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Role of Signaling Pathways in Acute Myeloid Leukemia

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1. Introduction

Acute myeloid leukemia (AML) is a cancer wherein dysregulated differentiation, uncontrolled growth and inhibition of apoptosis lead to accumulation of immature myeloid progenitor cells and progression of oncogenic expression (Lowenberg et al., 1999). AML is now seen to be initiated and maintained from a small, self-renewing population of leukemic stem cells (LSCs), which give rise to a progeny of more mature and highly cycling progenitors (colony forming unit-leukemia, CFU-L). CFU-Ls do not self-renew, however they are committed to proliferation and limited differentiation. By doing so, they originate a population of blast cells which constitute the majority of leukemic cells in both the bone marrow and peripheral blood of patients. The exact phenotype of LSCs is still debated, but they are comprised in the CD34+/CD38-/low population (Lane et al., 2009). CD34+/CD38+ leukemic cells were unable to initiate leukemia in immunodeficient mice. It should be noted that only about 50% of AML are able to initiate leukemia in NOD/SCID mice (Testa et al., 2007)

2. Leukemogenesis

The pathogenesis of leukemia may be explained by two classes of alterations of oncogenic genes as a result of chromosomal aberrations. C*lass I* mutations confers a proliferative and/or survival advantage to the cells. The current list of known leukemogenic class I mutations consists of more than 10 different protein tyrosine kinases (PTK) that undergo constitutive activation either by being fused to different N-terminal partner proteins providing an oligomerization domain, or by activating mutations such as point mutations in their kinase domain or internal tandem repeats (length mutations) in the juxtamembrane domain (Flt3, Kit). Most of these alterations are associated with chronic myeloproliferative disorders such as chronic myeloid leukemia/chronic myelomonocytic leukemia (CML/CMML) or Philadelphia negative myeloproliferative disorders, except activating mutations of Flt3 and Kit which are found almost exclusively in acute leukemia. Flt3 ITD (internal tandem duplication) mutants constitutively activate MAPK, AKT and STAT5, leading to Pim-1 activation and Bcl-xL (B-cell lymphoma) hyperexpression (Minami et al., 2003; Kim et al., 2005). Extracellular c-Kit mutations resulted in c-Kit receptor

