Biochimica et Biophysica Acta 1803 (2010) 991–1002

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

The emerging role of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling network in normal myelopoiesis and leukemogenesis

Alberto M. Martelli [a,b,*], Camilla Evangelisti [a], Francesca Chiarini [a], Cecilia Grimaldi [a], Alessandra Cappellini [c], Andrea Ognibene [d], James A. McCubrey [e]

[a] Department of Human Anatomy, University of Bologna, via Irnerio 48, 40126 Bologna, Italy
[b] IGM-CNR, Sezione di Bologna c/o I.O.R., via di Barbiano 1/10, 40136 Bologna, Italy
[c] Department of Health Sciences, University of Cassino, viale Bonomi, 03043 Cassino, Italy
[d] Musculoskeletal Cell Biology Laboratory, I.O.R., via di Barbiano 1/10, 40136 Bologna, Italy
[e] Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University, 600 Moye Boulevard, Greenville, NC 27834, USA

ARTICLE INFO

Article history:
Received 5 March 2010
Received in revised form 6 April 2010
Accepted 6 April 2010
Available online 23 April 2010

Keywords:
PI3K/Akt/mTOR
Hematopoietic stem cell
Signal transduction
Proliferation
Differentiation
Leukemia

ABSTRACT

The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway mediates diverse and important physiological cell functions which include proliferation, differentiation, survival, motility, autophagy, and metabolism. However, dysregulated PI3K/Akt/mTOR signaling has been documented in a wide range of neoplasias, including malignant hematological disorders. It is now emerging that this signaling network plays a key role during normal hematopoiesis, a tightly regulated process resulting in the formation of all blood lineages. Blood cell development encompasses a complex series of events which are mainly regulated by actions of cytokines, a family of extracellular ligands which stimulate many biological responses in a wide array of cell types. Hematopoiesis is strictly dependent on the correct function of the bone marrow microenvironment (BMM), as BMM cells secrete most of the cytokines. Several of these cytokines activate the PI3K/Akt/mTOR signaling network and regulate proliferation, survival, and differentiation events during hematopoiesis. Here, we review the evidence that links the signals emanating from the PI3K/Akt/mTOR cascade with the functions of hematopoietic stem cells and the process of myelopoiesis, including lineage commitment. We then highlight the emerging role played by aberrant PI3K/Akt/mTOR signaling during leukemogenesis.

1. Introduction

The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling cascade is crucial to widely divergent physiological processes, which include cell cycle progression, differentiation, transcription, translation, apoptosis, endocytosis, motility, autophagy, and metabolism [1]. Moreover, a clear link between this pathway and cancer has been established already in the 1980s, and in recent years it has become apparent that this signal transduction network is one of the most frequently aberrantly regulated pathways in human tumors [1]. Since therapeutic targeting of the PI3K/Akt/mTOR axis is being considered as an option for innovative treatment of several types of cancers, including hematopoietic malignancies [2,3], it becomes of critical importance to establish the role, if any, of this network in normal myelopoiesis. Indeed, myelosuppression is often a dose-limiting effect of traditional cytotoxic drugs. Therefore, the aim of this review is to highlight the relevance of PI3K/Akt/mTOR signaling during normal myelopoiesis in the adult. Moreover, we will also discuss the emerging evidence that links pathway activation with leukemogenesis. However, we shall begin with a general overview outlining the mechanisms which regulate the PI3K/Akt/mTOR signal transduction network.

2. The PI3K/Akt/mTOR pathway

2.1. PI3K

PI3Ks belong to a conserved family of intracellular lipid kinases that catalyze the phosphorylation of the D3 position of inositol lipids. PI3K products then act as second messengers and mediate reversible membrane localization of cytoplasmic proteins. There are three different PI3K classes: I, II, and III. Class I PI3K phosphorylates both phosphatidylinositol (PtdIns) 4 phosphate and PtdIns 4,5 bisphosphate [PtdIns (4,5)P_2] to yield PtdIns (3,4)P_2 and PtdIns 3,4,5 trisphosphate [PtdIns (3,4,5)P_3], respectively. However, the in vivo preferred substrate of class I PI3K is PtdIns (4,5)P_2 [4]. PtdIns (3,4)P_2 and PtdIns (3,4,5)P_3 recruit to the plasma membrane pleckstrin homology (PH) domain-containing proteins, including Akt and phosphoinositide-dependent protein kinase 1 (PDK1). Class I PI3K is

* Corresponding author. Dipartimento di Scienze Anatomiche Umane, Università di Bologna, 40126 Bologna, Italy. Tel.: +39 051 2091580; fax: +39 051 2091695.
E-mail address: alberto.martelli@unibo.it (A.M. Martelli).

0167-4889/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2010.04.005

divided further into A and B subtypes. Class IA PI3Ks are dimers comprising a regulatory (p85α, p85β, p55α, p55γ, and p50α) and a catalytic (p110α, p110β, and p110δ) subunits. They act downstream of both tyrosine kinase receptors (TKRs) and G protein-coupled receptors (GPCRs) (Fig. 1). The single class IB PI3K comprises a p101 regulatory and a p110γ catalytic subunits and is activated downstream of GPCRs [5]. p110α and p110β PI3Ks are ubiquitously expressed in mammalian tissues/organs and play key roles during development and cell growth. Therefore, their homozygous knockout is embryonic-lethal [6]. In contrast, p110γ and p110δ PI3Ks are highly enriched in leukocytes, so that their knockdowns lead to impaired immune responses [7].

Class II PI3Ks, which comprise the PI3K-C2α, -C2β, and -C2γ isoforms, preferentially phosphorylate PtdIns to yield PtdIns (3)P. They are downstream of TKRs and GPCRs, although their mechanism of activation most likely differs from that of Class I isoenzymes [8]. Class II PI3Ks are widely expressed in mammalian tissues/organs, however, their importance in cell signaling and biology, relative to that of class I PI3Ks, is not clear at the moment [9]. They could be involved in cell motility and exocytosis [8]. Vacuolar protein sorting 34 (vps34), is the only class III PI3K and exists as a heterodimer bound to the vps15 regulatory subunit (formerly called p150 in mammals). Vps34 has been implicated in autophagy, endocytosis, protein synthesis, and nutrient signaling [10,11].

2.2. Akt

Akt is a 57-kDa enzyme, belonging to the AGC protein kinase family. Akt, also referred to as protein kinase B (PKB), is the cellular homolog of the v-akt oncogene. The Akt family includes three highly conserved isoforms: Akt1/α, Akt2/β, and Akt3/γ. Whereas Akt1 and Akt2 are ubiquitously expressed, Akt3 displays a more restricted tissue distribution and is found abundantly in nervous tissue [4]. All Akt isoforms share structural homology, including PH domain, ATP binding site, and two phosphorylation sites. Genetic studies have disclosed that Akt isoforms exert non-redundant functions, despite their high sequence similarity. While Akt1 null mice have overall growth retardation, Akt2 null mice develop insulin-resistance and diabetes. In contrast, Akt3 null mice display reduced brain size [12].

Akt recruitment at the plasma membrane by PtdIns $(3,4)P_2$ and PtdIns $(3,4,5)P_3$ results in a conformational change [4], that enables the activation segment of Akt to be phosphorylated on Thr308 by PDK1, while Ser473 in the hydrophobic motif is targeted by a kinase that has been identified as the mTOR complex 2 (mTORC2) (Fig. 1), although other kinases could also be involved. These kinases include integrin-linked kinase, and DNA-dependent kinase [13]. Full Akt activation requires both the phosphorylation steps. Active Akt migrates to the cytosol, the mitochondria, and the nucleus. Nuclear Akt fulfils important anti-apoptotic roles [14]. However, the relative

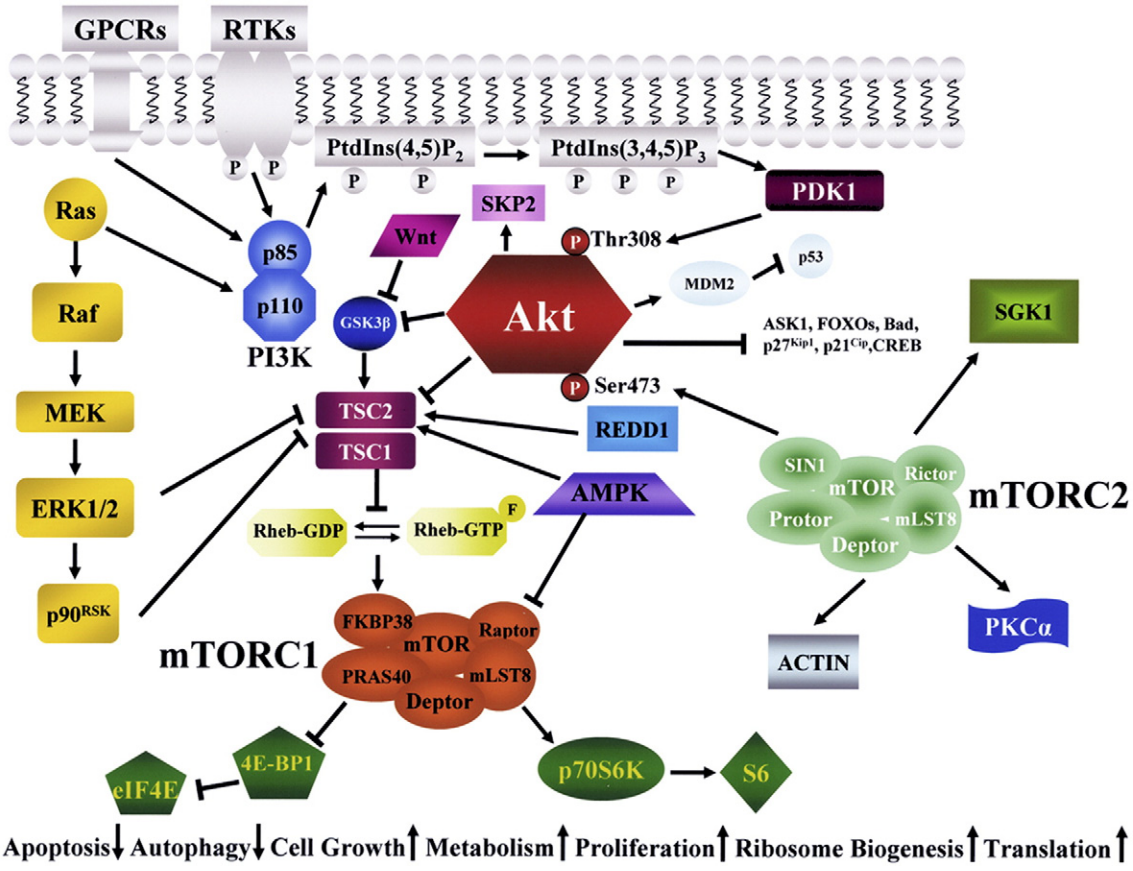

Fig. 1. The PI3K/Akt/mTOR pathway. GPCRs, RTKs, and Ras stimulate class I PI3K activity. PI3K generates PtdIns $(3,4,5)P_3$ from PtdIns $(4,5,)P_2$. PtdIns $(3,4,5)P_3$ attracts to the plasma membrane PDK1 that phosphorylates Akt on Thr308. Full Akt activation requires Ser473 phosphorylation by mTORC2. Once activated, Akt phosphorylates several substrates, including GSK3β, FOXO transcription factors, CREB, SKP2, MDM2, and Bad. Active Akt inhibits TSC2 activity through direct phosphorylation. TSC2 is a GAP that functions in association with TSC1 to inactivate the small G protein Rheb. Akt-driven TSC1/TSC2 complex inactivation allows Rheb to accumulate in a GTP-bound state. Rheb-GTP then upregulates, through a mechanism not yet fully elucidated, the protein kinase activity of mTORC1. However, other signals impinge on mTORC1, including the Ras/Raf/MEK/ERK1/2/$p90^{RSK}$ pathway, the AMPK network, REDD1, and Wnt/GSK3β. mTORC1 targets p70S6K and 4E-BP1 which are critical for translation. Ribosomal S6 protein is a target of p70S6K. mTORC2 regulates actin polymerization and phosphorylates PKCα and SGK1. The regulation of mTORC2 activity is still unclear. Arrows indicate activating events, whereas perpendicular lines highlight inhibitory events.

contribution of Akt signaling at the various cell domains remains to be established.

