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Review

The phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin signaling network and the control of normal myelopoiesis

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Summary. The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway plays a central role in cell growth, proliferation, differentiation, and survival under physiological conditions. Aberrant regulation of the PI3K/Akt/mTOR signal transduction network has been observed in a wide range of neoplasias, including malignant hematological disorders. This observation suggests that this signaling cascade could also play a critical role during normal hematopoiesis, a highly regulated process which results in the formation of all blood lineages. The development of blood cells comprises a complex series of events which are mainly regulated through the actions of cytokines, a large family of extracellular ligands than can stimulate many biological responses in a wide array of cell types. Several of these cytokines are known to activate the PI3K/Akt/mTOR signal transduction network and thus regulate proliferation, survival, and differentiation events during hematopoiesis. Moreover, hematopoiesis is strictly dependent on the correct functions of the bone marrow microenvironment. Here, we review the evidence which links the signals emanating from the PI3K/Akt/mTOR cascade with the functions of hematopoietic stem cells and the process of lineage commitment, which then gives rise to myeloid lineage-restricted cells. We then further highlight the key role played by the PI3K/Akt/mTOR network during erythropoiesis, megakaryocytopoiesis, and granulo-cytopoiesis/monocytopoiesis.

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Introduction

The phosphatidylinositol 3-kinase (PI3K, a family of lipid kinases)/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, and metabolism (Yuan and Cantley, 2008). Moreover, this pathway has been implicated in cancer since its discovery 20 years ago, and in recent years it has become apparent that it is one of the most frequently targeted pathways in sporadic human tumors (Yuan and Cantley, 2008). Pathway activation confers leukemogenic potential to mouse hematopoietic cells (Guo et al., 2008; Horn et al., 2008). Since therapeutic targeting of PI3K/Akt/mTOR axis is being considered as an option for molecularly targeted treatment of several types of cancers, including hematopoietic malignancies, it becomes of critical importance to establish the role, if any, of this network in normal myelopoiesis, as myelosuppression is quite often a dose-limiting effect of cytotoxic drugs. The aim of this review is to give the reader an updated overview of the relevance of PI3K/Akt/mTOR signaling during normal myelopoiesis in the adult. However, we shall begin with a general overview outlining the mechanisms which govern the PI3K/Akt/mTOR signal transduction

network.

The PI3K/Akt/mTOR pathway

PI3K

The family of PI3K enzymes is characterized by the ability to phosphorylate the 3'-OH group of inositol lipids and comprises three different classes, I, II, and III. Class I PI3K phosphorylates both phosphatidylinositol (PtdIns) 4 phosphate and PtdIns 4,5 bisphosphate [PtdIns (4,5)P_2] which are phosphorylated to yield PtdIns (3,4)P_2 and PtdIns 3,4,5 trisphosphate [PtdIns (3,4,5)P_3]. The in vivo preferred substrate of class I PI3K is PtdIns (4,5)P_2 (Brazil et al., 2004). 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). It is worth mentioning here that some PH domains require PtdIns (3,4,5)P_3 while others also bind PtdIns (3,4)P_2 (DiNitto and Lambright, 2006). Class I PI3K is divided further into A [activated by receptor tyrosine kinases (RTKs), Ras, and G-protein coupled receptors (GPCRs)] and B (activated by GPCRs) subtype (Fig. 1). Class IA PI3Ks are heterodimeric enzymes composed of a regulatory (p85α, p85ß, p55α, p55γ, p50α) and of catalytic (p110α, p110ß, p110δ) subunit. Class IB PI3K comprises a p101 regulatory and a p110γ catalytic subunit (Franke, 2008). p110α and p110ß PI3Ks are ubiquitously expressed and play a fundamental role in development and cell growth, so that their homozygous knockout is embryonic-lethal (Jia et al., 2009). On the other hand, p110α and 110δ PI3Ks are highly enriched in leukocytes and mostly related to the immune system functions, so that their knock-down leads to defective immune responses (Fruman and Bismuth, 2009).

Class II PI3Ks, which comprise the PI3K-C2α, -C2ß, and -C2γ isoforms, preferentially phosphorylate PtdIns to produce PtdIns (3)P. Although class II PI3Ks are widely expressed in mammalian tissues and organs, their importance in cell signaling and biology, relative to that of class I PI3Ks, is not clear at the moment (Kok et al., 2009).

Vacuolar protein sorting 34 (vps34), is the unique class III PI3K and exists as a heterodimer bound to the vps15 regulatory subunit (formerly called p150 in mammals). Vps34 has been implicated in endocytosis, autophagy, and nutrient signaling (Backer, 2008).

Akt

Akt is a 57-kDa serine/threonine protein kinase which is the cellular homolog of the v-akt oncogene. The Akt family includes three highly conserved isoforms: Akt1/α, Akt2/ß, and Akt3/γ, which share a high degree of sequence homology (Brazil et al., 2004). Functional differences exist between Akt isoforms, as Akt2 is critically involved in insulin-mediated glucose uptake (Bae et al., 2003) and in cell motility/invasion/metastatic potential of cancer cells (Arboleda et al., 2003).

Akt contains an NH_2-terminal PH domain, which interacts with PtdIns (3,4,5)P_3. Akt recruitment at the plasma membrane results in a conformational change, which enables its activation loop to be phosphorylated on Thr308 by PDK1 and on Ser473 in the COOH-terminus by a kinase which has been identified as the mTOR complex 2 (mTORC2), although other kinases could also be involved (Bozulic and Hemmings, 2009) (Fig. 1). Full Akt activation requires both the phosphorylation steps. Active Akt migrates to both the cytosol and the nucleus. Nuclear Akt may fullfil important anti-apoptotic roles (Martelli et al., 2006). Nevertheless, the relative contribution of Akt signaling at the plasma membrane, the cytosol, and the nucleus remains to be determined.

At present, over 100 Akt substrates have been identified (Manning and Cantley, 2007). Around 40 substrates which mediate the pleiotropic Akt functions have been characterized, including caspase-9, Bad, murine double minute 2, proline-rich Akt substrate 40 (PRAS40), FOXO family of Forkhead transcription factors, Raf, $p27^{Kip1}$, $p21^{Cip1/WAF1}$, glycogen synthase kinase 3ß (GSK3ß), endothelial nitric oxide synthase. Each of these substrates has a key role in the regulation of cell proliferation, survival, and differentiation, either directly or through an intermediary (Downward, 2004; Franke, 2008).

mTOR

mTOR is a 289-kDa enzyme which belongs to the PI3K-related kinase family and bears a COOH-terminal catalytic domain with significant sequence homology to PI3K. In spite of this homology, mTOR functions exclusively as a protein kinase (Memmott and Dennis, 2009). mTOR exists as two complexes, referred to as mTOR complex 1 (mTORC1) and mTORC2. mTORC1 is composed of mTOR/Raptor/mLST8/PRAS40/FKBP38/Deptor and is sensitive to rapamycin and its derivatives. mTORC2 comprises mTOR/Rictor/mLST8/SIN1/Protor/Deptor and is generally considered as being insensitive to rapamycin, even if long-term treatment of several cell lines with rapamycin indirectly inhibits mTORC2, by inducing its dissociation (Dunlop and Tee, 2009). mTORC1 regulates translation in response to nutrients/growth factors by phosphorylating components of the protein synthesis machinery, including p70S6 kinase (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 results in the release of the eukaryotic initiation factor 4E (eIF4E), allowing eIF4E to participate in the assembly of a translational initiation complex which recruits ribosomes to mRNA (Ma and Blenis, 2009). Therefore, mTORC1 regulates a variety of steps involved in protein synthesis, but in particular it controls

the expression of molecules such as c-Myc, cyclin D1, cyclin-dependent kinase 2, retinoblastoma protein, hypoxia-inducible factor-1α, ornithine decarboxylase, $p27^{Kip1}$, and vascular endothelial growth factor, which are key determinants of cell proliferation, survival, and angiogenesis (Dunlop and Tee, 2009; Mamane et al., 2006). mTORC1 also inhibits autophagy, a non-apoptotic form of cell death (Crazzolara et al., 2009).