hyperactivation in response to Kit ligand, with subsequent strong activation of MAPK and PI3K, while codon 816 c-Kit mutations induced constitutive STAT-3 activation and upregulation of Bcl-xL and c-myc (Schnittger et al., 2006)). Other class I alterations are gain of function mutations of the three main RAS isoforms (N-Ras, Ki-Ras, Ha-Ras) which are frequently seen in different myeloid malignancies (Beaupre and Kurzrock, 1999). N-Ras mutations lead to increased activity of the Ras pathway, resulting in increased proliferation and decreased apoptosis (Testa et al., 2007). Overexpression of *class I* mutations is generally sufficient to transform hematopoietic cells to growth-factor independence *in vitro* and to induce a lethal *leukemialike* myeloproliferative disorder in mice (Ilaria, 2004).
(Flt- FMS-Like Tyrosine Kinase 3, STAT- Signal Transducer and Activator of Transcription, MAPK-Mitogen-Activated Protein Kinase)
In contrast to class I mutations, there is a large group of genetic alterations mostly associated with acute leukemia, referred to as *class II* mutations, which impair differentiation of hematopoietic cells and subsequent apoptosis but do not directly provide proliferative and/or survival advantage. Many of them are loss of function mutations (either through fusion formation or point mutations) of transcriptional regulators that are critical for normal hematopoietic development and differentiation. Transcription factor fusion genes include CBF, RAR, MLL, HOX and CBP while loss of function mutations occur in AML1, CEBP/a, PU.1, GATA1 and IKAROS (reviewed in Chalandon and Schwaller, 2005). Via mediators of apoptosis, fusion proteins send anti-apoptotic signals that favor the preferential survival of leukemic cells:PML/RAR-α or CBF/SMMHC through the p53 pathway and AML1/ETO through the Bcl2-related pathway (Klampfer et al., 1996; Britos-bray et al., 1998; Pandolfi, 2001). PML/RARα fusion protein was also shown to exert an anti-apoptotic activity by downmodulating the expression of some death-inducing genes, such as TNF-R1 (Testa et al., 1998) and TRAIL-R1/-R2 (Ricioni et al., 2005). Nucleophosmin acts as a cellular p53 negative regulator to protect hematopoietic cells from stress-induced apoptosis (Lambert and Buckle, 2006). These mutations are usually not sufficient to mimic the human disease in transgenic mice since they do not readily induce a leukemia phenotype. However, after a long latency period, signs of myelodysplasia are often seen with a variable propensity to develop an immature and clonal hematologic disorder closely resembling human AML (reviewed in Chalandon and Schwaller, 2005). Additional mutations, occurring at the level of signal transduction molecules (the receptor tyrosine kinases Flt3 or c-Kit, NRas and Ki-Ras), are required for the generation of disease (reviewed in Testa et al., 2007). This hypothesis is supported by the analysis of unselected blood samples from neonates which showed that about 1% have class II genetic alterations that are detectable by PCR (Greaves et al., 2003).
(CBF-core binding factor, RAR –retinoic acid receptor, MLL-mixed lineage leukemia, HOX-homeobox, CBP-CREB binding protein, CEBP/a-CCAAT/enhancer binding protein, PML-promyelocyte leukemia, SMMHC-smooth muscle myosin heavy chain, TRAIL-tumor necrosis factor–related apoptosis-inducing ligand)
In the same light, being a heterogeneous disease, relapsed AML is unlikely to emanate from one predominant mechanism; instead, there are likely to be multiple biologic factors at play that allow for clinical relapse to occur. These factors likely include multidrug resistance proteins, aberrant signal transduction pathways, survival of leukemia stem cells, microenvironmental interactions, and immune tolerance. Many conditions in the environment select for the development of these target mechanisms, ranging from chemotherapeutic modalities, to signal transduction inhibitors, to upregulation of antileukemic immune responses (reviewed in Lancet and Karp, 2009)

PTK involved	Fusion gene	Chromosomal aberration	Disease phenotype
A. Fusion genes			
ABL (9q34)	BCR/ABL	t(9;22)(q34;q11)	CML
(ABL1)	TEL/ABL	t(9;12)(q34;p13)	Atypical CML
ARG (1q24)	BCR/ARG	t(1;22)(q24;q11)	Atypical CML
(ABL2)	TEL/ARG	t(1;12)(q24;p13)	Atypical CML
PDGF R (5q33)	TEL/PDGF R	t(5;12)(q33;p13)	CMML, atypical CML
	HIP1/PDGF R	t(5;7)(q33;q11)	CMML, atypical CML
	RAB5/PDGF R	t(5;17)(q33;p13)	CMML, atypical CML
	H4/PDGF R	t(5;10)(q33;q21)	CMML,atypical CML
	Myomegalin/PDGF R	t(1;5)(q23;q33)	CMML,atypical CML
	CEV14/PDGF R	t(5;14)(q33;q32)	relapse AML
	NIN1/PDGF R	t(1;5)(q23;q33)	atypical CML
	HCMOGT/PDGF R	t(5;17)(q33;p11)	juvenile CMML
	TP53BP1/PDGF R	t(5;15)(q33;q22)	atypical CML
PDGF R (4q12)	BCR/PDGF R	t(4;22)(q12;q11)	Atypical CML
AK2 (9p24)	BCR/JAK2	t(9;22)(p24;q11)	CML, atypical CML
	TEL/JAK2	t(9;12)(p24;p13)	Atypical CML, ALL, AML
	PCM1/JAK2	t(8;9)(p21-22;p23-24)	Atypical CML, AML, ALL
TRKC (15q25)	TEL/TRKC	t(12;15)(p13;q25)	AML
GFR3 (4p16)	TEL/FGFR3	t(4;12)(p16;p13)	AML
FRK(6q21)	TEL/FRK	t(6;12)(q21;p13)	AML
B. Gain of function mutations			
FLT3 (13q12)	ITD (80%), activation loop kinase domain (15%)		AML
KIT (4q12)	JM region, activation loop kinase domain		AML
JAK2 (9p21)	JAK2 V617F mutation	9pLOH	PV, ET, myelofibrosis
C. Deregulated expression			
FLT3 (13q12)	Overexpression	MLL alterations	ALL/AML

