D.M.; Koremoto, M.; Sakano, S. & Bathia, M. (2000). The Notch ligand Jagged-1 represents a novel growth factor of human hematopoietic stem cells. *J Exp Med*, Vol.192, No.9, (November 2000), pp. 1365-1372, ISSN 1540-9538.

Karanu, F.N.; Murdoch, B.; Miyabayashi, T.; Ohno, M.; Koremoto, M.; Gallacher, L.; Wu, D.; Itoh, A.; Sakano, S. & Bathia, M. (2001). Human homologues of Delta-1 and Delta-4 function as mitogenic regulators of primitive human hematopoietic cells. *Blood*, Vol.97, pp. 1960-1967, ISSN 0006-4971.

Kelly, OG., Pinson, KI. & Skarnes, W.C. (2004) The Wnt co-receptors Lrp5 and Lrp6 are essential for gastrulation in mice. *Development* , Vol.131, No.12, (June 2004), pp. 2803-2815, ISSN 09501991.

Kestler, H.A. & Kühl, M. (2008) From individual Wnt pathways towards a Wnt signaling network. *Philos Trans R Soc Lond B Biol Sci*, Vol.363, No.1495, (April 2008), pp. 1333-47, ISSN 0080-4622

Kiel, M.J.; Yilmaz, O.H.; Iwashita, T.; Yilmaz, O.H.; Terhorst, C. & Morrison S.J. (2005). SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*, Vol.121, No.7, (July 2005), pp. 1109-1121, ISSN 0092-8674.

Kim, K.A.; Kakitani, M.; Zhao, J.; Oshima, T.; Tang, T.; Binnerts, M.; Liu, Y.; Boyle, B.; Park, E.; Emtage, P.; Funk, W.D. & Tomizuka K. (2005). Mitogenic influence of human R-spondin1 on the intestinal epithelium. *Science*, Vol. 309, No. 5738, (August 2005), pp. 1256-1259, ISSN 0036-8075

Kirstetter, P., Anderson, K., Porse, BT., Jacobsen, SE. & Nerlov, C. (2006) Activation of the canonical Wnt pathway leads to loss of hematopoietic stem cell repopulation and multilineage differentiation block. *Nat Immunol* , Vol.7, No.10, (October 2006), pp.1048-1056, ISSN 15292916.

Koch, U., Wilson, A., Cobas, M., Kemler, R., Macdonald, H.R. & Radtke, F. (2008) Simultaneous loss of beta- and gamma-catenin does not perturb hematopoiesis or lymphopoiesis. *Blood,* Vol.111, No.1, (January 2008), pp.160-164, ISSN 15280020.

Kopan, R. & Ilagan, M. X. (2009) The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell,* Vol.137, No.2, (April 2009), pp. 216–233, ISSN 0092-8674

Korinek, V.; Barker, N.; Moerer, P.; van Donselaar, E.; Huls, G.; Peters, P.J. & Clevers, H. (1998). Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat Genet*., Vol.19, No.4, (August 1998), pp. 379-383, ISSN 1061-4036

Kuhl, M.; Sheldahl, L.C.; Malbon, C.C. & Moon, R.T. (2000) Ca^{2+}/calmodulin-dependent protein kinase II is stimulated by Wnt and Frizzled homologs and promotes ventral cell fates in Xenopus, *J. Biol. Chem*., Vol.275, No.17, (April 2000), pp. 12701–12711, ISSN 0021-9258

Kuhnert, F.; Davis, C.R.; Wang, H.T.; Chu, P.; Lee, M.; Yuan, J.; Nusse, R. & Kuo, C.J. (2004). Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. *Proc Natl Acad Sci U S A*, Vol.101, No.1, (January 2004), pp. 266-271, ISSN 1091-6490

Lai, E.C.(2002) Keeping a good pathway down: transcriptional repression of Notch pathway target genes by CSL proteins, *EMBO Rep*, Vol.3, No.9, (September 2002), pp. 840-845, ISSN 1469-221X.