Akt-mediated regulation of mTORC1 activity is a multi-step phenomenon. Akt inhibits TSC2 (Tuberous Sclerosis 2 or hamartin) function through direct phosphorylation. TSC2 is a GTPase-activating protein (GAP) that functions in association with TSC1 (Tuberous Sclerosis 1 or tuberin) to inactivate the small G protein Rheb (Ras homolog enriched in brain). TSC2 phosphorylation by Akt represses the GAP activity of the TSC1/TSC2 complex, allowing Rheb to accumulate in a GTP-bound state (Fig. 1). The mechanism by which Rheb-GTP activates mTORC1 has not been fully elucidated yet, although Rheb requires to be farnesylated for activating mTORC1 (Mavrakis et al., 2008). Akt also phosphorylates PRAS40, an inhibitor of mTORC1, and by doing so, it prevents PRAS40 ability to suppress mTORC1 signaling (Wang et al., 2008). Thus, this could be yet another way by which Akt activates mTORC1. 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 downstream signaling of mTORC1 (Fonseca et al., 2007).

Also, Ras/Raf/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) 1/2 signaling positively impinge on mTORC1. Indeed, both $p90^{RSK}$ and ERK 1/2 phosphorylate TSC2, thus suppressing its inhibitory function (Ma et al., 2005) (Fig. 1). mTORC1 signal transduction is inhibited by the master metabolic regulator, energy-sensing AMP-dependent protein kinase (AMPK), because AMPK phosphorylates and activates TSC2, thus inhibiting Rheb function (Hong et al., 2003).

mTORC2 activation is only partially understood however, it requires PI3K and the TSC1/TSC2 complex, but is independent of Rheb (Huang and Manning, 2009) and is largely insensitive to either nutrients or energy conditions. mTORC2 phosphorylates Akt on Ser473 which enhances subsequent Akt phosphorylation on Thr308 by PDK1 (Sarbassov et al., 2005). mTORC2 plays a role in cytoskeleton organization by controlling

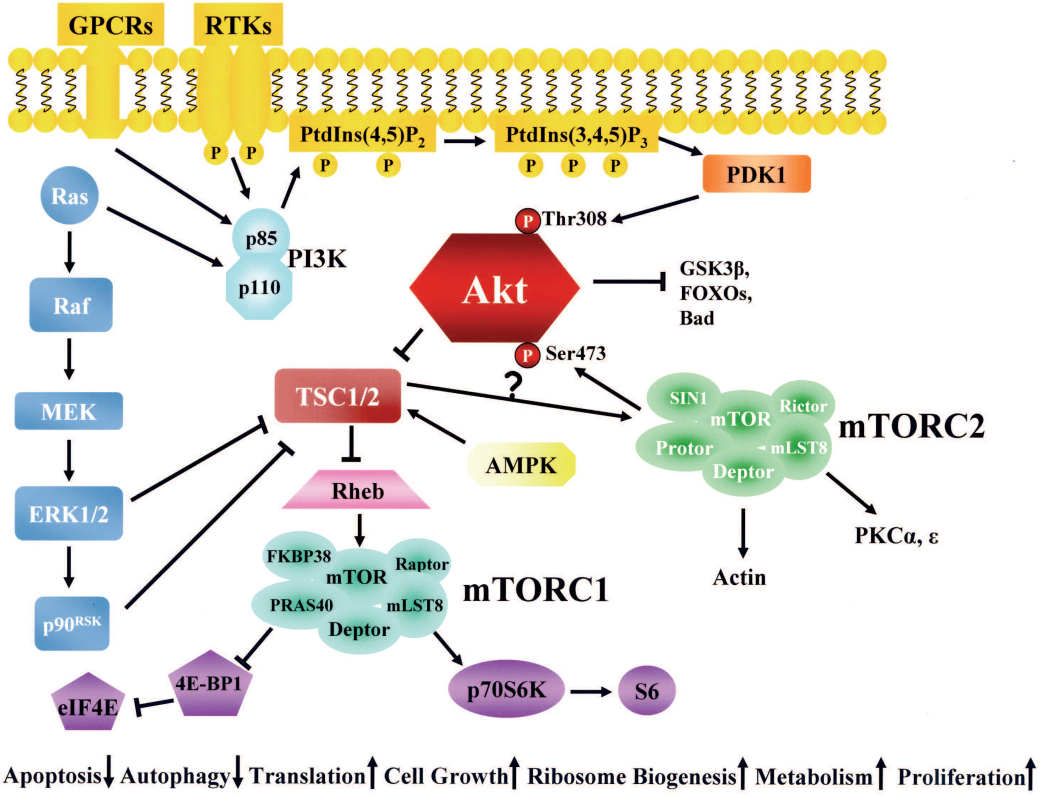

Fig. 1. The PI3K/Akt/mTOR pathway. GPCRs, receptor tyrosine kinases RTKs, and Ras stimulate class I PI3K activity. PI3K generates PtdIns $(3,4,5)P_3$ from PtdIns $(4,5,)P_2$. PtdIns $(3,4,5)P_3$ attracts to the plasma membrane PDK1 which phosphorylates Akt on Thr308. Full Akt activation requires Ser473 phosphorylation, which is effected by mTORC2. Once activated, Akt phosphorylates and inactivates several substrates, including GSK3ß, FOXO transcription factors, 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 up-regulates, through a mechanism not yet fully elucidated, the protein kinase activity of mTORC1. mTORC1 targets p70S6K and 4E-BP1 which are critical for translation. Ribosomal S6 protein is a target of p70S6K. TSC1/2 complex (but not Rheb) is required to activate mTORC2. mTORC2 regulates actin polymerization and phosphorylates PKCα and ε. However, other signaling cascades impinge on mTORC1, including the Ras/Raf/MEK/ERK1/2/ $p90^{RSK}$ pathway and the AMPK network. Arrows indicate activating events, whereas perpendicular lines highlight inhibitory events.

actin polymerization (Jacinto et al., 2004) and phosphorylates protein kinase C (PKC) α and ε (Huang and Manning, 2009).

Akt and mTORC1/2 are linked to each other via positive and negative regulatory circuits, which restrain their simultaneous hyperactivation through a mechanism involving p70S6K and PI3K. Assuming that an equilibrium exists between mTORC1 and mTORC2, when mTORC1 is formed, it could antagonize the formation of mTORC2 and reduce Akt activity. Indeed, once Akt activates mTORC1, the latter elicits a negative feedback loop to inhibit Akt activity (Dunlop and Tee, 2009). This negative regulation of Akt activity by mTORC1 is attributed to the effect of p70S6K on insulin receptor substrate (IRS) 1, downstream of insulin receptor and/or insulin-like growth factor-1 receptor (Shah et al., 2004; Bhaskar and Hay, 2007). Indeed, p70S6K phosphorylates and inactivates IRS1. Moreover, mTORC1 is capable of downregulating IRS2 expression by enhancing its proteosomal degradation (Sriburi et al., 2004). Consistently, mTORC1 inhibition by the rapamycin analogue, RAD001, increased IRS2 expression and Akt phosphorylation levels (Tamburini et al., 2008).

Deptor is a recently identified component of both mTORC1 and mTORC2, which acts as negative regulator of their protein kinase activity (Peterson et al., 2009). In agreement with its inhibitory role on mTORC1, Deptor overexpression characterizes a subset of multiple myeloma which displays high levels of phosphorylated Akt (Peterson et al., 2009).