At present, over 100 Akt substrates have been identified [15]. Around 40 substrates which mediate the pleiotropic Akt functions have been characterized, including caspase-9, Bad, murine double minute 2 (MDM2, a negative regulator of p53), proline-rich Akt substrate 40 (PRAS40), FOXO family of Forkhead transcription factors, apoptosis signal-regulated kinase 1 [ASK1, a negative regulator of pro-apoptotic c-Jun N-terminal kinase (JNK)], Raf, S-phase kinase-associated protein 2 (SKP2), $p27^{Kip1}$, $p21^{Cip1}$, glycogen synthase kinase 3β (GSK3β), and cAMP response element-binding protein (CREB). Each of these substrates plays a key role in the regulation of cell proliferation, differentiation, and survival, either directly or through an intermediary [5,16,17].

2.3. mTOR

mTOR is an atypical 289-kDa serine/threonine kinase, originally identified in the yeast *Saccharomyces cerevisiae*, that belongs to the PI3K-related kinase family and displays a COOH-terminal catalytic domain with a high sequence homology to PI3K. In spite of this homology, mTOR functions exclusively as a protein kinase [18]. mTOR signaling is conserved in eukaryotes from plants and yeasts to mammals. mTOR exists as two multi-protein complexes, referred to as mTOR complex 1 (mTORC1) and mTORC2 (Fig. 1). mTORC1 is comprised of mTOR/Raptor/mLST8/PRAS40/FKBP38/Deptor and is sensitive to rapamycin and its derivatives (rapalogs). mTORC2 is composed of mTOR/Rictor/mLST8/SIN1/Protor/Deptor and is generally described as being insensitive to rapamycin/rapalogs, although long-term treatment of about 20% of cancer cell lines with rapamycin/rapalogs leads to dissociation of mTORC2 [19,20]. Recently, a short (80-kDa) mTOR splicing variant, referred to as mTORβ, has been identified. mTORβ interacts with both Raptor and Rictor. It has been proposed that mTORβ, but not the full-length mTORα, is responsible for coordinating cell cycle progression and cell proliferation. Moreover, mTORβ was tumorigenic in nude mice and could be a protooncogene [21]. Deptor is a recently-identified, intriguing component of both mTORC1 and mTORC2 [22]. Loss of Deptor activated p70S6 kinase (p70S6K), Akt, and serum- and glucocorticoid-induced protein kinase 1 (SGK1, that belongs to the AGC kinase family), promoted cell growth and survival, and upregulated both mTORC1 and mTORC2 kinase activities. Deptor overexpression suppressed p70S6K but, by relieving feedback inhibition from mTORC1 to PI3K signaling (see later on), activated Akt. Consistent with many human cancers having activated mTORC1 and mTORC2 pathways, Deptor expression is low in most neoplasias [22]. Surprisingly, Deptor was found highly overexpressed in a subset of multiple myelomas harboring chromosomal translocations. However, in these cells, high Deptor expression was required to maintain PI3K/Akt activation and a reduction in Deptor levels led to apoptosis.

mTORC1 signaling integrates environmental clues (oxygen levels, growth factors, hormones, nutrients, and stressors) and information from the cell metabolic status. Thus, mTORC1 controls anabolic processes for promoting protein synthesis and cell growth [23]. mTORC1 regulates translation in response to nutrients/growth factors by phosphorylating components of the protein synthesis machinery, including p70S6K and 4E-BP1 (eukaryotic initiation factor 4E-binding protein 1). p70S6K phosphorylates the 40S ribosomal protein, S6, leading to active translation of mRNAs, while 4E-BP1 phosphorylation by mTORC1 on several amino acidic residues (Thr37; Thr46; Ser65; Thr70) results in the release of the eukaryotic initiation factor 4E (eIF4E). eIF4E is a key component for translation of 5′ capped mRNAs, which include transcripts encoding growth and survival promoting molecules, such as c-Myc, cyclin D1, cyclin-dependent kinase 2, retinoblastoma protein, $p27^{Kip1}$, vascular endothelial growth factor (VEGF), and signal activator and transducer of transcription 3 (STAT3) [19,24].

Besides protein synthesis, it is now beginning to emerge that mTORC1 could control lipid synthesis as well as mitochondrial metabolism and biogenesis [25]. Furthermore, mTORC1 negatively regulates autophagy, a catabolic process which is important in organelle degradation and protein turnover [26,27].

Akt-mediated regulation of mTORC1 activity involves several mechanisms. Akt phosphorylates Tuberous Sclerosis 2 (TSC2 or hamartin) at multiple sites, which include Ser 939, Ser 981, and Thr 1462. TSC2 is a GTPase-activating protein (GAP) that associates with Tuberous Sclerosis 1 (TSC1 or tuberin) for inactivating the small G protein Ras homolog enriched in brain (Rheb). When phosphorylated by Akt, TSC2 binds 14-3-3 protein [28]. This then reduces the GAP activity of the TSC1/TSC2 complex, allowing Rheb to accumulate in a GTP-bound state. The mechanism by which Rheb-GTP activates mTORC1 has not been fully elucidated yet, although Rheb requires to be farnesylated for activating mTORC1 [29]. Akt also phosphorylates PRAS40, an inhibitor of the interactions between mTORC1 and its substrates, and by doing so, prevents PRAS40 ability to suppress mTORC1 signaling [30]. Moreover, PRAS40 is a substrate of mTORC1 itself, and it has been demonstrated that mTORC1-mediated phosphorylation of PRAS40 facilitates the removal of its inhibition on mTORC1 [31]. Furthermore, Ras/Raf/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) 1/2 signaling positively regulates mTORC1 activity, as both ERK 1/2 and p90 ribosomal S6 kinase ($p90^{RSK}$) phosphorylate TSC2, thus suppressing its inhibitory function on Rheb [32]. Another signaling pathway which impinges on mTORC1 is the Wnt/GSK3β cascade [33], as it has been documented that Wnt stimulation could activate mTORC1 by inhibiting the TSC2 phosphorylation driven by GSK3β [34] (Fig. 1). mTORC1 signal transduction is inhibited by the master metabolic regulator, energy-sensing AMP-dependent protein kinase (AMPK), as AMPK phosphorylates TSC2 on Ser 1345. This phosphorylative event primes TSC2 for subsequent phosphorylation by GSK3β on Ser 1337 and Ser 1341 [35]. Indeed, the coordinated phosphorylation of TSC2 by AMPK and GSK3β is required for maximal activation of TSC2 and inhibition of mTORC1 [18,34]. However, AMPK also phosphorylates Raptor and this phosphorylation induces 14-3-3 protein binding to Raptor. The phosphorylation of Raptor by AMPK is necessary for the inhibition of mTORC1 and cell cycle arrest induced by energy stress [36].

For the purposes of this review, it is also important to emphasize here that hypoxia inhibits mTORC1 activity through induction of REDD1 (Regulated in Development and DNA damage responses) and REDD1-mediated dissociation of growth factor-stimulated TSC2/14-3-3-complex formation [37].

The mechanisms for TORC2 regulation have only begun to be revealed. However, mTORC2 activation requires PI3K and the TSC1/TSC2 complex, but is independent of Rheb and is largely insensitive to either nutrients or energy conditions [38]. mTORC2 phosphorylates Akt on Ser473 that enhances subsequent Akt phosphorylation on Thr308 by PDK1 [39]. Moreover, mTORC2 plays a role in cytoskeleton organization by controlling actin polymerization [40] and phosphorylates protein kinase C (PKC) α [38]. The oncogenetic role of mTORC2 has been recently highlighted by an investigation that documented the importance of mTORC2 in the development and progression of prostate cancers induced in mice by PTEN (phosphatase and tensin homolog deleted on chromosome 10) loss [41]. Another downstream target of mTORC2 is SGK1 [42]. There is now evidence that mTORC2 could facilitate cell proliferation through SGK1 and not Akt, at least in some experimental models [17].

Akt and mTORC1/2 are linked to each other via positive and negative regulatory feedback circuits, which restrain their simultaneous hyperactivation through mechanisms involving p70S6K and PI3K. Once mTORC1 is activated through Akt, the former elicits a negative feedback loop for inhibiting Akt activity [19]. This negative regulation of Akt activity by mTORC1 is a consequence of p70S6K-mediated phosphorylation of insulin receptor substrate (IRS) 1 adapter protein,

downstream of insulin receptor and/or insulin-like growth factor-1 receptor [43–45]. Indeed, IRS1 phosphorylation on Ser307 and Ser636/639 by p70S6K targets the adapter protein to proteasomal degradation [46]. Therefore, at least in principle, inhibition of mTORC1 activity by rapamycin/rapalogs could result in hyperactivation of both Akt and its downstream targets. Such a phenomenon has been documented to occur both in vitro and in vivo [47,48]. mTORC1 is capable of downregulating also IRS2 expression by enhancing its proteosomal degradation [49]. Recent work has also highlighted a p70S6K-mediated phosphorylation of Rictor on Thr 1135. This phosphorylative event exerted a negative regulatory effect on the mTORC2-dependent phosphorylation of Akt in vivo [50]. Thus, both mTORC1 and mTORC2 control Akt activation.

3. Negative regulation of PI3K/Akt/mTOR signaling

Phosphorylated inositol lipids are not hydrolyzed by any known phospholipase. Therefore, the activity of several phosphatases is instrumental to counterbalance PI3K/Akt/mTOR signaling activation. PTEN is a dual specificity lipid and protein phosphatase that preferentially removes the 3′-phosphate mainly from PtdIns (3,4,5)P_3, but is also active on PtdIns (3,4,)P_2, thereby antagonizing network signaling [51,52]. PTEN inactivating mutations or silencing occur in a wide variety of human cancers and this results in Akt/mTOR upregulation. Two other phosphatases, SHIP1 and SHIP2 (for Src homology domain-containing inositol phosphatase), remove the 5-phosphate from PtdIns (3,4,5)P_3 to yield PtdIns (3,4,)P_2 [53]. While SHIP1 is predominantly expressed in hematopoietic cells, SHIP2 is more ubiquitous. Protein phosphatase 2A (PP2A) downregulates Akt activity directly, by dephosphorylating Thr308 [54]. Additionally, Thr308 and Ser473 residues of Akt are targeted by the two isoforms (1 and 2) of PH domain leucine-rich repeat protein phosphatase (PHLPP) [55].

4. Normal hematopoiesis

Since most blood cells display a very short life, every day billions of mature blood cells are replenished in humans, during steady-state hematopoiesis. Moreover, the hematopoietic system also needs to quickly respond when physiological demands (e.g. bleeding and infection) dictate [56]. The integrity of hematopoiesis is strictly dependent on the existence and persistence in the adult bone marrow of the rare hematopoietic stem cells (HSCs) which display both an extensive self-renewal capacity and the ability to sustain all blood cell lineages throughout lifetime [57]. HSCs are defined operationally by their capacity to reconstitute the entire blood system of a recipient [58,59]. At least two types of HSC have been identified, long-term HSCs (LT-HSCs) and short-term HSCs (ST-HSCs) [60,61]. LT-HSCs can provide long-term (>4 months) hematopoietic reconstitution in the recipient and possess a high self-replicating activity (Fig. 2).

The prevailing model for adult hematopoietic lineage commitment is considered as a pyramidal/hierarchical model, implying that the first lineage commitment step of HSCs results in a strict separation of lymphopoiesis and myelopoiesis, giving origin to common lymphoid (CLP) and myeloid (CMP) progenitors. These progenitors are capable of exponential proliferation, as well as continuing the differentiation process [58,62]. While CLP will ultimately develop into natural killer (NK), T, and B lymphocytes, CMP will evolve into megakaryocyte/erythroid (MEP) and granulocyte/monocyte (GMP) committed progenitors [63,64], which then give rise to myeloid lineage committed cells [65] (Fig. 2). Therefore, myeloid cells comprise erythrocytes, megakaryocytes, and granulocytes/monocytes. An alternative commitment route entails the existence of progenitors which are restricted to lymphocytes and granulocytes/monocytes, but displaying little or no megakaryocyte/erythroid potential [66].

It is widely assumed that the dividing HSCs in bone marrow undergo asymmetric cell division, in which a HSC gives rise to two non-identical daughter cells, one keeping the HSC identity to maintain their number constant and the other becoming a more differentiated progenitor cell. HSC activity needs to be tightly regulated so as to meet physiological demands, but also to protect HSCs from physical, chemical, and oncogenetic damage [67]. The site or physical microenvironment that regulates self-renewal, proliferation, and differentiation of HSCs is referred to as the “HSC niche” [68,69]. Currently, two types of HSC niches have been identified in bone marrow, the endosteal niche located on the surface of trabecular bone, and the vascular niche at the bone marrow sinusoids, which are low-pressure blood vessels with a fenestrated endothelium located in the center of the bone marrow [70]. While the vascular niche is permissive for proliferation and differentiation, the endosteal niche facilitates HSC maintenance and quiescence [71]. The vascular niche constitutes a barrier somehow similar to the basement membrane for regulating the release of only the most mature hematopoietic elements, such as erythrocytes, mature granulocytes/monocytes, and platelets [72].