CML: chronic myeloid leukemia; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; CMML: chronic myelomonocytic leukemia; EMS; ITD: internal tandem duplication; JM: juxtamembrane; PV: polycythemia vera; ET: essential thrombocythemia; LOH: loss of heterozygosity

Table 1. Deregulated protein tyrosine kinases in myeloid leukemias (taken from Chalandon and Schwaller, 2005)

3. Signal Transduction Pathways (STP)

Signal transduction is the primary means by which eukaryotic cells respond to external signals from their environment and coordinate complex cellular changes. Extracellular signal is transduced into the cell through ligand-receptor binding, followed by the activation

of intracellular signaling pathways that involve a series of protein phosphorylation and dephosphorylation, protein-protein interaction, and protein-small molecules interaction (Liu and Zhou, 2004). Cytokines interact with cell-surface receptors initiating signaling cascades that promote cell growth and division, while inhibiting the pathways of apoptotic cell death. The JAK/STAT, Raf/ MEK/ERK and PI3K/Akt signaling pathways are activated by a variety of cytokines that function to potentiate or inhibit hematopoiesis. These include IL (interleukin)-3, IL-7, SCF (stem cell factor), G (granulocyte)-CSF, type I interferons (IFN) and TGF- (transforming growth factor)- beta (Steelman et al., 2004).

The phosphatidylinositol 3-kinase (PI3K)

PI3K /protein kinase B (Akt)/mammalian target of rapamycin (mTOR) (a family of lipid kinases) signaling cascade is crucial to many widely divergent physiological processes which include cell cycle progression, transcription, translation, differentiation, apoptosis, motility, and metabolism (Yuan and Cantley, 2008). The family of PI3K enzymes phosphorylates inositol lipids and comprises three different classes, I, II, and III. Phosphorylated phosphatidylinositol 3,4,5trisphosphate [PtdIns (3,4,5)P3] recruits to the plasma membrane pleckstrin homology (PH) domain-containing proteins, which include phosphoinositide-dependent protein kinase 1 (PDK1) and Akt. The phospholipid products of PI3K activate downstream targets, including PDK, Akt and PKC (Palmer et al., 1995; Toker et al., 1994; Nakanishi et al., 1993).
Class I PI3K is further classified as A [activated by receptor tyrosine kinases (RTKs), Ras, and G-protein coupled receptors (GPCRs)] and B (activated by GPCRs) subtype. Class IA and 1B PI3Ks are heterodimeric enzymes composed of a regulatory and of catalytic subunits (Martelli et al., 2010).

Phosphoinositide-dependent kinase (PDK)

PDK requires the phospholipid product of PI3K for activation. There are believed to be two members of the PDK family – PDK1 and PDK2. Association of Akt with phosphoinositides produces a conformational change allowing Ser473 to be phosphorylated by PDK1 (Scheid et al., 2002).