Negative regulation of PI3K/Akt/mTOR signaling

Phosphorylated inositol lipids are not hydrolyzed by any known phospholipase. Instead, they are regulated by phosphatases to control PI3K/Akt/mTOR-dependent signaling. 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 (Keniry and Parsons, 2008; Stiles, 2009). PTEN inactivating mutations or silencing occur in a wide variety of human cancers and this results in Akt/mTOR up-regulation. Therefore, PTEN is a tumor suppressor acting up-stream of Akt. Two other phosphatases, SHIP-1 and SHIP-2 (for Src homology domain-containing inositol phosphatase), are capable of removing the 5-phosphate from PtdIns (3,4,5)P_3 to yield PtdIns (3,4,)P_2 (Kalesnikoff et al., 2003). While SHIP-1 is predominantly expressed in hematopoietic cells, SHIP-2 is more ubiquitous. An important role for SHIP-1 in normal hematopoiesis has been recently described (Ong et al., 2007; Hazen et al., 2009). Protein phosphatase 2A (PP2A), which is rapidly emerging as a new oncosuppressor, down-regulates Akt activity, through dephosphorylation of Thr308 (Eichhorn et al., 2009). Finally, Thr308 and Ser473 residues of Akt are targeted by the two isoforms (1 and 2) of PH domain leucine-rich repeat protein phosphatase (PHLPP), another candidate tumor suppressor (Brognard and Newton, 2008).

Normal hematopoiesis

Since most blood cells display a very short half-life, every day billions of mature blood cells are replenished in humans, during steady-state hematopoiesis. Moreover, the hematopoietic system also quickly responds to physiological stresses, such as bleeding and infection (Robb, 2007). The integrity of hematopoiesis is strictly dependent on the existence and persistence in the adult bone marrow of the rare uncommitted hematopoietic stem cells (HSCs) which display extensive self-renewal capacity, as well as the ability to commit and develop towards all blood cell lineages (Luc et al., 2008). HSCs are defined operationally by their capacity to reconstitute the entire blood system of a recipient (Orkin and Zon, 2008; Weissman and Shizuru, 2008). At least two types of HSC have been identified, long-term HSCs (LT-HSCs) and short-term HSCs (ST-HSCs). 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 rise to common lymphoid (CLP) and myeloid (CMP) progenitors which are capable of exponential proliferation, as well as continuing the differentiation process (Reya et al., 2001; Orkin and Zon, 2008). 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 (Akashi et al., 2000; Kondo et al., 1997), which then give rise to myeloid lineage committed cells (Kaushansky, 2006) (Fig. 2). Thus, myeloid cells include 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 (Montecino-Rodriguez et al., 2001).

It is widely assumed that the dividing HSCs in bone marrow undergo asymmetric cell division, in which an individual HSC gives rise to 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 (Shiozawa et al., 2008). The site or physical microenvironment that regulates self-renewal, proliferation, and differentiation of HSCs is referred to as the "HSC niche" (Schofield, 1978; Raaijmakers and Scadden, 2008). 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 bone marrow (Huang et al., 2007).

The niches are composed of different cells, which include osteoblasts, osteoclasts, endothelial cells, fibroblasts, and adipocytes (Kiel and Morrison, 2008). These cells, which are part of the bone marrow microenvironment, produce the factors that are necessary for supporting HSC functions. Basal and emergency hematopoiesis are mostly regulated by cytokines, a large family of extracellular ligands than can stimulate a wide array of biological responses in many cell types (Baker et al., 2007). 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 that promote the proliferation of hematopoietic cells in culture, and support the in vitro maintenance of HSCs (Huang et al., 2007). 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 (Robb, 2007).

PI3K/Akt/mTOR signaling and normal myelopoiesis

HSC maintenance

Most (70%) adult HSCs are in a quiescent state, i.e. they are in the G0 phase of the cell cycle, while only a small (5%) subset is dividing (Wilson et al., 2008). It has been hypothesized that quiescence is one of the major mechanisms to keep HSC function (Orford and Scadden, 2008). The soluble factors which could be involved in HSC quiescence are unknown. Recent findings suggest that HSCs are maintained in a quiescent state through interactions with thrombopoietin (TPO)-producing osteoblasts (Yoshihara et al., 2007). Nevertheless, other groups have provided evidence that TPO works in synergy with IL-3 and SCF to promote proliferation in vitro of both murine and human HSCs (Kobayashi et al., 1996; Sitnicka et al., 1996). 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 in HSCs (Seita et al., 2007).

Studies from independent laboratories have documented that if HSCs undergo active proliferation, they lose their long-term functions. This could also be due to environmental changes (Siminovitch et al., 1964; Harrison et al., 1978). It is now emerging that

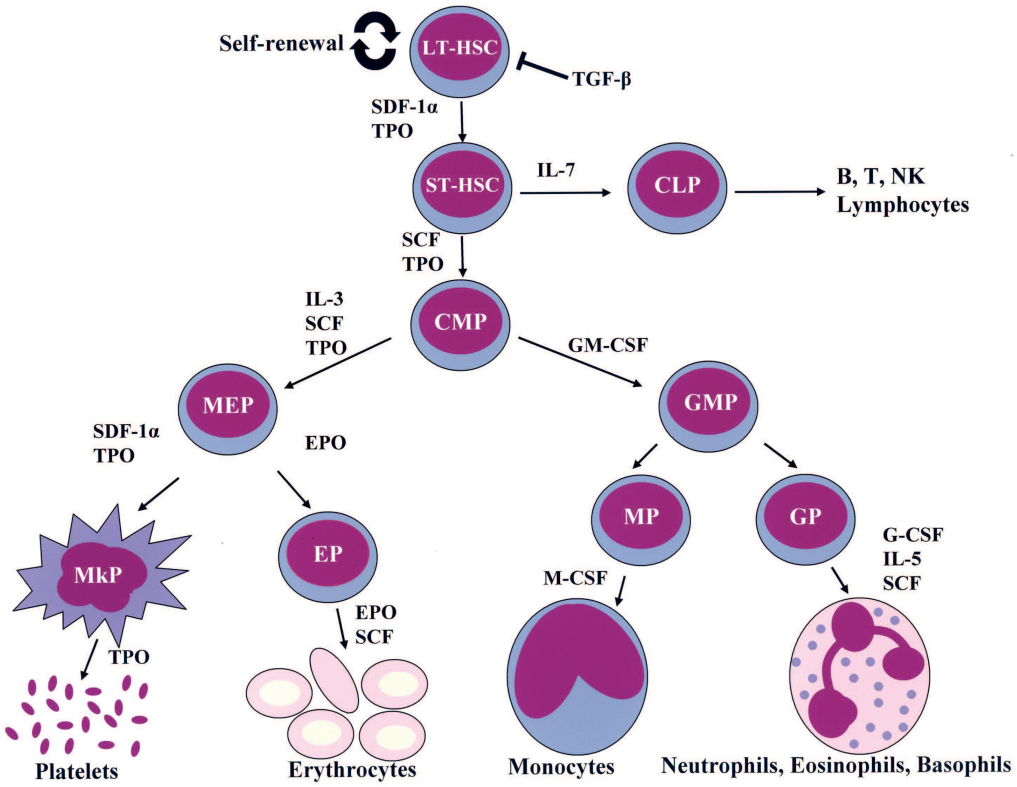

Fig. 2. Schematic of current model for adult hematopoiesis indicating intermediates in the hierarchy of hematopoietic differentiation. Hematopoiesis progresses from LT-HSCs (which are capable of self-renewal) to ST-HSCs that differentiate into multilineage committed CMP and CLP. These cells then give 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 known cytokines regulating the proliferation/differentiation processes are indicated in the figure. Arrows indicate activating events, whereas perpendicular lines highlight inhibitory events.

PI3K/Akt/mTORC1 signaling play important roles in HSC maintenance. Conditional deletion of PTEN in adult murine HSCs resulted in an initial expansion of HSCs followed by a depletion of LT-HSCs. This transient increase in HSC number was due to increased cell cycle progression, which, however, ultimately resulted in exhaustion of the HSC population (Zhang et al., 2006). Rapamycin reverted the phenotype of PTEN-deficient HSCs, suggesting that mTORC1 signaling was responsible for this phenotype (Yilmaz et al., 2006). Accordingly, TSC1 deficient mice also displayed initial increased proliferation, leading to short-term expansion but long-term depletion of HSCs (Chen et al., 2008; Gan et al., 2008). TSC1 deletion resulted in higher mTORC1 activity which promoted HSC proliferation. These proliferating HSCs displayed dramatically elevated reactive oxygen species (ROS) levels which led to their depletion (Chen et al., 2008). Indeed, ROS dramatically limit the lifespan of HSCs (Ito et al., 2006) and the bone marrow HSC niche is thought to be a low-oxygenic environment which impairs ROS production (Jang and Sharkis, 2007).