The niches are composed of different cells, which include osteoblasts, osteoclasts, endothelial cells, fibroblasts, and adipocytes [73]. These cells, which are part of the bone marrow microenvironment (BMM), produce the factors which are necessary for supporting HSC functions.

Basal and emergency hematopoiesis is mostly regulated by cytokines, a large family of extracellular ligands which can stimulate a wide array of biological responses in many cell types [74]. Several studies have strongly suggested that osteoblasts residing on the bone surface are key components of the endosteal HSC niche, as osteoblast cell lines secrete many cytokines which promote the proliferation of hematopoietic cells in culture, and support the in vitro maintenance of HSCs [70]. Cytokines can be arranged in a hierarchical system with broadly acting cytokines such as stem cell factor (SCF, the ligand for c-Kit tyrosine kinase receptor) and interleukin (IL) -2, -3, or -7 acting on multipotential cells, and lineage-specific cytokines, such as erythropoietin (EPO), acting on specific lineages [56] (Fig. 2).

5. PI3K/Akt/mTOR signaling and normal myelopoiesis

5.1. HSC maintenance

Most adult HSCs are in a quiescent state, i.e. they are in the G_0 phase of the cell cycle, while only a small (5%) subset is dividing [75]. In mice, it has been estimated that HSCs experience a single cell division every 2–4 weeks [76]. It has been hypothesized that quiescence is one of the major mechanisms to keep HSC function and protect them from environmental clues [77]. Our knowledge of the soluble factors which could be involved in HSC quiescence is limited. Recent findings suggest that HSCs could be maintained in a quiescent state through interactions with thrombopoietin (TPO)-producing osteoblasts [78]. Nevertheless, other investigators have documented that TPO works in synergy with IL-3 and SCF to promote proliferation in vitro of both murine and human HSCs [79,80]. It is therefore plausible that a delicate balance in positive and negative signals downstream from the TPO receptor plays a role in the regulation of the probability of self-renewal of HSCs [81].

Studies from independent laboratories have demonstrated that if HSCs undergo uncontrolled proliferation, they lose their long-term functions. It is now emerging that PI3K/Akt/mTORC1 signaling plays important roles in HSC maintenance. Indeed, it has been reported that lipid-raft clustering induced by SCF and TPO could be critically involved in mouse LT-HSC re-entry into the cell cycle by upregulation of Akt that thereby decreased FOXO3a transcription factor activity [82]. Also, acute treatment with interferon α (INFα) increased Akt1 phosphorylation in mice HSCs and this correlated with their entering an active cell cycle. However, chronic INFα exposure, impaired HSC function in repopulation assays [83].

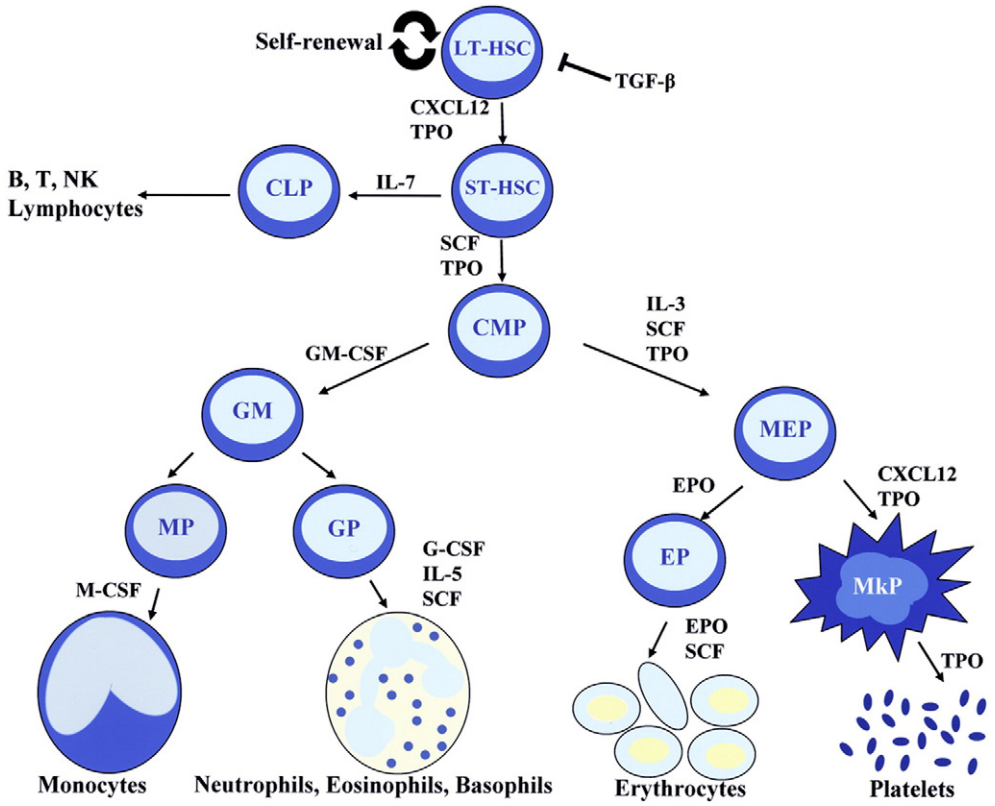

Fig. 2. Schematic of the current model for adult hematopoiesis highlighting intermediates in the hematopoietic differentiation hierarchy. Hematopoiesis progresses from LT-HSCs (which are capable of self-renewal) to ST-HSCs which differentiate into multilineage committed CMP and CLP. ST-HSCs then give rise to more differentiated dual progenitors, including those committed to myelopoiesis, i.e. megakaryocyte and erythroid progenitor cells (MEP), as well as granulocyte and macrophage progenitor cells (GMP). Ultimately, these dual progenitors originate unilineage committed progenitors for megakayocytes (MkP), erythrocytes (EP), monocytes (MP), and granulocytes (GP). Some of the best characterized cytokines regulating the proliferation/differentiation processes are indicated in the figure. Arrows indicate activating events, whereas perpendicular lines highlight inhibitory events.

Conditional deletion of PTEN in adult murine HSCs resulted in an initial expansion followed by a depletion of LT-HSCs. This transient increase in HSC number was due to enhanced cell cycle progression, that, however, ultimately resulted in exhaustion of the HSC population [84]. Rapamycin reverted the phenotype of PTEN-deficient HSCs, suggesting that mTORC1 signaling was responsible for this phenotype [85]. These results have been confirmed by others, who, however, highlighted that Wnt/β-catenin/GSK3β signaling could be upstream of mTORC1 activity in the regulation of HSC homeostasis in mice [86].

Different genetic strategies for activating mTORC1 signaling have led to essentially similar results. TSC1 deficient mice also displayed initial increased HSC proliferation, leading to short-term expansion but long-term depletion of HSCs [87,88]. TSC1 deletion resulted in higher mTORC1 activity that promoted HSC proliferation. These proliferating HSCs displayed dramatically elevated reactive oxygen species (ROS) levels which were responsible for the depletion, as a ROS-scavenger (N-acylcysteine) significantly rescued the repopulating activity of the mutant HSCs [87]. Indeed, it is well established that ROS dramatically limit the lifespan of HSCs [89] and the bone marrow HSC niche is thought to be a low-oxygenic environment that impairs ROS production [90]. ROS overproduction that characterizes $TSC1^{-/-}$ HSCs most likely has a mitochondrial origin, as it has been found that the mitochondrial mass and mitochondrial DNA were approximately two-fold increased in these genetically-modified HSCs. Moreover, the expression levels of mitochondrial oxidative genes were also significantly upregulated in TSC1-deficient HSCs, suggesting high levels of mitochondrial oxidative activity and ROS generation [87].

Also overexpression of Rheb2 caused a transient expansion of mouse HSCs, but these cells were significantly impaired in their ability to repopulate primary and secondary congenic transplant recipients [91]. At least another mTORC1 regulator plays a similar role in the maintenance of HSC properties. Promyelocytic leukemia (PML) gene deficient mice exhibited short-term increased but long-term decreased HSC repopulating activity [92]. HSCs with PML knockout exhibited mTORC1 hyperactivation and rapamycin rescued the repopulation defect observed in these mutant mice. In a previous study, this group had demonstrated that PML sequestered mTOR into nuclear bodies and inhibited mTORC1 functions under hypoxic conditions [93]. Therefore, when PML was deleted, signaling downstream of mTORC1 was upregulated, leading to HSC exhaustion.

Intriguingly, recent findings have documented that mTORC1 activity was higher in HSCs from old mice compared to those from young mice [94]. If mTORC1 was activated in young mice HSCs by conditional deletion of TSC1, these cells displayed an impaired capacity to reconstitute the hematopoietic system. In old mice, rapamycin increased the life span and restored the self-renewal/hematopoietic activity of HSCs, suggesting that mTORC1 inhibitors may have the potential to improve hematopoiesis in the elderly, who frequently displays anemia. Thus, these findings are in agreement with the emerging theory that dampening mTORC1 activity could lead to widespread protection from an array of age-related disorders [95].

Members of the FOXO family of transcription factors, FOXO1, FOXO3, and FOXO4 are substrates of Akt, that inactivates their transcriptional activity by increasing FOXO 14-3-3 protein dependent export into the cytoplasm [96]. It has been reported that FOXO factors are critical for the long-term maintenance of HSCs. Mice in which FOXO1, FOXO3, and FOXO4 were conditionally and concomitantly deleted in the adult hematopoietic system, displayed a marked reduction of HSC number and function in response to physiologic

oxidative stress [97]. Notably, there was a marked context-dependent increase in ROS levels in FOXO-deficient HSC compared with wild-type HSC. This correlated with changes in expression of several genes which regulate ROS production, including GADD45 (Growth Arrest and DNA Damage 45), catalase, and superoxide dismutase (Sod), Sod1 and Sod3. Furthermore, aged FOXO3a knockout animals also displayed a reduction of the HSC pool and an impaired repopulating capacity in serial transplantation assays, accompanied by elevated p38 mitogen-activated protein kinase activity and ROS levels [98]. Increased exit from quiescence and enhanced apoptosis, two of the features observed in FOXO-deficient mutants, could act together to decrease the pool size of HSCs available for self-renewal [99]. Intriguingly, these findings were in agreement with an earlier observation that documented the importance of PI3K/Akt/FOXO3 signaling for the survival of Lin^- mouse hematopoietic progenitor cells challenged with SCF [100]. These observations beg the question of which factors could regulate HSC quiescence and proliferation. It has been proposed that CXCL12 (for CXC chemokine ligand 12, previously referred to as stroma-derived factor-1α or SDF-1α) and transforming growth factor β (TGFβ) play key roles in the regulation of HSC cell cycle status [101]. CXCL12 is abundantly secreted by the osteoblasts which line the HSC niche, while HSCs express high levels of the CXCL12 receptor, CXCR4 [67]. CXCL12 acted as a survival and proliferation factor for human $CD34^+$ cells by upregulating proteins which accelerated cell cycle progression, while TGFβ blocked progression through the G_1 phase of the cell cycle. Interestingly, CXCL12 treatment of human $CD34^+$ cells isolated from the peripheral blood, resulted in activation of PI3K/Akt/mTORC1 signaling, while TGFβ opposed pathway upregulation [101]. In this human model, FOXO3a was identified as an important mediator of the opposing effects of the two cytokines on HSCs, as CXCL12 increased FOXO3a phosphorylation, whereas TGFβ downregulated it. Indeed, in $CD34^+$ cells overexpressing a non-phosphorylatable form of FOXO3a, CXCL12 did not promote cell cycle progression [101]. Another clue to the involvement of PI3K/Akt/mTORC1 signaling in HSC functions comes from the observation that SHIP1 deletion, that leads to pathway upregulation, initially resulted in higher proliferation of LT-HSCs, but reduced their long-term repopulation capacity [102]. However, SHIP1 is also expressed in cells comprising the HSC niche, so that SHIP1 deletion also profoundly altered the functions of these cells, including their chemokine production and their ability to control HSC proliferation and retention. Therefore, it has been hypothesized that while PTEN is probably the dominant inositol phosphatase that restrains PI3K signaling in HSCs, SHIP1 could be the dominant inositol phosphatase in the cells of the bone marrow niche which support HSCs [103].