Lasagni, L.; Ballerini, L.; Angelotti, M.L.; Parente, E.; Sagrinati, C.; Mazzinghi, B.; Peired, A.; Ronconi, E.; Becherucci, F.; Bani, D.; Gacci, M.; Carini, M.; Lazzeri, E. & Romagnani, P. (2010) Notch activation differentially regulates renal progenitors proliferation and differentiation toward the podocyte lineage in glomerular disorders. *Stem Cells*, Vol.28, No.9, (September 2010), pp. 1674-1685, ISSN 066-5099

Li, L. & Xie, T. (2005) Stem cell niche: structure and function, *Annu Rev Cell Dev Biol*., Vol.21, (November 2005), pp. 605-31, ISSN 1081-0706

Lindgren, D.; Boström, A.K.; Nilsson, K.; Hansson, J.; Sjölund, J.; Möller, C.; Jirström, K.; Nilsson, E.; Landberg, G.; Axelson, H. & Johansson, M.E. (2011) Isolation and characterization of progenitor-like cells from human renal proximal tubules, *Am J Pathol*, Vol.178, No.2, (February 2011), pp. 828-837, ISSN 0002-9440

Liu, J.; Sato, C.; Cerletti, M. & Wagers, A. (2010).Notch signaling in the regulation of stem cell self-renewal and differentiation. *Curr Top Dev Biol*, Vol.92, No.7, (April 2001), pp. 367-409, ISSN 0070-2153.

Liu, P., Wakamiya, M., Shea, MJ., Albrecht, U., Behringer, RR. & Bradley, A. (1999). Requirement for Wnt3 in vertebrate axis formation. *Nat Genet* , Vol.22, No.4, (August 1999), pp. 361-365, ISSN 10614036.

Logan, C.Y. & Nusse, R. (2004) The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol*., Vol.20, (July 2004), pp. 781–810, ISSN 1081-0706

Lopez-Garcia, C. Klein, A.M. Simons, B.D. Winton, D.J. (2010). Intestinal stem cell replacement follows a pattern of neutral drift. *Science*, Vol.330, No.6005, (November 2010), pp. (822-825), ISSN 0036-8075

MacDonald, B.T.; Tamai, K. & He, X. (2009) Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell,* Vol.17, No.1, (July 2009), pp. 9-26, ISSN. 1534-5807

Malhotra, S. & Kincade, P.W. (2009). Wnt-related molecules and signaling pathway equilibrium in hematopoiesis. *Cell Stem Cell,* Vol.4, No.1, (January 2009), pp.27–36, ISSN, 19345909.

Mao, B.; Wu, W.; Davidson, G.; Marhold, J.; Li, M.; Mechler, B.M.; Delius, H.; Hoppe, D.; Stannek, P.; Walter, C.; Glinka, A. & Niehrs, C. (2002) Kremen proteins are Dickkopf receptors that regulate Wnt/β-catenin signaling, *Nature,* Vol. 417, No.6889, (June 2002), pp. 664–667, ISSN 0028-0836

Medema, JP. Vermeulen, L. (2011). Microenvironmental regulation of stem cells in intestinal homeostasis and cancer. *Nature,* Vol.474, No.7351, (June 2011), pp. 318-326, ISSN 0028-0836

Medyouf, H.; Alcade, H.; Berthier, C.; Guillemain, M.C.; dos Santos, N.R.; Janin, A.; Decaudin, D.; de Thé, H. & Ghysdael, J. (2007). Targeting calcineurin activation as a therapeutic strategy for T-cell acute lymphoblastic leukemia. *Nature Medicine*, Vol.13, No.6, (June 2007), pp. 736-741, ISSN 1078-8956.