Members of the FOXO family of transcription factors, FOXO1, FOXO3, and FOXO4 are substrates of Akt, which inactivates their transcriptional activity by increasing FOXO 14-3-3 protein dependent export into the cytoplasm (Greer and Brunet, 2005). Recently, 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 (Tothova et al., 2007). Notably, there was a marked context-dependent increase in ROS levels in FOXO-deficient HSC compared with wild-type HSC, which correlated with changes in expression of genes which regulate ROS production, including GADD45, catalase, and superoxide dismutase Sod1 and Sod3. Furthermore, aged FOXO3a knockout animals also showed a reduction of the HSC pool and a deficient repopulating capacity in serial transplantation assays, accompanied by elevated p38 mitogen activated protein kinase activity and ROS levels (Miyamoto et al., 2007). Increased exit from quiescence and enhanced apoptosis, two of the features observed in FOXO-deficient mutants, could act in concert to decrease the pool size of HSCs available for self-renewal (Tothova and Gilliland, 2007). Interestingly, these findings are in agreement with an earlier observation which highlighted the importance of PI3K/Akt/FOXO3 signaling for the survival of Lin- mouse hematopoietic progenitor cells in response to SCF (Engstrom et al., 2003). These observations beg the question of which factors could be involved in the regulation of HSC quiescence and proliferation. A recent manuscript by Chabanon et al. (2008) suggests that stromal cell-derived factor-1α (SDF-1α, also referred to as CXCL12) and transforming growth factor-ß (TGF-ß) play an important role in the regulation of HSC cell cycle status. SDF-1α acts as a survival and proliferating factor for human $CD34^+$ cells by upregulating proteins which accelerate progression through the cell cycle, while TGF-ß blocks progression through the G1 phase of the cell cycle. Intriguingly, SDF-1α treatment of human $CD34^+$ cells from the peripheral blood resulted in activation of PI3K/Akt/mTOR signaling, while TGF-ß opposed pathway activation. In this human model, FOXO3a was identified as an important mediator of the opposing effects of the two cytokines on HSCs, as SDF-1α increased FOXO3a phosphorylation, whereas TGF-ß decreased it. Indeed, in $CD34^+$ cells with forced expression of a non-phosphorylatable form of FOXO3a, SDF-1α did not promote progression through the cell cycle (Chabanon et al., 2008). Another clue to the involvement of PI3K/Akt/mTOR signaling in HSC maintenance comes from the observation that SHIP1 deletion, which leads to PI3K/Akt/mTOR up-regulation, initially resulted in higher proliferation of LT-HSCs but reduced their long term repopulation ability, thus indicating again that higher levels of proliferation exhausted the LT-HSC population (Helgason et al., 2003).

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 (Luc et al., 2008). Transplantation of sublethally irradiated ß2 microglobuiln $^{-/-}$ NOD/SCID mice with $CD34^+$ cells ectopically expressing constitutively active Akt through retroviral transduction resulted in enhanced neutrophil and monocyte development, whereas dominant negative Akt induced eosinophil development in vivo (Buitenhuis et al., 2008). Intriguingly, it was found that both pharmacological inhibition of PI3K/Akt signaling and ectopic expression of a dominant negative Akt resulted in increased phosphorylation of CCAATT/enhancer binding protein α (C/EPBα) in neutrophil progenitors, whereas GSK3ß inhibition lowered C/EPBα phosphorylation levels. Moreover, expression of constitutively active Akt resulted in a dramatic decrease in the expression levels of Jun B, a transcriptional target of C/EPBα (Buitenhuis et al., 2008). It was then concluded that Akt can control C/EPBα phosphorylation through GSK3ß, thus influencing lineage choice decisions during myelopoiesis. It should be reminded here that C/EPBα is one of the key transcription factors that regulate lineage choices during myelopoiesis (Friedman, 2007) and its phosphorylation on Ser21 can inhibit granulocytic differentiation (Ross et al., 2004).

Erythropoiesis

Erythrocyte production is tightly regulated by the cytokines SCF and EPO, which support the survival,

proliferation, and differentiation of erythroid progenitors (Haseyama et al., 1999; Arcasoy and Jiang, 2005; Elliott et al., 2008; Hong et al., 2008). EPO and SCF activate common signaling pathways which include Ras/Raf/MEK/ERK, JAK/STAT-5, and PI3K/Akt/mTOR (Bakker et al., 2007). Compared with EPO, SCF is a much more potent activator of Akt (von Lindern et al., 2001). Nevertheless, SCF is unable to induce cell survival in the absence of EPO (Dolznig et al., 2001). Instead, SCF signaling delays differentiation, which is enhanced by the PI3K inhibitor LY294002 (von Lindern et al., 2001). 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 (Myklebust et al., 2002; Ghaffari et al., 2006). However, EPO could also 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 (Bouscary et al., 2003). Gab could then impinge upon Ras signaling, which is known to be very important for EPO effects on erythropoiesis (Zhang and Lodish, 2005). FOXO3a seems to play a critical role in these EPO- and SCF-evoked phenomena (Kashii et al., 2000), as its inactivation by Akt could result in downregulation of proapoptotic genes (Uddin et al., 2000) and of the cyclin-dependent kinase inhibitor, $p27^{Kip1}$ (Bouscary et al., 2003). Furthermore, Akt directly phosphorylated and activated the transcription factor GATA-1, which plays a fundamental role in erythroid differentiation (Kadri et al., 2005; Lowry and Mackay, 2006; Zhao et al., 2006). The involvement of mTORC1 in EPO signaling was supported by increased levels of phosphorylated p70S6K after EPO challenging (Bouscary et al., 2003). However, the consequences of mTORC1 activity on erythropoiesis are unclear, but could be related to enhanced cell cycle progression. 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, and which play an important role during their proliferation/differentiation. These included: cyclin D3, E and A, as well as c-Kit and CDH1 (E-cadherin) (Sivertsen et al., 2006). In another more recent study, gene expression profiling downstream of mTORC1 was investigated at the polysomal level, using immortalized erythroblasts challenged with EPO/SCF. Nine genes were identified that required EPO/SCF stimulation for polysome recruitment and were down-regulated during erythroid differentiation. One of the target genes, Immunoglobulin binding protein 1 (Igbp1), is a regulatory subunit of PP2A (the α4 subunit of PP2A) which sustains PI3K/Akt/mTOR signaling. Constitutive expression of Igbp1 impaired erythroid differentiation, maintained 4E-BP1 and p70S6K phosphorylation, and enhanced polysome recruitment of multiple eIF4E-sensitive mRNAs. Thus, PI3K-dependent polysome recruitment of Igbp1 acts as a positive feedback mechanism on translation initiation underscoring the important regulatory role of selective mRNA recruitment to polysomes in the balance between proliferation and maturation of erythroblasts (Grech et al., 2008).

Megakaryocytopoiesis

Megakaryocytes differentiation is characterized by endomitosis without nuclear and cellular division, thereby increasing their 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 (Fuhler et al., 2009). In this process, a complex network of hematopoietic growth factors are involved, among which TPO is the most thoroughly investigated regulator of megakaryocytes growth and differentiation. However, in addition to TPO, other cytokines have non-negligible effects on megakaryocytopoiesis, including SDF-1α (Majka et al., 2000) and BMP4, a member of the TGF-ß family (Jeanpierre et al., 2008).