5.2. Lineage commitment

Lineage commitment is the process by which a multipotent stem or progenitor cell becomes increasingly restricted in its lineage fate options, to eventually (or directly) develop into a fully committed progenitor of a single cell lineage [57]. Transplantation of sublethally irradiated β2 microglobulin$^{-/-}$ NonObese Diabetic/Severe Combined Immunodeficient (NOD/SCID) mice with $CD34^+$ cells overexpressing constitutively active Akt, resulted in enhanced monocyte and neutrophil development, whereas a dominant negative Akt construct induced eosinophil development in vivo [104]. Interestingly, it has been reported that either pharmacological inhibition of PI3K/Akt signaling or ectopic expression of a dominant negative Akt resulted in upregulated phosphorylation of CCAATT/enhancer binding protein α (C/EPBα) in neutrophil progenitors, whereas GSK3β inhibition lowered C/EPB α phosphorylation levels. Furthermore, the expression of constitutively active Akt caused a marked decrease in the expression levels of Jun B, a C/EPBα transcriptional target [104]. This would imply that Akt could control C/EPB α phosphorylation through GSK3β, thus influencing lineage choice decisions during myelopoiesis. It should be emphasized here that C/EPB α is one of the key transcription factors which regulate lineage choices during myelopoiesis [105] and its phosphorylation on Ser21 can inhibit granulocytic differentiation [106]. The importance of PI3K/Akt/GSK3β and Wnt/GSK3β signaling pathway cross-talks during myelopoiesis has been emphasized by others [107].

5.3. Erythropoiesis

Erythrocyte production is tightly regulated by the cytokines EPO and SCF, which sustain the proliferation, survival, and differentiation of erythroid progenitors [108–111]. EPO and SCF activate common signaling pathways which include JAK/STAT5, Ras/Raf/MEK/ERK, and PI3K/Akt/mTOR [112]. When compared with EPO, SCF is a much more potent Akt activator [113]. However, SCF could not induce cell survival in the absence of EPO [114]. Instead, SCF signaling delayed differentiation, that was increased by the PI3K inhibitor, LY294002 [113]. Upon EPO binding to its receptor (EPOR), the EPOR is tyrosine phosphorylated and recruits Src homology-2 (SH2) domain-containing proteins, thereby activating different signaling cascades (see above) [115,116]. Alternatively, EPO could lead to PI3K/Akt/mTOR activation through phosphorylation of the growth factor-bound protein (Grb)-associated binder (Gab), or through phosphorylation of the IRS2 adaptor protein [117]. Gab could then impinge upon Ras signaling, which is known to be critical for EPO effects on erythropoiesis [118]. The Akt substrate FOXO3a played a critical role in these EPO- and SCF-evoked phenomena [119], as its inactivation by Akt resulted in decreased expression of pro-apoptotic genes [120] and of the cyclin-dependent kinase inhibitor, $p27^{Kip1}$ [117]. Furthermore, Akt directly phosphorylated and activated the transcription factor GATA-1 (Globin transcription factor-1), a key regulator of erythroid differentiation [121–123]. The involvement of mTORC1 in EPO signaling was supported by the increased levels of phosphorylated p70S6K observed in response to EPO challenging [117]. However, the consequences of upregulated mTORC1 activity on erythropoiesis are unclear, but could be related to cell cycle progression. In this context it is important to emphasize how gene expression profiling studies have highlighted several genes which are under the control of the PI3K/Akt pathway in human early erythroid progenitors ($CD34^+CD71^+CD45RA^-GPA^-$), incubated with EPO. The upregulated genes played an important role during erythroid proliferation/differentiation and included: cyclin D3, E and A, as well as c-Kit and CDH1 (E-cadherin) [124]. In another more recent study, gene expression profiles downstream of mTORC1 were investigated at the polysomal level, using immortalized erythroblasts co-stimulated with EPO plus SCF. Nine genes were identified which required EPO/SCF stimulation for polysome recruitment and were downregulated during erythroid differentiation. One of these genes, Immunoglobulin binding protein 1 (Igbp1), is a regulatory subunit of PP2A (the α4 subunit of PP2A) that sustains PI3K/Akt/mTORC1 signaling. Constitutive expression of Igbp1 impaired erythroid differentiation, maintained high levels of 4E-BP1 and p70S6K phosphorylation, and enhanced polysome recruitment of multiple eIF4E-sensitive mRNAs. Therefore, it was inferred that PI3K-dependent polysome recruitment of Igbp1 acted as a positive feedback mechanism on translation initiation, underscoring the important regulatory role of selective mRNA recruitment to polysomes for finely tuning the balance between proliferation and maturation of erythroblasts [125].

5.4. Megakaryocytopoiesis

Megakaryocyte differentiation is characterized by endomitosis in the absence of nuclear and cellular division, thereby increasing DNA and cytoplasmic content. This leads to the generation of large polyploid cells with a dramatically increased cytoplasmic volume, the function of which is to produce and shed platelets [126]. During this process, a

complex network of hematopoietic cytokines/growth factors is involved. TPO is the most thoroughly investigated regulator of megakaryocyte growth and differentiation. Nevertheless, in addition to TPO, other cytokines have non-negligible effects on megakaryocytopoiesis, including CXCL12 [127] and bone morphogenetic protein 4 (BMP4), a member of the TGFβ family [128].

TPO binds its cognate receptor, the cellular protooncogene c-Mpl, i.e. the homolog of the murine myeloproliferative leukemia virus oncogene, v-Mpl [129]. Once activated by TPO engagement, c-MPL stimulates a series of signal transductions cascades, including PI3K/AKT/mTOR. [130–132]. Blocking this pathway resulted in inhibition of TPO-dependent megakaryocyte survival. Nevertheless, PI3K/AKT/mTOR signaling was necessary but not sufficient for TPO-induced cell cycle progression in primary megakaryocyte progenitors [133], implying that other pathways play important roles in the regulation of cell cycle.

Identified Akt substrates in megakaryocytes include FOXO3a [134], $p27^{Kip1}$ [135], and GSK3β [136]. One of the fundamental biological activities of TPO is the prevention of megakaryocyte apoptosis. Akt activation mediated by TPO, was instrumental for blunting caspase-3-mediated cleavage of anti-apoptotic Bcl-X_L protein. This could be one of the mechanisms by which PI3K/Akt signaling counteracts apoptosis during megakaryocytopoiesis [137]. mTORC1 and its downstream substrates, p70S6K and 4E-BP1, are critically involved in TPO-induced proliferation of megakaryocyte progenitors [138], as well as in the late stages of megakaryocyte differentiation [139]. A recent investigation has tried to unravel the functions of both mTORC1 and mTORC2 in MO7e megakaryoblastic cells. By exploiting lentiviral constructs encoding short hairpin RNA sequences to either Raptor or Rictor, it was documented that mTORC1 regulated cell growth and size by inhibiting autophagy, whereas mTORC2 was involved in cell cycle progression and nuclear ploidity [126]. Nevertheless, it remains to be established if these findings would apply also to primary megakaryocyte progenitors. Indeed, a previous study had highlighted that in $CD34^+$ cells, purified from human peripheral blood and treated with TPO, rapamycin (that should mainly target mTORC1) strongly inhibited cell polyploidization [140].

5.5. Granulocytopoiesis/monocytopoiesis

Granulocyte/macrophage-colony stimulating factor (GM-CSF) [141], granulocyte-colony stimulating factor (G-CSF), and macrophage-colony stimulating factor (M-CSF) are key factors for granulocytopoiesis/monocytopoiesis, and there is evidence for an involvement of PI3K/Akt signaling in these phenomena [141,142]. However, a very recent report has documented that mTORC1 signaling is not essential for myeloid progenitor differentiation [143].

6. PI3K/Akt/mTOR signaling and leukemogenesis

It is generally thought that leukemogenesis involves a series of alterations, which ultimately transform a normal HSC or a committed hematopoietic progenitor, into a leukemic stem cell (LSC) capable of propagating the malignant clone [144]. Over the last 15 years, remarkable progress has been made in the elucidation of the molecular pathogenesis of leukemias. This is especially true of acute myelogenous leukemia (AML) [145,146]. A 'two hits' model has suggested that AML development requires multiple genetic/epigenetic changes which deregulate different cell programs. Transcription factor fusion proteins such as AML1/ETO (Acute Myeloid Leukemia1/Eight Twenty One), PML-RARα (Retinoic Acid Receptor α), CBFβ/MYH11 (Core Binding Factor β/MYosin Heavy chain locus 11) or MLL/AF9 (Mixed-Lineage Leukemia/Acute lymphoblastic leukemia 1 Fused gene from chromosome 9) block myeloid cell differentiation by repressing target genes, thus providing one necessary event for leukemogenesis [146]. Disordered cell growth and upregulation of cell survival genes is a proposed necessary second event. Mutations in growth regulatory genes such as FLT3 (Fms-Like Tyrosine kinase 3), Ras, and c-Kit are common in AML patients, resulting in activation of multiple signal trasduction pathways which include PI3K/Akt/mTORC1 [147–149]. There is evidence of great interdependence between the two classes of molecular events. Indeed, changes in the transcriptional control in hematopoietic cells could modify the arrays of signal transduction effectors available for growth factor receptors, whereas activating mutations in signal transduction molecules induce alterations in the activity and expression of several transcription factors which are essential for normal myeloid differentiation [150].

LSCs share some properties with HSCs, as they are for the most part quiescent and capable of self-renewing. The quiescence of LSCs could explain, at least in part, the difficulties in eradicating this cell population by conventional polychemotherapy and the relapses which characterize AML [151].

There are some papers which have demonstrated that dysregulated PI3K/Akt signaling is leukemogenic in mice. For example, hematopoietic cells expressing mutated (active) p110α PI3K forms generated an acute leukemia-like disease characterized by anemia, neoplastic infiltration of hematopoietic organs, and 90% mortality within 5 weeks, when injected in a syngenic mouse model [152]. A rare, oncogenetic, activating mutation (E17K) in the PH domain of Akt1 that has been detected in some types of solid cancers (breast, colon, ovary), was leukemogenic in an in vivo murine model [153]. Intriguingly, this mutation has been identified also in pediatric patients with T-cell acute lymphoblastic leukemia (T-ALL) [154].

Some recent manuscripts have focused on the effects of PI3K/Akt/mTORC1 signaling activation in HSCs in relationship with the development of malignant hematological disorders, including leukemias. HSCs without functional PTEN, started to move out of the bone marrow, colonizing distant organs, and originating first a myeloproliferative disorder (MPD) and then an acute myeloid/lymphoid leukemic-like disease [84,85]. Rapamycin prevented the development of leukemia, implying an important role for mTORC1 in leukemogenesis.

A myristoylated allele of Akt1 (myr-Akt1) was introduced into murine HSCs via retroviral transduction. HSCs in the myr-Akt1 mice displayed transient expansion and increased cycling, which, however, were associated with impaired engraftment and subsequent depletion of the HSC pool [155]. Expression of myr-Akt1 was sufficient to induce by 6–8 weeks an MPD and a T-cell lymphoma with high frequency (90% and 65%, respectively), and an AML with a lower penetrance (10%, without any evidence of preexisting MPD). The importance of mTORC1 signaling in T-cell lymphoma (but not MPD or AML) pathogenesis was suggested by the significantly increased survival observed when myr-Akt1 mice were treated with rapamycin. Interestingly, in this mice model no ROS elevation was detected, suggesting that oxidative stress may not be the only important mechanism for HSC depletion.

Another mTORC1 regulator potentially involved in leukemogenesis is TSC1, as TSC1 knockout mice developed an MPD [88]. However, in another mouse model where TSC1 was deleted, no MPD was observed. Instead, the authors reported a reduction of myeloid development [87]. These conflicting results have been related to different deletor strains used in these two studies [76]. However, it may also be that there are TSC1/mTORC1 independent mechanisms mediating PI3K/PTEN/Akt signaling functions in HSCs that could be involved in leukemogenesis. In any case, it is very important to emphasize here that mTORC1 upregulation exerted a potent pro-survival effect in human LSCs transplanted in NOD/SCID mice [156]. These findings indicated that therapeutical targeting of mTORC1 has the potential for eradicating AML.

In contrast, no AML was observed in the conditional FOXO deletion model [97], despite strong similarities in the HSC phenotype with the myr-Akt1 mice or the conditional PTEN deletion model. Thus, these findings would imply that FOXO transcription factors contribute to maintenance of normal HSC homeostasis, but are not involved in

leukemogenesis. This suggests that alternative or additional downstream targets of Akt, such as mTORC1, are required for leukemic transformation. Nevertheless, recent findings have documented the importance of TGF-β/AKt/FOXO3a signaling for the maintenance of LSCs in a chronic myelogenous leukemia-like disease mouse model [157]. Therefore, the importance of FOXO transcription factors in the pathogenesis of leukemias should be further investigated.