Moeller, M.J.; Soofi, A.; Hartmann, I.; Le Hir, M.; Wiggins, R.; Kriz, W. & Holzman, L.B. (2004) Podocytes populate cellular crescents in a murine model of inflammatory glomerulonephritis. *J Am Soc Nephrol*, Vol.15, No.1, (January 2004), pp. 61-67, ISSN 1046-6673

Montcouquiol, M.; Crenshaw, E.B. 3r &, Kelley MW (2006) Noncanonical Wnt signaling and neural polarity. *Annu Rev Neurosci*., Vol.29, (July 2006), pp. 363-86, ISSN 0147-006X

Montgomery, R.K. Carlone, D.L. Richmond, C.A. Farilla, L. Kranendonk, M.E. Henderson, D.E. Baffour-Awuah, N.Y. Ambruzs, D.M. Fogli, L.K. Algra, S. Breault, D.T. (2011). Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. *Proc Natl Acad Sci U S A*, Vol. 108, No. 1, (January 2011), pp. (179-184), ISSN 1091-6490

Morgan, T. (1917) The theory of the gene, *Am. Nat.*, Vol.51, No.609, (September 1917), pp. 513-544. ISSN 00030147

Mori-Akiyama, Y.; van den Born, M.; van Es, J.H.; Hamilton, S.R.; Adams, H.P.; Zhang, J.; Clevers, H. & de Crombrugghe, B. (2007). SOX9 is required for the differentiation of paneth cells in the intestinal epithelium. *Gastroenterology*, Vol.133, No.2, (August 2007), pp. 539-546, ISSN 0016-5085

Morrison, S.J. & Kimble J. (2006) Asymmetric and symmetric stem-cell division in development and cancer, *Nature*, Vol.441, No.7097, (June 2006), pp. 1068-1074, ISSN 0028-0836

Muncan, V.; Sansom, O.J.; Tertoolen, L.; Phesse, T.J.; Begthel, H.; Sancho, E.; Cole, A.M.; Gregorieff, A.; de Alboran, I.M.; Clevers, H.; Clarke, A.R. (2006). Rapid loss of intestinal crypts upon conditional deletion of the Wnt/Tcf-4 target gene c-Myc. *Mol Cell Biol*, Vol.26, No.22, (November 2006), pp. 8418-8426, ISSN 1098-5549

Niida, A.; Hiroko, T.; Kasai, M.; Furukawa, Y.; Nakamura, Y.; Suzuki, Y.; Sugano, S. & Akiyama, T. (2004) DKK1, a negative regulator of Wnt signaling, is a target of the beta-catenin/TCF pathway, *Oncogene*, Vol.23, No.52, (November 2004), pp. 8520-6, ISSN 0950-9232

Okada, S.; Nakauchi, H.; Nagayoshi, K.; Nishikawa, S.; Miura, Y. & Suda, T. (1992). In vivo and in vitro stem cell function of c-kit- and Sca-1-positive murine hematopoietic cells. *Blood*, Vol.80, No.12, (December 1992), pp. 3044-3050, ISSN 1528-0020.

Paganin, M. & Ferrando, A. (2011). Molecular pathogenesis and targeted therapies for NOTCH1-induced T-cell acute lymphoblastic leukemia. *Blood*, Vol.25, No.2, (March 2011), pp. 83-90, ISSN 1528-0020.

Palomero, T.; Lim, W.K.; Odom, D.T.; Sulis, M.L.; Real, P.J.; Margolin, A.; Barnes, K.C.; O'Neil, J.; Neuberg, D.; Weng, A.P.; Aster, J.C.; Sigaux, F.; Soulier, J.; Look, A.T.; Young, R.A.; Califano, A. & Ferrando, A.A. (2006). NOTCH1 directly regulates c-MYC and activates a fee-forward-loop transcriptional network promoting leukemic cell growth. *Proc Natl Acad Sci*, *USA* , Vol.103, No.48, (November 2008), pp. 18261-18266, ISSN 1091-6490.

Palomero, T.; Sulis, M.L.; Cortina, M.; Real, P.J.; Barnes, K.; Ciofani, M.; Caparros, E; Buteau, J.; Brown, K.; Perkins, S.L.; Bhagat, G.; Mishra, A.; Basso, G.; Parsons, R.; Zúñiga-Pflücker, J.C.; Dominguez, M. & Ferrando A.A. (2007). Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. *Nature medicine*, Vol.13, No.10, (October 2007), pp. 1203-1210, ISSN 1074-7613.