TPO binds its cognate receptor, the cellular protooncogene c-Mpl, i.e. the homolog of the murine myeloproliferative leukemia virus oncogene, v-Mpl (Kaushansky, 2005). Once c-Mpl is activated by TPO engagement, it stimulates a series of signaling cascades which include PI3K/AKT/mTOR (Bouscary et al., 2001; Miyakawa et al., 2001; Rojnuckarin et al., 2001). Blocking this pathway resulted in inhibition of TPO-dependent megakaryocytes survival. Nevertheless this signaling cascade is necessary but not sufficient for TPO-induced cell cycle progression in primary megakaryocyte progenitors (Geddis et al., 2001), hinting that other pathways play an important role in cell cycle regulation.

Identified Akt substrates in megakaryocytes include FOXO3a (Tanaka et al., 2001), $p27^{Kip1}$ (Nakao et al., 2008), and GSK3ß (Soda et al., 2008). One of the fundamental biological activities of TPO is to prevent the apoptosis of megakaryocytes. TPO-mediated Akt activation was important to blunt caspase-3-mediated cleavage of anti-apoptotic $Bcl\text{-}X_L$ protein. This could constitute a mechanism by which PI3K/Akt signaling counteracts apoptosis during megakaryocytopoiesis (Kozuma et al., 2007).mTOR and its downstream substrates, p70S6K and 4E-BP1, have been demonstrated to be critically involved in TPO-induced proliferation of megakaryocyte progenitors (Drayer et al., 2006), as well as in the late stages of megakaryocyte differentiation (Raslova et al., 2006). A recent paper has unraveled the functions of mTORC1 and mTORC2 in MO7e megakaryoblastic cells. Using lentiviral constructs encoding short hairpin RNA sequences to either Rictor or Raptor, it was possible to document that mTORC1 regulated cell growth and size by inhibiting autophagy, while mTORC2 was involved in cell cycle progression and nuclear ploidity (Fuhler et al., 2009). However, it remains to be established whether these findings would apply also to primary megakaryocyte

progenitors. Indeed, a previous report had highlighted that in $CD34^+$ cells, purified from human peripheral blood and treated with TPO to differentiate into megakaryocytic progeny, rapamycin (which should mainly target mTORC1) strongly inhibited cell polyploidization (Guerriero et al., 2006).

Granulocytopoiesis/monocytopoiesis

Granulocyte/macrophage-colony stimulating factor (GM-CSF) (Zhu et al., 2006), granulocyte-colony stimulating factor (G-CSF), and macrophage-colony stimulating factor (M-CSF) play important roles during granulocytopoiesis/monocytopoiesis, and there is evidence for the involvement of PI3K/Akt signaling in these phenomena (Liu et al., 2006; Zhu et al., 2006). Interestingly, however, a very recent article has highlighted that mTOR signaling appears not to be essential for differentiation of myeloid progenitors (Geest et al., 2009).

Conclusions and future perspectives

It is clear that a correct regulation of PI3K/Akt/mTOR signaling is required for the fine tuning of multiple processes involved in blood cell production. This finding has a deep clinical relevance, as inhibitors of this pathway are being tested in clinical trails for the treatment of cancer patients or have already been approved for clinical use in some types of neoplasias, including adavanced renal cell carcinoma (Yap et al., 2008). Therefore, chronic inhibition of PI3K/Akt signaling may lead to issues of hematological insufficiency in the long term, as suggested, for example, by the thrombocytopenia which is detected in patients treated with mTOR inhibitors (Campone et al., 2009). Another potential disadvantage of mTOR inhibitors is that in some studies they have caused an increase in active Akt, via feedback loops involving p70S6K and IRS1 (see above). Nevertheless, the extent to which disruption of a negative feedback mechanism actually limits the therapeutic effects of mTOR inhibitors in cancer patients remains to be determined. Remarkably, it is now beginning to emerge that aberrantly activated PI3K/Akt/mTOR signaling is also detectable in cancer stem cells from different kinds of neoplasm (Eyler et al., 2008; Dubrovska et al., 2009; Guertin et al., 2009) and that cancer stem cells displayed preferential sensitivity to pathway inhibition when compared to healthy cells (Bleau et al., 2009; Gallia et al., 2009). This seems to be true also of HSCs and leukemia stem cells, at least in mice (Yilmaz et al., 2006). This observation provides the proof-of-principle that functional differences in signaling pathways between cancer stem cells and healthy stem cells can be identified and therapeutically exploited so that it may be possible to devise new pharmacological strategies that will prove more effective against cancer and less toxic to normal stem cells.

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References

Akashi K., Traver D., Miyamoto T. and Weissman I.L. (2000). A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature 404, 193-197.

Arboleda M.J., Lyons J.F., Kabbinavar F.F., Bray M.R., Snow B.E., Ayala R., Danino M., Karlan B.Y. and Slamon D.J. (2003). Overexpression of AKT2/protein kinase B, leads to up-regulation of beta1 integrins, increased invasion, and metastasis of human breast and ovarian cancer cells. Cancer Res. 63, 196-206.

Arcasoy M.O. and Jiang X. (2005). Co-operative signalling mechanisms required for erythroid precursor expansion in response to erythropoietin and stem cell factor. Br. J. Haematol. 130, 121-129.

Backer J.M. (2008). The regulation and function of Class III PI3Ks: novel roles for Vps34. Biochem. J. 410, 1-17.

Bae S.S., Cho H., Mu J. and Birnbaum M.J. (2003). Isoform-specific regulation of insulin-dependent glucose uptake by Akt/protein kinase B. J. Biol. Chem. 278, 49530-49536.

Baker S.J., Rane S.G. and Reddy E.P. (2007). Hematopoietic cytokine receptor signaling. Oncogene 26, 6724-6737.

Bakker W.J., van Dijk T.B., Parren-van Amelsvoort M., Kolbus A., Yamamoto K., Steinlein P., Verhaak R.G., Mak T.W., Beug H., Lowenberg B. and von Lindern M. (2007). Differential regulation of Foxo3a target genes in erythropoiesis. Mol. Cell. Biol. 27, 3839-3854.

Bhaskar P.T. and Hay N. (2007). The two TORCs and Akt. Dev. Cell 12, 487-502.

Bleau A.M., Hambardzumyan D., Ozawa T., Fomchenko E.I., Huse J.T., Brennan C.W. and Holland E.C. (2009). PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma tumor stem-like cells. Cell Stem Cell 4, 226-235.

Bouscary D., Lecoq-Lafon C., Chretien S., Zompi S., Fichelson S., Muller O., Porteu F., Dusanter-Fourt I., Gisselbrecht S., Mayeux P. and Lacombe C. (2001). Role of Gab proteins in phosphatidylinositol 3-kinase activation by thrombopoietin (Tpo). Oncogene 20, 2197-2204.

Bouscary D., Pene F., Claessens Y.E., Muller O., Chretien S., Fontenay-Roupie M., Gisselbrecht S., Mayeux P. and Lacombe C. (2003). Critical role for PI 3-kinase in the control of erythropoietin-induced erythroid progenitor proliferation. Blood 101, 3436-3443.

Bozulic L. and Hemmings B.A. (2009). PIKKing on PKB: regulation of PKB activity by phosphorylation. Curr. Opin. Cell Biol. 21, 256-261.

Brazil D.P., Yang Z.Z. and Hemmings B.A. (2004). Advances in protein kinase B signalling: AKTion on multiple fronts. Trends Biochem. Sci. 29, 233-242.

Brognard J. and Newton A.C. (2008). PHLiPPing the switch on Akt and protein kinase C signaling. Trends Endocrinol. Metab. 19, 223-230.

Buitenhuis M., Verhagen L.P., van Deutekom H.W., Castor A., Verploegen S., Koenderman L., Jacobsen S.E. and Coffer P.J. (2008). Protein kinase B (c-akt) regulates hematopoietic lineage choice decisions during myelopoiesis. Blood 111, 112-121.

Campone M., Levy V., Bourbouloux E., Berton Rigaud D., Bootle D., Dutreix C., Zoellner U., Shand N., Calvo F. and Raymond E. (2009). Safety and pharmacokinetics of paclitaxel and the oral mTOR inhibitor everolimus in advanced solid tumours. Br. J. Cancer 100, 315-321.