How we could reconcile the finding that enhanced HSC proliferation followed by their exhaustion leads to leukemia in mouse models? Cancer development requires the combination of several genetic/epigenetic alterations, which aid cell transformation. The preceding HSC proliferation phase caused by an oncogenetic alteration (in our case PI3K/Akt/mTORC1 upregulation) may be the key feature that promotes leukemogenesis along with other cooperating hits which rescue HSC exhaustion. Indeed, there are several genetic alterations which lead to HSC exhaustion in mice models and cause leukemia. These include, among the others, Runx1, PU.1, PML, and Rb deletions (see [158] for a comprehensive and updated review on this topic). This model of various hits causing leukemia is supported by an investigation that has documented that PTEN deletion in mouse HSC led to an MPD, followed by T-ALL. In this model, LSCs showed increased levels of unphosphorylated β-catenin. Conditional ablation of one allele of the β-catenin gene substantially decreased the incidence and delayed the occurrence of T-ALL caused by PTEN loss, suggesting that activation of the β-catenin pathway may contribute to the formation or expansion of the LSC population [159]. Moreover, a recurring chromosomal translocation, T(14;15), resulted in aberrant overexpression of the c-myc oncogene in T-ALL LSCs, recapitulating a subset of human T-ALL. Therefore, this intriguing study clearly indicated that multiple genetic or molecular alterations could contribute cooperatively to HSC malignant transformation and T-ALL development.

There are, however, mouse models which display HSC exhaustion (for example, knockouts of CDC42, CXCR4 and SHIP1) but have not been associated with leukemia [158]. Thus, HSC proliferation/depletion could not invariably precede malignant transformation. This may depend on how long the aberrant HSCs would persist in the body, so that additional hits could rescue them before complete HSC exhaustion takes place. Therefore, the time window for HSC exhaustion might dictate the incidence of individual hematological neoplasias in humans [158].

7. Conclusions and future perspectives

Over the last decade, extensive studies carried out in many laboratories have considerably increased our understanding of the molecular mechanisms which are essential for normal myelopoiesis in the adult.

The findings reviewed in this article strongly suggest that a correct regulation of PI3K/Akt/mTORC1 signaling is required for the fine tuning of multiple processes involved in blood cell production, as well as in the control of HSC functions. Indeed, a very recent report has highlighted how both Akt1 and Akt2, by regulating the intracellular levels of ROS, are necessary for the maintenance of mouse LT-HSCs [160]. There also is increasing evidence that an aberrant regulation of the PI3K/Akt/mTORC1 network could contribute to leukemogenesis.

Pharmacological inhibitors of this network are being tested in many clinical trials for the treatment of cancer patients. Some inhibitors (rapalogues) have already been approved for clinical use in some types of neoplasia, including advanced renal cell carcinoma [161]. Chronic inhibition of PI3K/Akt/mTORC1 signaling may lead to issues of hematological insufficiency in the long term, as suggested, for example, by the thrombocytopenia that is sometimes (but not always) detected in patients treated with mTORC1 inhibitors [162].

However, many of the studies regarding PI3K/Akt/mTORC1 signaling and hematopoiesis were performed using in vitro models and may not necessarily reflect the situation in vivo. Although it seems that the PI3K/Akt/mTORC1 network is important for the generation of erythrocytes, granulocytes/monocytes, and platelets, several lines of evidence suggest that pathway inhibitors are much less toxic to normal myelopoiesis than they are to leukemic cells [163]. This observation could be explained by putting forward the so called “oncogenic addiction” hypothesis, where only neoplastic cells are highly reliant on signaling pathways which have been upregulated during disease progression enabling their survival [164]. Moreover, multiple signaling pathways are activated by cytokines. These pathways are often redundant and display extensive cross-talks. Thus, it might be that during normal myelopoiesis other signaling cascades could substitute for PI3K/Akt activation, such as the MAPK (MAP kinase) signaling pathways [165].

In this context, it is intriguing that bone marrow mononuclear cells from healthy donors did not display PI3K/Akt/mTORC1 pathway activation, whereas signaling upregulation was easily detected in the same fraction prepared from patients with neoplastic hematological disorders, including AML (see Fig. 3 and Refs. [166–168]). It could be that during normal myelopoiesis activation of this signaling pathway is a short-lived and more discrete phenomenon, whereas in leukemias the phenomenon is long-lasting and widespread.

Remarkably, it is now beginning to emerge that aberrantly activated PI3K/Akt/mTORC1 signaling is also detectable in cancer stem cells from different kinds of neoplasia [41,169,170] and that cancer stem cells displayed preferential sensitivity to pathway inhibition when compared to healthy stem cells [171,172]. This seems to be true also of HSCs and LCSs, at least in mice [85].

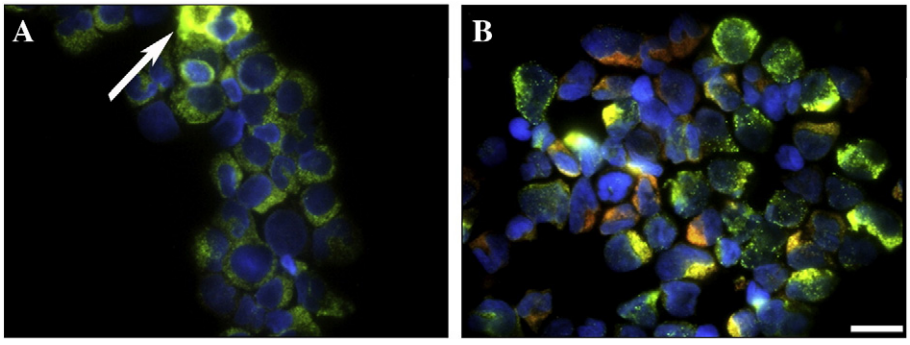

Fig. 3. Akt is activated in leukemic, but not in healthy, bone marrow mononuclear cells (BMMCs). BMMCs were isolated from a healthy donor (A) and from an AML patient (B). Cells were cytocentrifuged, fixed, and permeabilized. They were then stained with antibodies to the myeloid differentiation antigen, CD33, and to Ser473 p-Akt. CD33 was revealed by an FITC-conjugated antibody, whereas Ser473 p-Akt was labeled with a rhodamine-conjugated secondary antibody. Note how only two of healthy BMMCs displayed positivity for both CD33 and Ser473 p-Akt (arrow), while in leukemic BMMCs a strong co-localization (yellow or orange) of the two signals was evident in many of the cells. Original magnification, ×600. Scale bar: 15 μM.

These observations provide the proof-of-principle that functional differences in signaling pathways between neoplastic stem cells and healthy stem cells could be identified.

Therefore, further identification of specific PI3K/Akt/mTOR substrates and of their roles in the quiescence, proliferation, survival, and differentiation of HSCs and LSCs could provide innovative pharmacological treatments for patients with malignant hematological disorders.

Acknowledgements

This work has been supported by grants from: Fondazione del Monte and Progetti Strategici Università di Bologna EF2006 (to AMM), Italian MIUR PRIN 2008 (to AMM), and National Institutes of Health (USA) (R01098195, to JAM).