Pellegrinet, L.; Rodilla, V.; Liu, Z.; Chen, S.; Koch, U.; Espinosa, L.; Kaestner, K.H.; Kopan, R.; Lewis, J. & Radtke, F. (2011). Dll1- and dll4-mediated notch signaling are required for homeostasis of intestinal stem cells. *Gastroenterology*, Vol.140, No.4, (April 2011), pp. 1230-1240, ISSN 0016-5085

Pinson K.I, Brennan J, Monkley S, Avery B.J, Skarnes W.C (2000) An LDL-receptor-related protein mediates Wnt signaling in mice. *Nature.* Vol.407, No.6803, (September 2000), pp. 535–538, ISSN 0028-0836

Pinto, D.; Gregorieff, A.; Begthel, H. & Clevers, H. (2003). Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. *Genes Dev*, Vol.17, No.14, (July 2003), pp. 1709-1713, ISSN 1549-5477

Radtke, F.; Fasnacht, N. & MacDonald, H.R. (2010). Notch Signaling in the Immune System. *Immunity*, Vol.32, No.1, (January 2010), pp. 14-27, ISSN 1074-7613.

Rawlins, E.L. & Hogan, B.L. (2006) Epithelial stem cells of the lung: privileged few or opportunities for many? *Development*, Vol.133, No.13, (July 2006), pp. 2455-2465, ISSN 1011-6370

Remuzzi, G.; Benigni, A. & Remuzzi A. (2006) Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J Clin Invest*. , Vol.116, No.2, (February 2006), pp. 288-296, ISSN 0021-9738

Renstrom, J.; Kroger, M., Peschel, C. & Oostendorp, R.A.J. (2010). How the niche regulates hematopoietic stem cells. *Chemico-Biological Interactions*, Vol.184, No.1-2, (March 2010), pp. 7-15, ISSN 0009-2797.

Reya, T., Duncan, AW., Ailles, L., Domen, J., Scherer, D.C., Willert, K., Hintz, L., Nusse, R. & Weissman, IL. (2003) A role for Wnt signaling in self-renewal of haematopoietic stem cells. *Nature,* Vol.423, No.6938, (May 2003), pp.409-414 ISSN 0028-0836.

Rhyu, M.S.; Jan, L.Y. & Jan, Y.N. (1994) Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells, *Cell*, Vol.76, No.3, (February 1994), pp. 477-491, ISSN 0092-8674

Ronconi, E.; Sagrinati, C.; Angelotti, M.L.; Lazzeri, E.; Mazzinghi, B.; Ballerini, L.; Parente, E.; Becherucci, F.; Gacci, M.; Carini, M.; Maggi, E.; Serio, M.; Vannelli, G.B.; Lasagni, L.; Romagnani, S. & Romagnani, P. (2009) Regeneration of glomerular podocytes by human renal progenitors. *J Am Soc Nephrol*., Vol.20, No.2, (February 2009), pp. 322-332, ISSN 1046-6673

Roszko, I.; Sawada, A. & Solnica-Krezel, L. (2009) Regulation of convergence and extension movements during vertebrate gastrulation by the Wnt/PCP pathway, *Semin Cell Dev Biol*., Vol.20, No.8, (October 2009), pp. 986-97, ISSN 1084-9521

Sagrinati, C.; Netti, G.S.; Mazzinghi, B.; Lazzeri, E.; Liotta, F.; Frosali, F.; Ronconi, E.; Meini, C.; Gacci, M.; Squecco, R.; Carini, M.; Gesualdo, L.; Francini, F.; Maggi, E.; Annunziato, F.; Lasagni, L.; Serio, M.; Romagnani, S. & Romagnani, P. (2006) Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. *J Am Soc Nephrol*., Vol.17, No.9, (September 2006), pp. 2443-2456, ISSN 1046-6673.