Protein kinase B (Akt)

Akt is a 57-kDa serine/threonine protein kinase central to cell signaling downstream of growth factors, cytokines, and other cellular stimuli. Activated Akt was originally isolated from cells of the leukemia and lymphoma prone AKR strain of mice (Staal, 1987). It comprises three highly conserved isoforms: Akt1/α, Akt2/β, and Akt3/γ which are functionally different (Staal, 1987; Nicolson and Anderson, 2002; Staal et al., 1988). Once Akt is recruited at the plasma membrane, its activation loop is phosphorylated on Thr308 by PDK1 while the mTOR complex 2 (mTORC2), activated by RTK, phosphorylates Ser473 in the Akt COOH-terminus. Full Akt activation requires both phosphorylation steps. Active Akt migrates to both the cytosol and the nucleus. Nuclear Akt may fulfill important anti-apoptotic roles. So far, over 100 Akt substrates have been identified (Manning and Cantley, 2007). Of these, about 40 which mediate the pleiotropic Akt functions have been characterized, including Bad, caspase-9, murine double minute 2 (MDM2), IκB kinase (IKK) α, proline-rich Akt substrate 40-kDa (PRAS40) 40, the Foxo family of Forkhead box-o transcription factors, apoptosis signal-regulated kinase 1 [ASK1, a negative regulator of pro-apoptotic c-Jun N-terminal kinase (JNK)], Raf, p27Kip1, p21Cip1, glycogen synthase kinase

3β (GSK3β). Each of these substrates has a key role in the regulation of cell survival and proliferation, either directly or through an intermediary.

The antiapoptotic effects of Akt occur through its phosphorylation of a wide variety of targets. The first antiapoptotic target identified was Bad, a member of the Bcl-2 family. Phosphorylation of Bad at S136 by Akt allows phosphorylated Bad to interact with 14-3-3 proteins, promoting cell survival (Datta et al., 1997; Andreeff et al., 1999). Interaction of Bad with 14-3-3 proteins inhibits the ability of Bad to interact with Bcl-2 and Bcl-xL. This allows Bcl-xL to bind to proapoptotic Bax molecules and prevent the formation of proapoptotic Bax homodimers. However, Bad is also phosphorylated on different sites by members of the Raf/MEK/ERK (S112) and PKA (S112, S155) pathways.

In human cells, Akt phosphorylates and inactivates caspase-9. Overexpression of Akt inhibits cytochrome c-induced activation of caspase-9 (Cardone et al., 1998). Phosphorylation of the Foxo family of transcription factors is also attributed to Akt (Biggs et al 1999); Brunet et al., 1999; Rena et al., 1999; Tang et al., 1999).This phosphorylation results in forkhead transcription factors translocation to the cytoplasm, thus inhibiting transcription of pro-apoptotic genes such as FasL (Brunet et al., 1999). Akt activates transcription of antiapoptotic genes through phosphorylation of IKK and regulation of nuclear factor-kappa B (NF-kB) (Ozes et al., 1999). Akt also promotes cell survival and cell cycle progression by its ability to phosphorylate MDM2 and GSK-3 (Fukumoto et al., 2001; Zhou et al., 2001). Once phosphorylated by Akt, MDM2 translocates to the nucleus and interacts with p300. p300 dissociates from p19ARF, resulting in the degradation of p53 and cell cycle progression. Akt phosphorylates GSK-3, inhibiting its activity. The decreased GSK-3 activity increases stability of catenin and enhances its association with lymphoid enhancer factor/T cell factor (LEF/TCF) (Fukumoto et al., 2001). The catenin–LEF/TCF complex increases transcription of proteins such as cyclin D1 and c-myc, promoting cell cycle progression (Fukumoto et al., 2001). Clearly, Akt can affect both cell cycle progression and apoptosis (reviewed in Steelman et al., 2004).

MTORC1 is a critical regulator of translation initiation and ribosome biogenesis and plays an evolutionarily conserved role in cell growth control (Wullschleger et al., 2006). The enhanced sensitivity of cancer cells and mouse tumor models exhibiting oncogenic activation of the PI3K-Akt pathway to mTORC1 inhibitors, such as rapamycin, illustrates the importance of mTORC1 activation downstream of Akt (Sabatini, 2006). One of the best-conserved functions of Akt is its role in promoting cell growth (i.e., an increase in cell mass). The predominant mechanism appears to be through activation of mTOR complex 1 (mTORC1 or the mTOR-raptor complex), which is regulated by both nutrients and growth factor signaling. mTORC1 signaling integrates environmental clues (growth factors, hormones, nutrients, stressors) and information from the cell metabolic status. Thus, mTORC1 controls anabolic processes for promoting protein synthesis and cell growth (Manning and Cantley, 2007).