Chabanon A., Desterke C., Rodenburger E., Clay D., Guerton B., Boutin L., Bennaceur-Griscelli A., Pierre-Louis O., Uzan G., Abecassis L., Bourgeade M.F., Lataillade J.J. and Le Bousse-Kerdiles M.C. (2008). A cross-talk between stromal cell-derived factor-1 and transforming growth factor-beta controls the quiescence/cycling switch of CD34(+) progenitors through FoxO3 and mammalian target of rapamycin. Stem Cells 26, 3150-3161.

Chen C., Liu Y., Liu R., Ikenoue T., Guan K.L., Liu Y. and Zheng P. (2008). TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. J. Exp. Med. 205, 2397-2408.

Crazzolara R., Cisterne A., Thien M., Hewson J., Baraz R., Bradstock K.F. and Bendall L.J. (2009). Potentiating effects of RAD001 (Everolimus) on vincristine therapy in childhood acute lymphoblastic leukemia. Blood 113, 3297-3306.

DiNitto J.P. and Lambright D.G. (2006). Membrane and juxtamembrane targeting by PH and PTB domains. Biochim. Biophys. Acta 1761, 850-867.

Dolznig H., Boulme F., Stangl K., Deiner E.M., Mikulits W., Beug H. and Mullner E.W. (2001). Establishment of normal, terminally differentiating mouse erythroid progenitors: molecular characterization by cDNA arrays. FASEB J. 15, 1442-1444.

Downward J. (2004). PI 3-kinase, Akt and cell survival. Semin. Cell Dev. Biol. 15, 177-182.

Drayer A.L., Olthof S.G. and Vellenga E. (2006). Mammalian target of rapamycin is required for thrombopoietin-induced proliferation of megakaryocyte progenitors. Stem Cells 24, 105-114.

Dubrovska A., Kim S., Salamone R.J., Walker J.R., Maira S.M., Garcia-Echeverria C., Schultz P.G. and Reddy V.A. (2009). The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations. Proc. Natl. Acad. Sci. USA 106, 268-273.

Dunlop E.A. and Tee A.R. (2009). Mammalian target of rapamycin complex 1: signalling inputs, substrates and feedback mechanisms. Cell. Signal. 21, 827-835.

Eichhorn P.J., Creyghton M.P. and Bernards R. (2009). Protein phosphatase 2A regulatory subunits and cancer. Biochim. Biophys. Acta 1795, 1-15.

Elliott S., Pham E. and Macdougall I.C. (2008). Erythropoietins: a common mechanism of action. Exp. Hematol. 36, 1573-1584.

Engstrom M., Karlsson R. and Jonsson J.I. (2003). Inactivation of the forkhead transcription factor FoxO3 is essential for PKB-mediated survival of hematopoietic progenitor cells by kit ligand. Exp. Hematol. 31, 316-323.

Eyler C.E., Foo W.C., LaFiura K.M., McLendon R.E., Hjelmeland A.B. and Rich J.N. (2008). Brain cancer stem cells display preferential sensitivity to Akt inhibition. Stem Cells 26, 3027-3036.

Fonseca B.D., Smith E.M., Lee V.H., MacKintosh C. and Proud C.G. (2007). PRAS40 is a target for mammalian target of rapamycin complex 1 and is required for signaling downstream of this complex. J. Biol. Chem. 282, 24514-24524.

Franke T.F. (2008). PI3K/Akt: getting it right matters. Oncogene 27, 6473-6488.

Friedman A.D. (2007). Transcriptional control of granulocyte and monocyte development. Oncogene 26, 6816-6828.

Fruman D.A. and Bismuth G. (2009). Fine tuning the immune response with PI3K. Immunol. Rev. 228, 253-272.

Fuhler G.M., Tyl M.R., Olthof S.G., Lyndsay Drayer A., Blom N. and Vellenga E. (2009). Distinct roles of the mTOR components Rictor and Raptor in MO7e megakaryocytic cells. Eur. J. Haematol. 83, 235-245.

Gallia G.L., Tyler B.M., Hann C.L., Siu I.M, Giranda V.L., Vescovi A.L., Brem H. and Riggins G.J. (2009). Inhibition of Akt inhibits growth of glioblastoma and glioblastoma stem-like cells. Mol. Cancer Ther. 8, 386-393.

Gan B., Sahin E., Jiang S., Sanchez-Aguilera A., Scott K.L., Chin L., Williams D.A., Kwiatkowski D.J. and DePinho R.A. (2008). mTORC1-dependent and -independent regulation of stem cell renewal, differentiation, and mobilization. Proc. Natl. Acad. Sci. USA 105, 19384-19389.

Geddis A.E., Fox N.E. and Kaushansky K. (2001). 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, 34473-34479.

Geest C.R., Zwartkruis F.J., Vellenga E., Coffer P.J. and Buitenhuis M. (2009). Mammalian target of rapamycin activity is required for expansion of CD34$^+$ hematopoietic progenitor cells. Haematologica 94, 901-910.

Ghaffari S., Kitidis C., Zhao W., Marinkovic D., Fleming M.D., Luo B., Marszalek J. and Lodish H.F. (2006). AKT induces erythroid-cell maturation of JAK2-deficient fetal liver progenitor cells and is required for Epo regulation of erythroid-cell differentiation. Blood 107, 1888-1891.

Grech G., Blazquez-Domingo M., Kolbus A., Bakker W.J., Mullner E.W., Beug H. and von Lindern M. (2008). Igbp1 is part of a positive feedback loop in stem cell factor-dependent, selective mRNA translation initiation inhibiting erythroid differentiation. Blood 112, 2750-2760.

Greer E.L. and Brunet A. (2005). FOXO transcription factors at the interface between longevity and tumor suppression. Oncogene 24, 7410-7425.

Guerriero R., Parolini I., Testa U., Samoggia P., Petrucci E., Sargiacomo M., Chelucci C., Gabbianelli M. and Peschle C. (2006). Inhibition of TPO-induced MEK or mTOR activity induces opposite effects on the ploidy of human differentiating megakaryocytes. J. Cell Sci. 119, 744-752.

Guertin D.A., Stevens D.M., Saitoh M., Kinkel S., Crosby K., Sheen J.H., Mullholland D.J., Magnuson M.A., Wu H. and Sabatini D.M. (2009). mTOR complex 2 is required for the development of prostate cancer induced by Pten loss in mice. Cancer Cell 15, 148-159.

Guo W., Lasky J.L., Chang C.J., Mosessian S., Lewis X., Xiao Y., Yeh J.E., Chen J.Y., Iruela-Arispe M.L., Varella-Garcia M. and Wu H. (2008). Multi-genetic events collaboratively contribute to Pten-null leukaemia stem-cell formation. Nature 453, 529-533.

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

Haseyama Y., Sawada K., Oda A., Koizumi K., Takano H., Tarumi T., Nishio M., Handa M., Ikeda Y. and Koike T. (1999). Phosphatidylinositol 3-kinase is involved in the protection of primary cultured human erythroid precursor cells from apoptosis. Blood 94,1568-1577.

Hazen A.L., Smith M.J., Desponts C., Winter O., Moser K. and Kerr W.G. (2009). SHIP is required for a functional hematopoietic stem cell niche. Blood 113, 2924-2933.

Helgason C.D., Antonchuk J., Bodner C. and Humphries R.K. (2003). Homeostasis and regeneration of the hematopoietic stem cell pool are altered in SHIP-deficient mice. Blood 102, 3541-3547.

Hong L., Ramdas B., Chen J., Harris C., Wojchowski D.M. and Kapur R. (2008). KIT associated intracellular tyrosines play an essential role in EpoR co-signaling. Cell. Signal. 20, 1513-1520.

Hong S.P., Leiper F.C., Woods A., Carling D. and Carlson M. (2003). Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. Proc. Natl. Acad. Sci. USA 100, 8839-8843.