Sander, G.R. & Powell, B.C. (2004). Expression of notch receptors and ligands in the adult gut. *J Histochem Cytochem*, Vol. 52, No. 4, (April 2004), pp. 509-516, ISSN 0022-1554

Saneyoshi, T.; Kume, S.; Amasaki, Y. & Mikoshiba, K. (2002) The Wnt/calcium pathway activates NF-AT and promotes ventral cell fate in Xenopus embryos. *Nature*, Vol.417, No.6886, (May 2002), pp. 295–299, ISSN 0028-0836

Sangiorgi, E. Capecchi, M.R. (2008). Bmi1 is expressed in vivo in intestinal stem cells. *Nat Genet*. Vol.40, No.7, (July 2008), pp. 915-920, ISSN 1061-4036

Sansom, O.J.; Meniel, V.S.; Muncan, V.; Phesse, T.J.; Wilkins, J.A.; Reed, K.R.; Vass, J.K.; Athineos, D.; Clevers, H. & Clarke, A.R. (2007). Myc deletion rescues Apc deficiency in the small intestine. *Nature*, Vol.446, No.7136, (April 2007), pp. 676-679, ISSN 0028-0836

Sansom, O.J.; Reed, K.R.; Hayes, A.J.; Ireland, H.; Brinkmann, H.; Newton, I.P.; Batlle, E.; Simon-Assmann, P.; Clevers, H.; Nathke, I.S.; Clarke, A.R. & Winton D.J. (2004). Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration. *Genes Dev*. Vol.18, No.2, (June 2004), pp. 1385-1390, ISSN 1549-5477

Sarmento, L.M.; Huang, H.; Limon, A.; Gordon, W.; Fernandes, J.; Tavares, M.J.; Miele, L.; Cardoso, A.A.; Classon, M. & Carlesso, N. (2005). Notch1 modulates timing of G1-S progression by inducing SKP2 transcription and p27 Kip1 degradation. Journal of Experimental Medicine, Vol.202, No.1, (July 2005), pp. 157-168, ISSN 1540-9538.

Sato, T. van Es, J.H. Snippert, H.J. Stange, D.E. Vries, R.G. van den Born, M. Barker, N. Shroyer, N.F. van de Wetering, M. Clevers, H. (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature*, Vol.469, No. 330, (January 2011), pp. 415-418, ISSN 0028-0836

Sato, T. Vries, R.G. Snippert, H.J. van de Wetering, M. Barker, N. Stange, D.E. van Es, J.H. Abo, A. Kujala, P. Peters, P.J. Clevers, H. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*, Vol.459, No.7244, (May 2009), pp. 262-265, ISSN 0028-0836

Schofield, R. (1978) The relationship between the spleen colony-forming cell and the haematopoietic stem cell, *Blood Cells*, Vol.4, No.1-2, pp. 7-25, ISSN 0340-4684

Schröder, N. & Gossler, A. (2002). Expression of Notch pathway components in fetal and adult mouse small intestine. *Gene Expr Patterns*, Vol.2, No.3-4, (December 2002), pp. 247-250, ISSN 1567-133X

Seto. E.S. & Bellen, H.J.(2006) Internalization is required for proper Wingless signaling in Drosophila melanogaster, *J. Cell Biol*, Vol.173, No.1, (April 2006), pp. 95–106, ISSN 0021-9525

Sheldahl, L.C.; Park, M.; Malbon, C.C. & Moon, R.T. (1999) Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner, *Curr. Biol*, Vol.9, No.13, (July 1999), pp. 695–698, ISSN 0960-9822

Singh, N.; Phillips, R.A.; Iscove, N.N. & Egan, S.E. (2000). Expression of notch receptors, notch ligands, and fringe genes in hematopoiesis. *Exp hematol*, Vol.28, No.5, (May 2000), pp. 527-534, ISSN 0301-472X.