References

[1] T.L. Yuan, L.C. Cantley, PI3K pathway alterations in cancer: variations on a theme, Oncogene 27 (2008) 5497–5510.
[2] J.A. Engelman, Targeting PI3K signalling in cancer: opportunities, challenges and limitations, Nat. Rev. Cancer 9 (2009) 550–562.
[3] P. Liu, H. Cheng, T.M. Roberts, J.J. Zhao, Targeting the phosphoinositide 3-kinase pathway in cancer, Nat. Rev. Drug Discov. 8 (2009) 627–644.
[4] D.P. Brazil, Z.Z. Yang, B.A. Hemmings, Advances in protein kinase B signalling: AKTion on multiple fronts, Trends Biochem. Sci. 29 (2004) 233–242.
[5] T.F. Franke, PI3K/Akt: getting it right matters, Oncogene 27 (2008) 6473–6488.
[6] S. Jia, T.M. Roberts, J.J. Zhao, Should individual PI3 kinase isoforms be targeted in cancer? Curr. Opin. Cell Biol. 21 (2009) 199–208.
[7] D.A. Fruman, G. Bismuth, Fine tuning the immune response with PI3K, Immunol. Rev. 228 (2009) 253–272.
[8] M. Falasca, T. Maffucci, Rethinking phosphatidylinositol 3-monophosphate, Biochim. Biophys. Acta 1793 (2009) 1795–1803.
[9] K. Kok, B. Geering, B. Vanhaesebroeck, Regulation of phosphoinositide 3-kinase expression in health and disease, Trends Biochem. Sci. 34 (2009) 115–127.
[10] J.M. Backer, The regulation and function of Class III PI3Ks: novel roles for Vps34, Biochem. J. 410 (2008) 1–17.
[11] E. Morselli, L. Galluzzi, O. Kepp, J.M. Vicencio, A. Criollo, M.C. Maiuri, G. Kroemer, Anti- and pro-tumor functions of autophagy, Biochim. Biophys. Acta 1793 (2009) 1524–1532.
[12] Y.R. Chin, A. Toker, Function of Akt/PKB signaling to cell motility, invasion and the tumor stroma in cancer, Cell. Signal. 21 (2009) 470–476.
[13] L. Bozulic, B.A. Hemmings, PIKKing on PKB: regulation of PKB activity by phosphorylation, Curr. Opin. Cell Biol. 21 (2009) 256–261.
[14] A.M. Martelli, I. Faenza, A.M. Billi, L. Manzoli, C. Evangelisti, F. Fala, L. Cocco, Intranuclear 3′-phosphoinositide metabolism and Akt signaling: new mechanisms for tumorigenesis and protection against apoptosis? Cell. Signal. 18 (2006) 1101–1107.
[15] B.D. Manning, L.C. Cantley, AKT/PKB signaling: navigating downstream, Cell 129 (2007) 1261–1274.
[16] J. Downward, PI 3-kinase, Akt and cell survival, Sem. Cell Devel. Biol. 15 (2004) 177–182.
[17] L.R. Pearce, D. Komander, D.R. Alessi, The nuts and bolts of AGC protein kinases, Nat. Rev. Mol. Cell Biol. 11 (2010) 9–22.
[18] R.M. Memmott, P.A. Dennis, Akt-dependent and -independent mechanisms of mTOR regulation in cancer, Cell. Signal. 21 (2009) 656–664.
[19] E.A. Dunlop, A.R. Tee, Mammalian target of rapamycin complex 1: signalling inputs, substrates and feedback mechanisms, Cell. Signal. 21 (2009) 827–835.
[20] M. Rosner, M. Hengstschlager, Cytoplasmic and nuclear distribution of the protein complexes mTORC1 and mTORC2: rapamycin triggers dephosphorylation and delocalization of the mTORC2 components rictor and sin1, Hum. Mol. Genet. 17 (2008) 2934–2948.
[21] G. Panasyuk, I. Nemazanyy, A. Zhyvoloup, V. Filonenko, D. Davies, M. Robson, R.B. Pedley, M. Waterfield, I. Gout, mTORβ splicing isoform promotes cell proliferation and tumorigenesis, J. Biol. Chem. 284 (2009) 30807–30814.
[22] T.R. Peterson, M. Laplante, C.C. Thoreen, Y. Sancak, S.A. Kang, W.M. Kuehl, N.S. Gray, D.M. Sabatini, DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival, Cell 137 (2009) 873–886.
[23] J. Tamburini, A.S. Green, N. Chapuis, V. Bardet, C. Lacombe, P. Mayeux, D. Bouscary, Targeting translation in acute myeloid leukemia: a new paradigm for therapy? Cell Cycle 8 (2009) 3893–3899.
[24] Y. Mamane, E. Petroulakis, O. LeBacquer, N. Sonenberg, mTOR, translation initiation and cancer, Oncogene 25 (2006) 6416–6422.
[25] M. Laplante, D.M. Sabatini, mTOR signaling at a glance, J. Cell Sci. 122 (2009) 3589–3594.
[26] C.H. Jung, S.H. Ro, J. Cao, N.M. Otto, D.H. Kim, mTOR regulation of autophagy, FEBS Lett. 584 (2010) 1287–1295.
[27] E.L. Eskelinen, P. Saftig, Autophagy: a lysosomal degradation pathway with a central role in health and disease, Biochim. Biophys. Acta 1793 (2009) 664–673.
[28] X. Bai, Y. Jiang, Key factors in mTOR regulation, Cell. Mol. Life Sci. 67 (2010) 239–253.
[29] K.J. Mavrakis, H. Zhu, R.L. Silva, J.R. Mills, J. Teruya-Feldstein, S.W. Lowe, W. Tam, J. Pelletier, H.G. Wendel, Tumorigenic activity and therapeutic inhibition of Rheb GTPase, Genes Dev. 22 (2008) 2178–2188.
[30] L. Wang, T.E. Harris, J.C. Lawrence Jr., Regulation of proline-rich Akt substrate of 40 kDa (PRAS40) function by mammalian target of rapamycin complex 1 (mTORC1)-mediated phosphorylation, J. Biol. Chem. 283 (2008) 15619–15627.
[31] B.D. Fonseca, E.M. Smith, V.H. Lee, C. MacKintosh, C.G. Proud, PRAS40 is a target for mammalian target of rapamycin complex 1 and is required for signaling downstream of this complex, J. Biol. Chem. 282 (2007) 24514–24524.
[32] L. Ma, Z. Chen, H. Erdjument-Bromage, P. Tempst, P.P. Pandolfi, Phosphorylation and functional inactivation of TSC2 by Erk: implications for tuberous sclerosis and cancer pathogenesis, Cell 121 (2005) 179–193.
[33] T. Jin, I. George Fantus, J. Sun, Wnt and beyond Wnt: multiple mechanisms control the transcriptional property of β-catenin, Cell. Signal. 20 (2008) 1697–1704.
[34] K. Inoki, H. Ouyang, T. Zhu, C. Lindvall, Y. Wang, X. Zhang, Q. Yang, C. Bennett, Y. Harada, K. Stankunas, C.Y. Wang, X. He, O.A. MacDougald, M. You, B.O. Williams, K.L. Guan, TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth, Cell 126 (2006) 955–968.
[35] A. Vazquez-Martin, C. Oliveras-Ferraros, E. Lopez-Bonet, J.A. Menendez, AMPK: evidence for an energy-sensing cytokinetic tumor suppressor, Cell Cycle 8 (2009) 3679–3683.
[36] D.M. Gwinn, D.B. Shackelford, D.F. Egan, M.M. Mihaylova, A. Mery, D.S. Vasquez, B.E. Turk, R.J. Shaw, AMPK phosphorylation of raptor mediates a metabolic checkpoint, Mol. Cell 30 (2008) 214–226.
[37] M.P. DeYoung, P. Horak, A. Sofer, D. Sgroi, L.W. Ellisen, Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling, Genes Dev. 22 (2008) 239–251.
[38] J. Huang, B.D. Manning, A complex interplay between Akt, TSC2 and the two mTOR complexes, Biochem. Soc. Trans. 37 (2009) 217–222.
[39] D.D. Sarbassov, D.A. Guertin, S.M. Ali, D.M. Sabatini, Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex, Science 307 (2005) 1098–1101.
[40] E. Jacinto, R. Loewith, A. Schmidt, S. Lin, M.A. Ruegg, A. Hall, M.N. Hall, Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive, Nat. Cell Biol. 6 (2004) 1122–1128.
[41] D.A. Guertin, D.M. Stevens, M. Saitoh, S. Kinkel, K. Crosby, J.H. Sheen, D.J. Mullholland, M.A. Magnuson, H. Wu, D.M. Sabatini, mTOR complex 2 is required for the development of prostate cancer induced by PTEN loss in mice, Cancer Cell 15 (2009) 148–159.
[42] J.M. Garcia-Martinez, D.R. Alessi, mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1), Biochem. J. 416 (2008) 375–385.
[43] O.J. Shah, Z. Wang, T. Hunter, Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies, Curr. Biol. 14 (2004) 1650–1656.
[44] P.T. Bhaskar, N. Hay, The two TORCs and Akt, Dev. Cell 12 (2007) 487–502.
[45] S.A. Lang, C. Hackl, C. Moser, S. Fichtner-Feigl, G.E. Koehl, H.J. Schlitt, E.K. Geissler, O. Stoeltzing, Implication of RICTOR in the mTOR inhibitor-mediated induction of insulin-like growth factor-I receptor (IGF-IR) and human epidermal growth factor receptor-2 (Her2) expression in gastrointestinal cancer cells, Biochim. Biophys. Acta 1803 (2010) 435–442.
[46] X. Xu, A. Sarikas, D.C. Dias-Santagata, G. Dolios, P.J. Lafontant, S.C. Tsai, W. Zhu, H. Nakajima, H.O. Nakajima, L.J. Field, R. Wang, Z.Q. Pan, The CUL7 E3 ubiquitin ligase targets insulin receptor substrate 1 for ubiquitin-dependent degradation, Mol. Cell 30 (2008) 403–414.
[47] Y. Shi, H. Yan, P. Frost, J. Gera, A. Lichtenstein, Mammalian target of rapamycin inhibitors activate the AKT kinase in multiple myeloma cells by up-regulating the insulin-like growth factor receptor/insulin receptor substrate-1/phosphatidylinositol 3-kinase cascade, Mol. Cancer Ther. 4 (2005) 1533–1540.
[48] M. Breuleux, M. Klopfenstein, C. Stephan, C.A. Doughty, L. Barys, S.M. Maira, D. Kwiatkowski, H.A. Lane, Increased AKT S473 phosphorylation after mTORC1 inhibition is rictor dependent and does not predict tumor cell response to PI3K/mTOR inhibition, Mol. Cancer Ther. 8 (2009) 742–753.
[49] R. Sriburi, S. Jackowski, K. Mori, J.W. Brewer, XBP1: a link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum, J. Cell Biol. 167 (2004) 35–41.
[50] C.C. Dibble, J.M. Asara, B.D. Manning, Characterization of Rictor phosphorylation sites reveals direct regulation of mTOR complex 2 by S6K1, Mol. Cell. Biol. 29 (2009) 5657–5670.
[51] M. Keniry, R. Parsons, The role of PTEN signaling perturbations in cancer and in targeted therapy, Oncogene 27 (2008) 5477–5485.
[52] B.L. Stiles, Phosphatase and tensin homologue deleted on chromosome 10: extending its PTENtacles, Int. J. Biochem. Cell Biol. 41 (2009) 757–761.
[53] J. Kalesnikoff, L.M. Sly, M.R. Hughes, T. Buchse, M.J. Rauh, L.P. Cao, V. Lam, A. Mui, M. Huber, G. Krystal, The role of SHIP in cytokine-induced signaling, Rev. Physiol. Biochem. Pharmacol. 149 (2003) 87–103.
[54] P.J. Eichhorn, M.P. Creyghton, R. Bernards, Protein phosphatase 2A regulatory subunits and cancer, Biochim. Biophys. Acta 1795 (2009) 1–15.
[55] J. Brognard, A.C. Newton, PHLiPPing the switch on Akt and protein kinase C signaling, Trends Endocrinol. Met. 19 (2008) 223–230.
[56] L. Robb, Cytokine receptors and hematopoietic differentiation, Oncogene 26 (2007) 6715–6723.
[57] S. Luc, N. Buza-Vidas, S.E. Jacobsen, Delineating the cellular pathways of hematopoietic lineage commitment, Semin. Immunol. 20 (2008) 213–220.
[58] S.H. Orkin, L.I. Zon, Hematopoiesis: an evolving paradigm for stem cell biology, Cell 132 (2008) 631–644.