Horn S., Bergholz U., Jucker M., McCubrey J.A., Trumper L., Stocking C. and Basecke J. (2008). Mutations in the catalytic subunit of class IA PI3K confer leukemogenic potential to hematopoietic cells. Oncogene 27, 4096-4106.

Huang J. and Manning B.D. (2009). A complex interplay between Akt, TSC2 and the two mTOR complexes. Biochem. Soc. Trans. 37, 217-222.

Huang X., Cho S. and Spangrude G.J. (2007). Hematopoietic stem cells: generation and self-renewal. Cell Death Differ. 14, 1851-1859.

Ito K., Hirao A., Arai F., Takubo K., Matsuoka S., Miyamoto K., Ohmura M., Naka K., Hosokawa K., Ikeda Y. and Suda T. (2006). Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nat. Med. 12, 446-451.

Jacinto E., Loewith R., Schmidt A., Lin S., Ruegg M.A., Hall A. and Hall M.N. (2004). Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat. Cell Biol. 6, 1122-1128.

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

Jeanpierre S., Nicolini F.E., Kaniewski B., Dumontet C., Rimokh R., Puisieux A. and Maguer-Satta V. (2008). BMP4 regulation of human megakaryocytic differentiation is involved in thrombopoietin signaling. Blood 112, 3154-3163.

Jia S., Roberts T.M. and Zhao J.J. (2009). Should individual PI3 kinase isoforms be targeted in cancer? Curr. Opin. Cell Biol. 21, 199-208.

Kadri Z., Maouche-Chretien L., Rooke H.M., Orkin S.H., Romeo P.H., Mayeux P., Leboulch P. and Chretien S. (2005). Phosphatidylinositol 3-kinase/Akt induced by erythropoietin renders the erythroid differentiation factor GATA-1 competent for TIMP-1 gene transactivation. Mol. Cell. Biol. 25, 7412-7422.

Kalesnikoff J., Sly L.M., Hughes M.R., Buchse T., Rauh M.J., Cao L.P., Lam V., Mui A., Huber M. and Krystal G. (2003). The role of SHIP in cytokine-induced signaling. Rev. Physiol. Biochem. Pharmacol. 149, 87-103.

Kashii Y., Uchida M., Kirito K., Tanaka M., Nishijima K., Toshima M., Ando T., Koizumi K., Endoh T., Sawada K., Momoi M., Miura Y., Ozawa K. and Komatsu N. (2000). 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, 941-949.

Kaushansky K. (2005). The molecular mechanisms that control thrombopoiesis. J. Clin. Invest. 115, 3339-3347.

Kaushansky K. (2006). Lineage-specific hematopoietic growth factors. N. Engl. J. Med. 354, 2034-2045.

Keniry M. and Parsons R. (2008). The role of PTEN signaling perturbations in cancer and in targeted therapy. Oncogene 27, 5477-5485.

Kiel M.J. and Morrison S.J. (2008). Uncertainty in the niches that maintain haematopoietic stem cells. Nat. Rev. Immunol. 8, 290-301.

Kobayashi M., Laver J.H., Kato T., Miyazaki H. and Ogawa M. (1996). Thrombopoietin supports proliferation of human primitive hematopoietic cells in synergy with steel factor and/or interleukin-3. Blood 88, 429-436.

Kok K., Geering B. and Vanhaesebroeck B. (2009). Regulation of phosphoinositide 3-kinase expression in health and disease. Trends Biochem. Sci. 34, 115-127.

Kondo M., Weissman I.L. and Akashi K. (1997). Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell 91, 661-672.

Kozuma Y., Kojima H., Yuki S., Suzuki H. and Nagasawa T. (2007). 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, 1274-1282.

Liu H., Qiu Y., Xiao L. and Dong F. (2006). Involvement of protein kinase Cγ in the negative regulation of Akt activation stimulated by granulocyte colony-stimulating factor. J. Immunol. 176, 2407-2413.

Lowry J.A. and Mackay J.P. (2006). GATA-1: one protein, many partners. Int. J. Biochem. Cell Biol. 38, 6-11.

Luc S., Buza-Vidas N. and Jacobsen S.E. (2008). Delineating the cellular pathways of hematopoietic lineage commitment. Semin. Immunol. 20, 213-220.

Ma L., Chen Z., Erdjument-Bromage H., Tempst P. and Pandolfi P.P. (2005). Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis. Cell 121, 179-193.

Ma X.M. and Blenis J. (2009). Molecular mechanisms of mTOR-mediated translational control. Nat. Rev. Mol. Cell Biol. 10, 307-318.

Majka M., Janowska-Wieczorek A., Ratajczak J., Kowalska M.A., Vilaire G., Pan Z.K., Honczarenko M., Marquez L.A., Poncz M. and Ratajczak M.Z. (2000). Stromal-derived factor 1 and thrombopoietin regulate distinct aspects of human megakaryopoiesis. Blood 96, 4142-4151.

Mamane Y., Petroulakis E., LeBacquer O. and Sonenberg N. (2006). mTOR, translation initiation and cancer. Oncogene 25, 6416-6422.

Manning B.D. and Cantley L.C. (2007). AKT/PKB signaling: navigating downstream. Cell 129, 1261-1274.

Martelli A.M., Faenza I., Billi A.M., Manzoli L., Evangelisti C., Fala F. and Cocco L. (2006). Intranuclear 3'-phosphoinositide metabolism and Akt signaling: new mechanisms for tumorigenesis and protection against apoptosis? Cell. Signal. 18, 1101-1107.

Mavrakis K.J., Zhu H., Silva R.L., Mills J.R., Teruya-Feldstein J., Lowe S.W., Tam W., Pelletier J. and Wendel H.G. (2008). Tumorigenic activity and therapeutic inhibition of Rheb GTPase. Genes Dev. 22, 2178-2188.

Memmott R.M. and Dennis P.A. (2009). Akt-dependent and -independent mechanisms of mTOR regulation in cancer. Cell. Signal. 21, 656-664.

Miyakawa Y., Rojnuckarin P., Habib T. and Kaushansky K. (2001). 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, 2494-2502.

Miyamoto K., Araki K.Y., Naka K., Arai F., Takubo K., Yamazaki S.,

Matsuoka S., Miyamoto T., Ito K., Ohmura M., Chen C., Hosokawa K., Nakauchi H., Nakayama K., Nakayama K.I., Harada M., Motoyama N., Suda T. and Hirao A. (2007). Foxo3a is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell 1, 101-112.

Montecino-Rodriguez E., Leathers H. and Dorshkind K. (2001). Bipotential B-macrophage progenitors are present in adult bone marrow. Nat. Immunol. 2, 83-88.

Myklebust J.H., Blomhoff H.K., Rusten L.S., Stokke T. and Smeland E.B. (2002). Activation of phosphatidylinositol 3-kinase is important for erythropoietin-induced erythropoiesis from CD34(+) hematopoietic progenitor cells. Exp. Hematol. 30, 990-1000.

Nakao T., Geddis A.E., Fox N.E. and Kaushansky K. (2008). 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, 257-266.

Ong C.J., Ming-Lum A., Nodwell M., Ghanipour A., Yang L., Williams D.E., Kim J., Demirjian L., Qasimi P., Ruschmann J., Cao L.P., Ma K., Chung S.W., Duronio V., Andersen R.J., Krystal G. and Mui A.L. (2007). Small-molecule agonists of SHIP1 inhibit the phosphoinositide 3-kinase pathway in hematopoietic cells. Blood 110, 1942-1949.

Orford K.W. and Scadden D.T. (2008). Deconstructing stem cell self-renewal: genetic insights into cell-cycle regulation. Nat. Rev. Genet. 9, 115-128.

Orkin S.H. and Zon L.I. (2008). Hematopoiesis: an evolving paradigm for stem cell biology. Cell 132, 631-644.

Peterson T.R., Laplante M., Thoreen C.C., Sancak Y., Kang S.A., Kuehl W.M., Gray N.S. and Sabatini D.M. (2009). DEPTOR is an mTOR inhibitor frequently opverexpressed in multiple myeloma cells and required for their survival. Cell 137, 873-886.