Slusarski, D.C.; Yang-Snyder, J.; Busa, W.B. & Moon, R.T. (1997) Modulation of embryonic intracellular Ca^{2+} signaling by Wnt-5A, *Dev. Biol*., Vol.182, No.1, (February 1997), pp. 114–120, ISSN 0012-1606

Smeets, B.; Angelotti, M.L.; Rizzo, P.; Dijkman, H.; Lazzeri, E.; Mooren, F.; Ballerini, L.; Parente, E.; Sagrinati, C.; Mazzinghi, B.; Ronconi, E.; Becherucci, F.; Benigni, A.; Steenbergen, E.; Lasagni, L.; Remuzzi, G.; Wetzels, J. & Romagnani, P. (2009) Renal progenitor cells contribute to hyperplastic lesions of podocytopathies and crescentic glomerulonephritis. *J Am Soc Nephrol*, Vol.20, No.12, (December 2009), pp. 2593-2603, ISSN 1046-6673

Smith, A.G. (2001). Embryo-derived stem cells: of mice and men, *Annu Rev Cell Dev Biol*., Vol.17, (November 2001), pp. 435-462, ISSN 1081-0706

Snippert, H.J. van der Flier, L.G. Sato, T. van Es, J.H. van den Born, M. Kroon-Veenboer, C. Barker, N. Klein, A.M. van Rheenen, J. Simons, B.D. Clevers, H. (2010). Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell*, Vol.143, No.1, (October 2010), pp. 134-144, ISSN 0092-8674

Staal, F.J. & Sen, J.M. (2008). The canonical Wnt signaling pathway plays an important role in lymphopoiesis and hematopoiesis. *Eur. J. Immunol,* Vol.38, No.7, (Jul 2008), pp.1788–1794, ISSN 00142980.

Tajbakhsh, S.; Rocheteau, P. & Le Roux, I. (2009) Asymmetric cell divisions and asymmetric cell fates, *Annu Rev Cell Dev Biol*, Vol.25, (November 2009), pp. 671-699, ISSN 1081-0706

Takashima, S.; Kadowaki, M.; Aoyama, K.; Koyama, M.; Oshima, T.; Tomizuka, K.; Akashi, K. & Teshima T. (2011). The Wnt agonist R-spondin1 regulates systemic graft-versus-host disease by protecting intestinal stem cells. *J Exp Med*, Vol.208, No.2, (February 2011), pp. 285-294, ISSN 0022-1007

Tanigaki, K. & Honjo, T. (2007). Regulation of lymphocyte development by Notch signaling. *Nature Immunology*, Vol.8, No.5, (May 2007), pp. 451-456, ISSN 1529-2916.

Thorner, P.S.; Ho, M.; Eremina, V.; Sado, Y. & Quaggin, S. (2008) Podocytes contribute to the formation of glomerular crescents. *J Am Soc Nephrol*, Vol.19, No.3, (March 2008), pp. 495-502, ISSN 1046-6673.

van de Wetering, M. Sancho, E. Verweij, C. de Lau, W. Oving, I. Hurlstone, A. van der Horn, K. Batlle, E. Coudreuse, D. Haramis, A.P. Tjon-Pon-Fong, M. Moerer, P. van den Born, M. Soete, G. Pals, S. Eilers, M. Medema, R. Clevers, H. (2002). The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell*, Vol.111, No.2, (October 2002), pp. (241-250), ISSN 0092-8674

Van Den Berg, DJ., Sharma, AK., Bruno, E. & Hoffman, R. (1998). Role of members of the Wnt gene family in human hematopoiesis. *Blood,* Vol.92, No.9, (November 1998), pp. 3189–3202, ISSN 15280020.

van Es, J.H.; van Gijn, M.E.; Riccio, O.; van den Born, M.; Vooijs, M.; Begthel, H.; Cozijnsen, M.; Robine, S.; Winton, D.J.; Radtke, F. & Clevers, H. (2005). Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature*, Vol.435, No.7044, (June 2005), pp. 959-963, ISSN 0028-0836

Veeman, M..T.; Axelrod, J.D. & Moon, R.T. A second canon. Functions and mechanisms of beta-catenin-independent Wnt signaling. *Dev. Cell.* Vol.5, No.3, (September 2003), pp. 367–377, ISSN 1534-5807