[59] I.L. Weissman, J.A. Shizuru, The origins of the identification and isolation of hematopoietic stem cells, and their capability to induce donor-specific transplantation tolerance and treat autoimmune diseases, Blood 112 (2008) 3543–3553.
[60] Y. Sharma, C.M. Astle, D.E. Harrison, Heterozygous kit mutants with little or no apparent anemia exhibit large defects in overall hematopoietic stem cell function, Exp. Hematol. 35 (2007) 214–220.
[61] W. Tong, Y.M. Ibarra, H.F. Lodish, Signals emanating from the membrane proximal region of the thrombopoietin receptor (mpl) support hematopoietic stem cell self-renewal, Exp. Hematol. 35 (2007) 1447–1455.
[62] T. Reya, S.J. Morrison, M.F. Clarke, I.L. Weissman, Stem cells, cancer, and cancer stem cells, Nature 414 (2001) 105–111.
[63] M. Kondo, I.L. Weissman, K. Akashi, Identification of clonogenic common lymphoid progenitors in mouse bone marrow, Cell 91 (1997) 661–672.
[64] K. Akashi, D. Traver, T. Miyamoto, I.L. Weissman, A clonogenic common myeloid progenitor that gives rise to all myeloid lineages, Nature 404 (2000) 193–197.
[65] K. Kaushansky, Lineage-specific hematopoietic growth factors, New Engl. J. Med. 354 (2006) 2034–2045.
[66] E. Montecino-Rodriguez, H. Leathers, K. Dorshkind, Bipotential B-macrophage progenitors are present in adult bone marrow, Nat. Immunol. 2 (2001) 83–88.
[67] Y. Shiozawa, A.M. Havens, K.J. Pienta, R.S. Taichman, The bone marrow niche: habitat to hematopoietic and mesenchymal stem cells, and unwitting host to molecular parasites, Leukemia 22 (2008) 941–950.
[68] R. Schofield, The relationship between the spleen colony-forming cell and the haemopoietic stem cell, Blood Cells 4 (1978) 7–25.
[69] M.H. Raaijmakers, D.T. Scadden, Evolving concepts on the microenvironmental niche for hematopoietic stem cells, Curr. Opin. Hematol. 15 (2008) 301–306.
[70] X. Huang, S. Cho, G.J. Spangrude, Hematopoietic stem cells: generation and self-renewal, Cell Death Differ. 14 (2007) 1851–1859.
[71] P.J. Quesenberry, J.M. Aliotta, The paradoxical dynamism of marrow stem cells: considerations of stem cells, niches, and microvesicles, Stem Cell Rev. 4 (2008) 137–147.
[72] F. Ayala, R. Dewar, M. Kieran, R. Kalluri, Contribution of bone microenvironment to leukemogenesis and leukemia progression, Leukemia 23 (2009) 2233–2241.
[73] M.J. Kiel, S.J. Morrison, Uncertainty in the niches that maintain haematopoietic stem cells, Nat. Rev. Immunol. 8 (2008) 290–301.
[74] S.J. Baker, S.G. Rane, E.P. Reddy, Hematopoietic cytokine receptor signaling, Oncogene 26 (2007) 6724–6737.
[75] A. Wilson, E. Laurenti, G. Oser, R.C. van der Wath, W. Blanco-Bose, M. Jaworski, S. Offner, C.F. Dunant, L. Eshkind, E. Bockamp, P. Lio, H.R. Macdonald, A. Trumpp, Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair, Cell 135 (2008) 1118–1129.
[76] B. Gan, R.A. DePinho, mTORC1 signaling governs hematopoietic stem cell quiescence, Cell Cycle 8 (2009) 1003–1006.
[77] K.W. Orford, D.T. Scadden, Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation, Nat. Rev. Genet. 9 (2008) 115–128.
[78] H. Yoshihara, F. Arai, K. Hosokawa, T. Hagiwara, K. Takubo, Y. Nakamura, Y. Gomei, H. Iwasaki, S. Matsuoka, K. Miyamoto, H. Miyazaki, T. Takahashi, T. Suda, Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche, Cell Stem Cell 1 (2007) 685–697.
[79] E. Sitnicka, N. Lin, G.V. Priestley, N. Fox, V.C. Broudy, N.S. Wolf, K. Kaushansky, The effect of thrombopoietin on the proliferation and differentiation of murine hematopoietic stem cells, Blood 87 (1996) 4998–5005.
[80] M. Kobayashi, J.H. Laver, T. Kato, H. Miyazaki, M. Ogawa, Thrombopoietin supports proliferation of human primitive hematopoietic cells in synergy with steel factor and/or interleukin-3, Blood 88 (1996) 429–436.
[81] J. Seita, H. Ema, J. Ooehara, S. Yamazaki, Y. Tadokoro, A. Yamasaki, K. Eto, S. Takaki, K. Takatsu, H. Nakauchi, Lnk negatively regulates self-renewal of hematopoietic stem cells by modifying thrombopoietin-mediated signal transduction, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 2349–2354.
[82] S. Yamazaki, A. Iwama, S. Takayanagi, Y. Morita, K. Eto, H. Ema, H. Nakauchi, Cytokine signals modulated via lipid rafts mimic niche signals and induce hibernation in hematopoietic stem cells, EMBO J. 25 (2006) 3515–3523.
[83] M.A. Essers, S. Offner, W.E. Blanco-Bose, Z. Waibler, U. Kalinke, M.A. Duchosal, A. Trumpp, IFNα activates dormant haematopoietic stem cells in vivo, Nature 458 (2009) 904–908.
[84] J. Zhang, J.C. Grindley, T. Yin, S. Jayasinghe, X.C. He, J.T. Ross, J.S. Haug, D. Rupp, K.S. Porter-Westpfahl, L.M. Wiedemann, H. Wu, L. Li, PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention, Nature 441 (2006) 518–522.
[85] O.H. Yilmaz, R. Valdez, B.K. Theisen, W. Guo, D.O. Ferguson, H. Wu, S.J. Morrison, PTEN dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells, Nature 441 (2006) 475–482.
[86] J. Huang, Y. Zhang, A. Bersenev, W.T. O'Brien, W. Tong, S.G. Emerson, P.S. Klein, Pivotal role for glycogen synthase kinase-3 in hematopoietic stem cell homeostasis in mice, J. Clin. Invest. 119 (2009) 3519–3529.
[87] C. Chen, Y. Liu, R. Liu, T. Ikenoue, K.L. Guan, Y. Liu, P. Zheng, TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species, J. Exp. Med. 205 (2008) 2397–2408.
[88] B. Gan, E. Sahin, S. Jiang, A. Sanchez-Aguilera, K.L. Scott, L. Chin, D.A. Williams, D.J. Kwiatkowski, R.A. DePinho, mTORC1-dependent and -independent regulation of stem cell renewal, differentiation, and mobilization, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 19384–19389.
[89] K. Ito, A. Hirao, F. Arai, K. Takubo, S. Matsuoka, K. Miyamoto, M. Ohmura, K. Naka, K. Hosokawa, Y. Ikeda, T. Suda, Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells, Nat. Med. 12 (2006) 446–451.
[90] Y.Y. Jang, S.J. Sharkis, A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche, Blood 110 (2007) 3056–3063.
[91] T.B. Campbell, S. Basu, G. Hangoc, W. Tao, H.E. Broxmeyer, Overexpression of Rheb2 enhances mouse hematopoietic progenitor cell growth while impairing stem cell repopulation, Blood 114 (2009) 3392–3401.
[92] K. Ito, R. Bernardi, A. Morotti, S. Matsuoka, G. Saglio, Y. Ikeda, J. Rosenblatt, D.E. Avigan, J. Teruya-Feldstein, P.P. Pandolfi, PML targeting eradicates quiescent leukaemia-initiating cells, Nature 453 (2008) 1072–1078.
[93] R. Bernardi, I. Guernah, D. Jin, S. Grisendi, A. Alimonti, J. Teruya-Feldstein, C. Cordon-Cardo, M.C. Simon, S. Rafii, P.P. Pandolfi, PML inhibits HIF-1α translation and neoangiogenesis through repression of mTOR, Nature 442 (2006) 779–785.
[94] C. Chen, Y. Liu, Y. Liu, P. Zheng, mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells, Sci. Signal. 2 (2009) ra75.
[95] M.N. Stanfel, L.S. Shamieh, M. Kaeberlein, B.K. Kennedy, The TOR pathway comes of age, Biochim. Biophys. Acta 1790 (2009) 1067–1074.
[96] E.L. Greer, A. Brunet, FOXO transcription factors at the interface between longevity and tumor suppression, Oncogene 24 (2005) 7410–7425.
[97] Z. Tothova, R. Kollipara, B.J. Huntly, B.H. Lee, D.H. Castrillon, D.E. Cullen, E.P. McDowell, S. Lazo-Kallanian, I.R. Williams, C. Sears, S.A. Armstrong, E. Passegue, R.A. DePinho, D.G. Gilliland, FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress, Cell 128 (2007) 325–339.
[98] K. Miyamoto, K.Y. Araki, K. Naka, F. Arai, K. Takubo, S. Yamazaki, S. Matsuoka, T. Miyamoto, K. Ito, M. Ohmura, C. Chen, K. Hosokawa, H. Nakauchi, K. Nakayama, K.I. Nakayama, M. Harada, N. Motoyama, T. Suda, A. Hirao, FoxO3a is essential for maintenance of the hematopoietic stem cell pool, Cell Stem Cell 1 (2007) 101–112.
[99] Z. Tothova, D.G. Gilliland, FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system, Cell Stem Cell 1 (2007) 140–152.
[100] M. Engstrom, R. Karlsson, J.I. Jonsson, Inactivation of the forkhead transcription factor FoxO3 is essential for PKB-mediated survival of hematopoietic progenitor cells by kit ligand, Exp. Hematol. 31 (2003) 316–323.
[101] A. Chabanon, C. Desterke, E. Rodenburger, D. Clay, B. Guerton, L. Boutin, A. Bennaceur-Griscelli, O. Pierre-Louis, G. Uzan, L. Abecassis, M.F. Bourgeade, J.J. Lataillade, M.C. Le Bousse-Kerdiles, A cross-talk between stromal cell-derived factor-1 and transforming growth factor-β controls the quiescence/cycling switch of CD34+ progenitors through FoxO3 and mammalian target of rapamycin, Stem Cells 26 (2008) 3150–3161.
[102] C.D. Helgason, J. Antonchuk, C. Bodner, R.K. Humphries, Homeostasis and regeneration of the hematopoietic stem cell pool are altered in SHIP-deficient mice, Blood 102 (2003) 3541–3547.
[103] A.L. Hazen, M.J. Smith, C. Desponts, O. Winter, K. Moser, W.G. Kerr, SHIP is required for a functional hematopoietic stem cell niche, Blood 113 (2009) 2924–2933.
[104] M. Buitenhuis, L.P. Verhagen, H.W. van Deutekom, A. Castor, S. Verploegen, L. Koenderman, S.E. Jacobsen, P.J. Coffer, Protein kinase B (c-akt) regulates hematopoietic lineage choice decisions during myelopoiesis, Blood 111 (2008) 112–121.
[105] A.D. Friedman, Transcriptional control of granulocyte and monocyte development, Oncogene 26 (2007) 6816–6828.
[106] S.E. Ross, H.S. Radomska, B. Wu, P. Zhang, J.N. Winnay, L. Bajnok, W.S. Wright, F. Schaufele, D.G. Tenen, O.A. MacDougald, Phosphorylation of C/EBPα inhibits granulopoiesis, Mol. Cell. Biol. 24 (2004) 675–686.
[107] G. Nteliopoulos, S.B. Marley, M.Y. Gordon, Influence of PI-3K/Akt pathway on Wnt signalling in regulating myeloid progenitor cell proliferation. Evidence for a role of autocrine/paracrine Wnt regulation, Br. J. Haematol. 146 (2009) 637–651.
[108] Y. Haseyama, K. Sawada, A. Oda, K. Koizumi, H. Takano, T. Tarumi, M. Nishio, M. Handa, Y. Ikeda, T. Koike, Phosphatidylinositol 3-kinase is involved in the protection of primary cultured human erythroid precursor cells from apoptosis, Blood 94 (1999) 1568–1577.
[109] M.O. Arcasoy, X. Jiang, Co-operative signalling mechanisms required for erythroid precursor expansion in response to erythropoietin and stem cell factor, Br. J. Haematol. 130 (2005) 121–129.
[110] L. Hong, B. Ramdas, J. Chen, C. Harris, D.M. Wojchowski, R. Kapur, KIT associated intracellular tyrosines play an essential role in EpoR co-signaling, Cell. Signal. 20 (2008) 1513–1520.
[111] S. Elliott, E. Pham, I.C. Macdougall, Erythropoietins: a common mechanism of action, Exp. Hematol. 36 (2008) 1573–1584.
[112] W.J. Bakker, T.B. van Dijk, M. Parren-van Amelsvoort, A. Kolbus, K. Yamamoto, P. Steinlein, R.G. Verhaak, T.W. Mak, H. Beug, B. Lowenberg, M. von Lindern, Differential regulation of Foxo3a target genes in erythropoiesis, Mol. Cell. Biol. 27 (2007) 3839–3854.
[113] M. von Lindern, E.M. Deiner, H. Dolznig, M. Parren-Van Amelsvoort, M.J. Hayman, E.W. Mullner, H. Beug, Leukemic transformation of normal murine erythroid progenitors: v- and c-ErbB act through signaling pathways activated by the EpoR and c-Kit in stress erythropoiesis, Oncogene 20 (2001) 3651–3664.
[114] H. Dolznig, F. Boulme, K. Stangl, E.M. Deiner, W. Mikulits, H. Beug, E.W. Mullner, Establishment of normal, terminally differentiating mouse erythroid progenitors: molecular characterization by cDNA arrays, FASEB J. 15 (2001) 1442–1444.
[115] J.H. Myklebust, H.K. Blomhoff, L.S. Rusten, T. Stokke, E.B. Smeland, Activation of phosphatidylinositol 3-kinase is important for erythropoietin-induced erythropoiesis from CD34+ hematopoietic progenitor cells, Exp. Hematol. 30 (2002) 990–1000.
[116] S. Ghaffari, C. Kitidis, W. Zhao, D. Marinkovic, M.D. Fleming, B. Luo, J. Marszalek, H.F. Lodish, AKT induces erythroid-cell maturation of JAK2-deficient fetal liver progenitor cells and is required for EPO regulation of erythroid-cell differentiation, Blood 107 (2006) 1888–1891.