Raaijmakers M.H. and Scadden D.T. (2008). Evolving concepts on the microenvironmental niche for hematopoietic stem cells. Curr. Opin. Hematol. 15, 301-306.

Raslova H., Baccini V., Loussaief L., Comba B., Larghero J., Debili N. and Vainchenker W. (2006). Mammalian target of rapamycin (mTOR) regulates both proliferation of megakaryocyte progenitors and late stages of megakaryocyte differentiation. Blood 107, 2303-2310.

Reya T., Morrison S.J., Clarke M.F. and Weissman I.L. (2001). Stem cells, cancer, and cancer stem cells. Nature 414, 105-111.

Robb L. (2007). Cytokine receptors and hematopoietic differentiation. Oncogene 26, 6715-6723.

Rojnuckarin P., Miyakawa Y., Fox N.E., Deou J., Daum G. and Kaushansky K. (2001). The roles of phosphatidylinositol 3-kinase and protein kinase C˙ for thrombopoietin-induced mitogen-activated protein kinase activation in primary murine megakaryocytes. J. Biol. Chem. 276, 41014-41022.

Ross S.E., Radomska H.S., Wu B., Zhang P., Winnay J.N., Bajnok L., Wright W.S., Schaufele F., Tenen D.G. and MacDougald O.A. (2004). Phosphorylation of C/EBP· inhibits granulopoiesis. Mol. Cell. Biol. 24, 675-686.

Sarbassov D.D., Guertin D.A., Ali S.M. and Sabatini D.M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307, 1098-1101.

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

Seita J., Ema H., Ooehara J., Yamazaki S., Tadokoro Y., Yamasaki A., Eto K., Takaki S., Takatsu K. and Nakauchi H. (2007). Lnk negatively regulates self-renewal of hematopoietic stem cells by modifying thrombopoietin-mediated signal transduction. Proc. Natl. Acad. Sci. USA 104, 2349-2354.

Shah O.J., Wang Z. and Hunter T. (2004). Inappropriate activation of the TSC/Rheb/mTOR/S6K cassette induces IRS1/2 depletion, insulin resistance, and cell survival deficiencies. Curr. Biol. 14, 1650-1656.

Shiozawa Y., Havens A.M., Pienta K.J. and Taichman R.S. (2008). The bone marrow niche: habitat to hematopoietic and mesenchymal stem cells, and unwitting host to molecular parasites. Leukemia 22, 941-950.

Siminovitch L., Till J.E. and McCulloch E.A. (1964). Decline in colony-forming ability of marrow cells subjected to serial transplantation into irradiated mice. J. Cell. Physiol. 64, 23-31.

Sitnicka E., Lin N., Priestley G.V., Fox N., Broudy V.C., Wolf N.S. and Kaushansky K. (1996). The effect of thrombopoietin on the proliferation and differentiation of murine hematopoietic stem cells. Blood 87, 4998-5005.

Sivertsen E.A., Hystad M.E., Gutzkow K.B., Dosen G., Smeland E.B., Blomhoff H.K. and Myklebust J.H. (2006). PI3K/Akt-dependent Epo-induced signalling and target genes in human early erythroid progenitor cells. Br. J. Haematol. 135,117-128.

Soda M., Willert K., Kaushansky K. and Geddis A.E. (2008). Inhibition of GSK-3, promotes survival and proliferation of megakaryocytic cells through a beta-catenin-independent pathway. Cell. Signal. 20, 2317-2323.

Sriburi R., Jackowski S., Mori K. and Brewer J.W. (2004). XBP1: a link between the unfolded protein response, lipid biosynthesis, and biogenesis of the endoplasmic reticulum. J. Cell Biol. 167, 35-41.

Stiles B.L. (2009). Phosphatase and tensin homologue deleted on chromosome 10: extending its PTENtacles. Int. J. Biochem. Cell Biol. 41, 757-761.

Tamburini J., Chapuis N., Bardet V., Park S., Sujobert P., Willems L., Ifrah N., Dreyfus F., Mayeux P., Lacombe C. and Bouscary D. (2008). Mammalian target of rapamycin (mTOR) inhibition activates phosphatidylinositol 3-kinase/Akt by up-regulating insulin-like growth factor-1 receptor signaling in acute myeloid leukemia: rationale for therapeutic inhibition of both pathways. Blood 111, 379-382.

Tanaka M., Kirito K., Kashii Y., Uchida M., Watanabe T., Endo H., Endoh T., Sawada K., Ozawa K. and Komatsu N. (2001). 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, 15082-15089.

Tothova Z. and Gilliland D.G. (2007). FoxO transcription factors and stem cell homeostasis: insights from the hematopoietic system. Cell Stem Cell 1, 140-152.

Tothova Z., Kollipara R., Huntly B.J., Lee B.H., Castrillon D.H., Cullen D.E., McDowell E.P., Lazo-Kallanian S., Williams I.R., Sears C., Armstrong S.A., Passegue E., DePinho R.A. and Gilliland D.G. (2007). FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell 128, 325-339.

Uddin S., Kottegoda S., Stigger D., Platanias L.C. and Wickrema A. (2000). Activation of the Akt/FKHRL1 pathway mediates the antiapoptotic effects of erythropoietin in primary human erythroid progenitors. Biochem. Biophys. Res. Commun. 275, 16-19.

von Lindern M., Deiner E.M., Dolznig H., Parren-Van Amelsvoort M., Hayman M.J., Mullner E.W. and Beug H. (2001). 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, 3651-3664.

Wang L., Harris T.E. and Lawrence J.C. Jr (2008). 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, 15619-15627.

Weissman I.L. and Shizuru J.A. (2008). 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, 3543-3553.

Wilson A., Laurenti E., Oser G., van der Wath R.C., Blanco-Bose W., Jaworski M., Offner S., Dunant C.F., Eshkind L., Bockamp E., Lio P., Macdonald H.R. and Trumpp A. (2008). Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell 135, 1118-1129.

Yap T.A., Garrett M.D., Walton M.I., Raynaud F., de Bono J.S. and Workman P. (2008). Targeting the PI3K-AKT-mTOR pathway: progress, pitfalls, and promises. Curr. Opin. Pharmacol. 8, 393-412.

Yilmaz O.H., Valdez R., Theisen B.K., Guo W., Ferguson D.O., Wu H. and Morrison S.J. (2006). Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. Nature 441, 475-482.

Yoshihara H., Arai F., Hosokawa K., Hagiwara T., Takubo K., Nakamura Y., Gomei Y., Iwasaki H., Matsuoka S., Miyamoto K., Miyazaki H., Takahashi T. and Suda T. (2007). Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. Cell Stem Cell 1, 685-697.

Yuan T.L. and Cantley L.C. (2008). PI3K pathway alterations in cancer: variations on a theme. Oncogene 27, 5497-5510.

Zhang J., Grindley J.C., Yin T., Jayasinghe S., He X.C., Ross J.T., Haug J.S., Rupp D., Porter-Westpfahl K.S., Wiedemann L.M., Wu H. and Li L. (2006). PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. Nature 441, 518-522.

Zhang J. and Lodish H.F. (2005). Identification of K-ras as the major regulator for cytokine-dependent Akt activation in erythroid progenitors in vivo. Proc. Natl. Acad. Sci. USA 102, 14605-14610.

Zhao W., Kitidis C., Fleming M.D., Lodish H.F. and Ghaffari S. (2006). Erythropoietin stimulates phosphorylation and activation of GATA-1 via the PI3-kinase/AKT signaling pathway. Blood 107, 907-915.

Zhu D., Hattori H., Jo H., Jia Y., Subramanian K.K., Loison F., You J., Le Y., Honczarenko M., Silberstein L., Luo H.R. (2006). Deactivation of phosphatidylinositol 3,4,5-trisphosphate/Akt signaling mediates neutrophil spontaneous death. Proc. Natl. Acad. Sci. USA 103, 14836-14841.

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