Vermeulen, L.; De Sousa, E.; Melo, F.; van der Heijden, M.; Cameron, K.; de Jong, J.H.; Borovski, T.; Tuynman, J.B.; Todaro, M.; Merz, C.; Rodermond, H.; Sprick, M.R.; Kemper, K.; Richel, D.J.; Stassi, G. & Medema, J.P. (2010). Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol*, Vol.12, No.5, (May 2010), pp. 468-476, ISSN 1097-6256

Vermeulen, L.; Sprick, M.R.; Kemper, K.; Stassi, G. & Medema, J.P. (2008). Cancer stem cells--old concepts, new insights. *Cell Death Differ*, Vol.15, No.6, (June 2008), pp. 947-958, ISSN 1350-9047

Vijayaragavan, K., Szabo, E., Bosse, M., Ramos-Mejia, V., Moon, R.T.& Bhatia, M. (2009). Noncanonical Wnt signaling orchestrates early developmental events toward hematopoietic cell fate from human embryonic stem cells. *Cell Stem Cell* , Vol.4, No.3, (March 2009), pp. 248-262, ISSN 19345909.

Vilimas, T.; Mascarenhas, J.; Palomero, T.; Mandal, M.; Buonamici, S.; Meng, F.; Thompson, B.; Spaulding, C.; Macaroun, S.; Alegre, M.L.; Kee, B.; Ferrandom, A.; Miele, L. & Aifantis, I. (2007). Targeting the NF-kappaB signaling pathway in Notch1-induced T-cell leukemia. *Nat Med*, Vol.13, No.1, (January 2007), pp. 70-77, ISSN 1078-8956.

Weng, A.P.; Ferrando, A.A.; Lee, W.; Morris, J.P., 4h; Silverman, L.B.; Sanchez-Irizarry, C; Blacklow, S.C.; Look, A.T. & Aster, J.C. (2004). Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science*, Vol.306, No.5694, (October 2004), pp. 269-271, ISSN 0036-8075.

Westfall, T.A.; Brimeyer, R.; Twedt, J.; Gladon, J.; Olberding, A.; Furutani-Seiki, M. & Slusarski, D.C. (2003) Wnt-5/pipetail functions in vertebrate axis formation as a negative regulator of Wnt/beta-catenin activity, *J. Cell Biol*., Vol.162, No.5, (September 2003), pp. 889–898, ISSN 0021-9525

Willert, K., Brown, J.D., Danenberg, E., Duncan, AW., Weissman, IL., Reya, T., Yates, JR. 3rd, Nusse, R. (2003). Wnt proteins are lipidmodified and can act as stem cell growth factors. *Nature*, Vol.423, No.6938, (May 2003), pp.448–452, ISSN 00280836.

Wodarz, A. & Nusse, R. (1998) Mechanisms of Wnt signaling in development. *Annu. Rev. Cell Dev. Biol*. Vol.14, (November 1998), pp. 59–88, ISSN 1081-0706

Yamashita YM, Yuan H, Cheng J, Hunt AJ. (2010) Polarity in stem cell division: asymmetric stem cell division in tissue homeostasis, *Cold Spring Harb Perspect Biol,* Vol.2, pp. a001313, ISSN 1943-0264

Yang, Q.; Bermingham, N.A.; Finegold, M.J. & Zoghbi, H.Y. (2001). Requirement of Math1 for secretory cell lineage commitment in the mouse intestine. *Science*, Vol.294, No.5549, (December 2001), pp. 2155-2158, ISSN 0036-8075

Zhao, C., Blum, J., Chen, A., Kwon, HY., Jung, SH., Cook, JM., Lagoo, A. & Reya T. (2007). Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. *Cancer Cell*, Vol.12, No.6, (December 2007), pp.528–41, ISSN 15356108.

Zhu, Y., Sun, Z., Han, Q., Liao, L., Wang, J., Bian, C., Li, J., Yan, X., Liu, Y., Shao, C., Zhao, RC.. (2009). Human mesenchymal stem cells inhibit cancer cell proliferation by secreting DKK-1. *Leukemia* , Vol.23, No.5, (May 2009), pp.925-33, ISSN 08876924.