[117] D. Bouscary, F. Pene, Y.E. Claessens, O. Muller, S. Chretien, M. Fontenay-Roupie, S. Gisselbrecht, P. Mayeux, C. Lacombe, Critical role for PI 3-kinase in the control of erythropoietin-induced erythroid progenitor proliferation, Blood 101 (2003) 3436–3443.
[118] J. Zhang, H.F. Lodish, Identification of K-ras as the major regulator for cytokine-dependent Akt activation in erythroid progenitors in vivo, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 14605–14610.
[119] Y. Kashii, M. Uchida, K. Kirito, M. Tanaka, K. Nishijima, M. Toshima, T. Ando, K. Koizumi, T. Endoh, K. Sawada, M. Momoi, Y. Miura, K. Ozawa, N. Komatsu, A member of Forkhead family transcription factor, FKHRL1, is one of the downstream molecules of phosphatidylinositol 3-kinase-Akt activation pathway in erythropoietin signal transduction, Blood 96 (2000) 941–949.
[120] S. Uddin, S. Kottegoda, D. Stigger, L.C. Platanias, A. Wickrema, Activation of the Akt/FKHRL1 pathway mediates the antiapoptotic effects of erythropoietin in primary human erythroid progenitors, Biochem. Biophys. Res. Commun. 275 (2000) 16–19.
[121] Z. Kadri, L. Maouche-Chretien, H.M. Rooke, S.H. Orkin, P.H. Romeo, P. Mayeux, P. Leboulch, S. Chretien, Phosphatidylinositol 3-kinase/Akt induced by erythropoietin renders the erythroid differentiation factor GATA-1 competent for TIMP-1 gene transactivation, Mol. Cell. Biol. 25 (2005) 7412–7422.
[122] J.A. Lowry, J.P. Mackay, GATA-1: one protein, many partners, Int. J. Biochem. Cell Biol. 38 (2006) 6–11.
[123] W. Zhao, C. Kitidis, M.D. Fleming, H.F. Lodish, S. Ghaffari, Erythropoietin stimulates phosphorylation and activation of GATA-1 via the PI3-kinase/AKT signaling pathway, Blood 107 (2006) 907–915.
[124] E.A. Sivertsen, M.E. Hystad, K.B. Gutzkow, G. Dosen, E.B. Smeland, H.K. Blomhoff, J.H. Myklebust, PI3K/Akt-dependent EPO-induced signalling and target genes in human early erythroid progenitor cells, Br. J. Haematol. 135 (2006) 117–128.
[125] G. Grech, M. Blazquez-Domingo, A. Kolbus, W.J. Bakker, E.W. Mullner, H. Beug, M. von Lindern, Igbp1 is part of a positive feedback loop in stem cell factor-dependent, selective mRNA translation initiation inhibiting erythroid differentiation, Blood 112 (2008) 2750–2760.
[126] G.M. Fuhler, M.R. Tyl, S.G. Olthof, A. Lyndsay Drayer, N. Blom, E. Vellenga, Distinct roles of the mTOR components Rictor and Raptor in MO7e megakaryocytic cells, Eur. J. Haematol. 83 (2009) 235–245.
[127] M. Majka, A. Janowska-Wieczorek, J. Ratajczak, M.A. Kowalska, G. Vilaire, Z.K. Pan, M. Honczarenko, L.A. Marquez, M. Poncz, M.Z. Ratajczak, Stromal-derived factor 1 and thrombopoietin regulate distinct aspects of human megakaryopoiesis, Blood 96 (2000) 4142–4151.
[128] S. Jeanpierre, F.E. Nicolini, B. Kaniewski, C. Dumontet, R. Rimokh, A. Puisieux, V. Maguer-Satta, BMP4 regulation of human megakaryocytic differentiation is involved in thrombopoietin signaling, Blood 112 (2008) 3154–3163.
[129] K. Kaushansky, The molecular mechanisms that control thrombopoiesis, J. Clin. Invest. 115 (2005) 3339–3347.
[130] Y. Miyakawa, P. Rojnuckarin, T. Habib, K. Kaushansky, Thrombopoietin induces phosphoinositol 3-kinase activation through SHP2, Gab, and insulin receptor substrate proteins in BAF3 cells and primary murine megakaryocytes, J. Biol. Chem. 276 (2001) 2494–2502.
[131] P. Rojnuckarin, Y. Miyakawa, N.E. Fox, J. Deou, G. Daum, K. Kaushansky, The roles of phosphatidylinositol 3-kinase and protein kinase Cζ for thrombopoietin-induced mitogen-activated protein kinase activation in primary murine megakaryocytes, J. Biol. Chem. 276 (2001) 41014–41022.
[132] D. Bouscary, C. Lecoq-Lafon, S. Chretien, S. Zompi, S. Fichelson, O. Muller, F. Porteu, I. Dusanter-Fourt, S. Gisselbrecht, P. Mayeux, C. Lacombe, Role of Gab proteins in phosphatidylinositol 3-kinase activation by thrombopoietin (TPO), Oncogene 20 (2001) 2197–2204.
[133] A.E. Geddis, N.E. Fox, K. Kaushansky, Phosphatidylinositol 3-kinase is necessary but not sufficient for thrombopoietin-induced proliferation in engineered Mpl-bearing cell lines as well as in primary megakaryocytic progenitors, J. Biol. Chem. 276 (2001) 34473–34479.
[134] M. Tanaka, K. Kirito, Y. Kashii, M. Uchida, T. Watanabe, H. Endo, T. Endoh, K. Sawada, K. Ozawa, N. Komatsu, Forkhead family transcription factor FKHRL1 is expressed in human megakaryocytes. Regulation of cell cycling as a downstream molecule of thrombopoietin signaling, J. Biol. Chem. 276 (2001) 15082–15089.
[135] T. Nakao, A.E. Geddis, N.E. Fox, K. Kaushansky, PI3K/Akt/FOXO3a pathway contributes to thrombopoietin-induced proliferation of primary megakaryocytes in vitro and in vivo via modulation of $p27^{Kip1}$, Cell Cycle 7 (2008) 257–266.
[136] M. Soda, K. Willert, K. Kaushansky, A.E. Geddis, Inhibition of GSK-3β promotes survival and proliferation of megakaryocytic cells through a β-catenin-independent pathway, Cell. Signal. 20 (2008) 2317–2323.
[137] Y. Kozuma, H. Kojima, S. Yuki, H. Suzuki, T. Nagasawa, Continuous expression of Bcl-xL protein during megakaryopoiesis is post-translationally regulated by thrombopoietin-mediated Akt activation, which prevents the cleavage of Bcl-xL, J. Thromb. Haemost. 5 (2007) 1274–1282.
[138] A.L. Drayer, S.G. Olthof, E. Vellenga, Mammalian target of rapamycin is required for thrombopoietin-induced proliferation of megakaryocyte progenitors, Stem Cells 24 (2006) 105–114.
[139] H. Raslova, V. Baccini, L. Loussaief, B. Comba, J. Larghero, N. Debili, W. Vainchenker, Mammalian target of rapamycin (mTOR) regulates both proliferation of megakaryocyte progenitors and late stages of megakaryocyte differentiation, Blood 107 (2006) 2303–2310.
[140] R. Guerriero, I. Parolini, U. Testa, P. Samoggia, E. Petrucci, M. Sargiacomo, C. Chelucci, M. Gabbianelli, C. Peschle, Inhibition of TPO-induced MEK or mTOR activity induces opposite effects on the ploidy of human differentiating megakaryocytes, J. Cell Sci. 119 (2006) 744–752.
[141] D. Zhu, H. Hattori, H. Jo, Y. Jia, K.K. Subramanian, F. Loison, J. You, Y. Le, M. Honczarenko, L. Silberstein, H.R. Luo, Deactivation of phosphatidylinositol 3, 4, 5-trisphosphate/Akt signaling mediates neutrophil spontaneous death, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 14836–14841.
[142] H. Liu, Y. Qiu, L. Xiao, F. Dong, Involvement of protein kinase Cε in the negative regulation of Akt activation stimulated by granulocyte colony-stimulating factor, J. Immunol. 176 (2006) 2407–2413.
[143] C.R. Geest, F.J. Zwartkruis, E. Vellenga, P.J. Coffer, M. Buitenhuis, Mammalian target of rapamycin activity is required for expansion of $CD34^+$ hematopoietic progenitor cells, Haematologica 94 (2009) 901–910.
[144] S.W. Lane, D.T. Scadden, D.G. Gilliland, The leukemic stem cell niche: current concepts and therapeutic opportunities, Blood 114 (2009) 1150–1157.
[145] J.K. Warner, J.C. Wang, K. Takenaka, S. Doulatov, J.L. McKenzie, L. Harrington, J.E. Dick, Direct evidence for cooperating genetic events in the leukemic transformation of normal human hematopoietic cells, Leukemia 19 (2005) 1794–1805.
[146] J.A. Kennedy, F. Barabe, Investigating human leukemogenesis: from cell lines to in vivo models of human leukemia, Leukemia 22 (2008) 2029–2040.
[147] C. Scholl, D.G. Gilliland, S. Frohling, Deregulation of signaling pathways in acute myeloid leukemia, Semin. Oncol. 35 (2008) 336–345.
[148] J.A. McCubrey, L.S. Steelman, S.L. Abrams, F.E. Bertrand, D.E. Ludwig, J. Basecke, M. Libra, F. Stivala, M. Milella, A. Tafuri, P. Lunghi, A. Bonati, A.M. Martelli, Targeting survival cascades induced by activation of Ras/Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways for effective leukemia therapy, Leukemia 22 (2008) 708–722.
[149] L.S. Steelman, S.L. Abrams, J. Whelan, F.E. Bertrand, D.E. Ludwig, J. Basecke, M. Libra, F. Stivala, M. Milella, A. Tafuri, P. Lunghi, A. Bonati, A.M. Martelli, J.A. McCubrey, Contributions of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways to leukemia, Leukemia 22 (2008) 686–707.
[150] M.A. Moore, Converging pathways in leukemogenesis and stem cell self-renewal, Exp. Hematol. 33 (2005) 719–737.
[151] N. Misaghian, G. Ligresti, L.S. Steelman, F.E. Bertrand, J. Basecke, M. Libra, F. Nicoletti, F. Stivala, M. Milella, A. Tafuri, M. Cervello, A.M. Martelli, J.A. McCubrey, Targeting the leukemic stem cell: the Holy Grail of leukemia therapy, Leukemia 23 (2009) 25–42.
[152] S. Horn, U. Bergholz, M. Jucker, J.A. McCubrey, L. Trumper, C. Stocking, J. Basecke, Mutations in the catalytic subunit of class IA PI3K confer leukemogenic potential to hematopoietic cells, Oncogene 27 (2008) 4096–4106.
[153] J.D. Carpten, A.L. Faber, C. Horn, G.P. Donoho, S.L. Briggs, C.M. Robbins, G. Hostetter, S. Boguslawski, T.Y. Moses, S. Savage, M. Uhlik, A. Lin, J. Du, Y.W. Qian, D.J. Zeckner, G. Tucker-Kellogg, J. Touchman, K. Patel, S. Mousses, M. Bittner, R. Schevitz, M.H. Lai, K.L. Blanchard, J.E. Thomas, A transforming mutation in the pleckstrin homology domain of AKT1 in cancer, Nature 448 (2007) 439–444.
[154] A. Gutierrez, T. Sanda, R. Grebliunaite, A. Carracedo, L. Salmena, Y. Ahn, S. Dahlberg, D. Neuberg, L.A. Moreau, S.S. Winter, R. Larson, J. Zhang, A. Protopopov, L. Chin, P.P. Pandolfi, L.B. Silverman, S.P. Hunger, S.E. Sallan, A.T. Look, High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia, Blood 114 (2009) 647–650.
[155] M.G. Kharas, R. Okabe, J.J. Ganis, M. Gozo, T. Khandan, M. Paktinat, D.G. Gilliland, K. Gritsman, Constitutively active AKT depletes hematopoietic stem cells and induces leukemia in mice, Blood 115 (2010) 1409–1415.
[156] Q. Xu, J.E. Thompson, M. Carroll, mTOR regulates cell survival after etoposide treatment in primary AML cells, Blood 106 (2005) 4261–4268.
[157] K. Naka, T. Hoshii, T. Muraguchi, Y. Tadokoro, T. Ooshio, Y. Kondo, S. Nakao, N. Motoyama, A. Hirao, TGF-β-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia, Nature 463 (2010) 676–680.
[158] B. Jacob, M. Osato, Stem cell exhaustion and leukemogenesis, J. Cell. Biochem. 107 (2009) 393–399.
[159] W. Guo, J.L. Lasky, C.J. Chang, S. Mosessian, X. Lewis, Y. Xiao, J.E. Yeh, J.Y. Chen, M.L. Iruela-Arispe, M. Varella-Garcia, H. Wu, Multi-genetic events collaboratively contribute to PTEN-null leukaemia stem-cell formation, Nature 453 (2008) 529–533.
[160] M.M. Juntilla, V.D. Patil, M. Calamito, R.P. Joshi, M.J. Birnbaum, G.A. Koretzky, AKT1 and AKT2 maintain hematopoietic stem cell function by regulating reactive oxygen species, Blood (2010) prepublished online March 30, 2010; DOI 10.1182/blood-2009-09-241000.
[161] T.A. Yap, M.D. Garrett, M.I. Walton, F. Raynaud, J.S. de Bono, P. Workman, Targeting the PI3K-AKT-mTOR pathway: progress, pitfalls, and promises, Curr. Opin. Pharmacol. 8 (2008) 393–412.
[162] M. Campone, V. Levy, E. Bourbouloux, D. Berton Rigaud, D. Bootle, C. Dutreix, U. Zoellner, N. Shand, F. Calvo, E. Raymond, Safety and pharmacokinetics of paclitaxel and the oral mTOR inhibitor everolimus in advanced solid tumours, Br. J. Cancer 100 (2009) 315–321.
[163] A.M. Martelli, C. Evangelisti, F. Chiarini, C. Grimaldi, L. Manzoli, J.A. McCubrey, Targeting the PI3K/AKT/mTOR signaling network in acute myelogenous leukemia, Exp. Opin. Invest. Drugs 18 (2009) 1333–1349.
[164] I.B. Weinstein, A. Joe, Oncogene addiction, Cancer Res. 68 (2008) 3077–3080.
[165] D.E. Johnson, Src family kinases and the MEK/ERK pathway in the regulation of myeloid differentiation and myeloid leukemogenesis, Adv. Enzyme Regul. 48 (2008) 98–112.
[166] M. Nyakern, P.L. Tazzari, C. Finelli, C. Bosi, M.Y. Follo, T. Grafone, P.P. Piccaluga, G. Martinelli, L. Cocco, A.M. Martelli, Frequent elevation of Akt kinase phosphorylation in blood marrow and peripheral blood mononuclear cells from high-risk myelodysplastic syndrome patients, Leukemia 20 (2006) 230–238.
[167] M.Y. Follo, S. Mongiorgi, C. Bosi, A. Cappellini, C. Finelli, F. Chiarini, V. Papa, M. Libra, G. Martinelli, L. Cocco, A.M. Martelli, The Akt/mammalian target of

rapamycin signal transduction pathway is activated in high-risk myelodysplastic syndromes and influences cell survival and proliferation, Cancer Res. 67 (2007) 4287–4294.
[168] J. Hsu, Y. Shi, S. Krajewski, S. Renner, M. Fisher, J.C. Reed, T.F. Franke, A. Lichtenstein, The AKT kinase is activated in multiple myeloma tumor cells, Blood 98 (2001) 2853–2855.
[169] C.E. Eyler, W.C. Foo, K.M. LaFiura, R.E. McLendon, A.B. Hjelmeland, J.N. Rich, Brain cancer stem cells display preferential sensitivity to Akt inhibition, Stem Cells 26 (2008) 3027–3036.
[170] A. Dubrovska, S. Kim, R.J. Salamone, J.R. Walker, S.M. Maira, C. Garcia-Echeverria, P. G. Schultz, V.A. Reddy, The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 268–273.
[171] G.L. Gallia, B.M. Tyler, C.L. Hann, I.M. Siu, V.L. Giranda, A.L. Vescovi, H. Brem, G.J. Riggins, Inhibition of Akt inhibits growth of glioblastoma and glioblastoma stem-like cells, Mol. Cancer Ther. 8 (2009) 386–393.
[172] A.M. Bleau, D. Hambardzumyan, T. Ozawa, E.I. Fomchenko, J.T. Huse, C.W. Brennan, E.C. Holland, PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells, Cell Stem Cell 4 (2009) 226–235.