Janus kinase/Signal Transducer and Activator of Transcription (JAK/STAT)

The JAK/STAT pathway consists of three families of genes: the JAK, or Janus family of tyrosine kinases, the STAT (signal transducers and activators of transcription) family and the CIS/SOCS family, which serves to downregulate the activity of the JAK/STAT pathway (Silvennoinen et al., 1993; Kisseleva et al., 2002; Krebs and Hilton, 2002; Fujitani et al., 1997). The JAK/STAT pathway involves signaling from the cytokine receptor to the nucleus. JAKs are stimulated by activation of a cytokine receptor. Stimulation of JAKs results in STAT

transcription factor activity. JAKs are a family of large tyrosine kinases, having molecular weights in the range of 120–140 kDa (1130–1142 aa). Four JAKs (JAK1, JAK2, JAK3 and Tyk2) have been identified in mammals. JAK3 expression is limited to hematopoietic cells (Steelman et al., 2004).

The STAT gene family consists of seven proteins (STAT1,STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6). Upregulation of STAT3 is detected with high frequency inhuman cancer. STAT3 is activated not only by cytokine receptors, such as the receptor for the IL-6 family cytokines, but also growth receptor tyrosine kinases, such as the EGFR family including Her2/Neu, and non-receptor tyrosine kinases such as Src and Abl(Turkson et al., 1998), and is also activated in response to stimulation of G-protein-coupled receptors (GPCR) (Pelletier et al., 2003). Classically, the receptor stimulation by ligand induces STAT3 binding to phosphotyrosine residues of receptors through its SH2 domain and its phosphorylation on a critical tyr705 residue by the receptor itself, or by associated Janus kinase (JAK, Jak1–3, Tyk2) or Src family tyrosine kinases (Yu et al., 2004).

Ras/Raf/MAPK kinase/extracellular signal-regulated kinase pathway (Ras/Raf/MEK/ERK)

The Ras/Raf/MEK/ERK pathway is a central signal transduction pathway, which transmits signals from multiple cell surface receptors to transcription factors in the nucleus (Chang et al., 2003a; Chang et al., 2003b; Chang et al., 2003c). This pathway is frequently referred to as the MAP kinase pathway as MAPK stands for mitogen-activated protein kinase indicating that this pathway can be stimulated by mitogens, cytokines and growth factors. The pathway can be activated by Ras stimulating the membrane translocation of Raf. This pathway also interacts with many different signal transduction pathways including PI3K/Akt and JAK/STAT.

Ras is a small GTP-binding protein, which is the common upstream molecule of several signaling pathways including Raf/MEK/ERK, PI3K/Akt and RalEGF/Ral (Chang et al., 2003a; Chang et al., 2003b; Chang et al., 2003c). There are three different Ras family members: Ha-Ras, Ki-Ras and N-Ras. The Ras proteins show varying abilities to activate the Raf/MEK/ERK and PI3K/Akt cascades, as Ki-Ras has been associated with Raf/MEK/ERK while Ha-Ras is associated with PI3K/Akt activation.

The Raf protein family consists of A-Raf, B-Raf and Raf-1, which are involved in the regulation of proliferation, differentiation and apoptosis induced after cytokine stimulation (Blalock et al., 1999; Mercer et al., 2003; Naumann et al., 1007, Pritchard et al., 1996; Mercer et al., 2002).Raf-1 has many effects on the regulation of apoptosis. Some of these effects occur at the mitochondrial membrane and are independent of MEK and ERK activity. It was observed that overexpression of activated A-Raf abrogates the cytokine dependence of hematopoietic cells. Overexpression of B-Raf in Rat-1 cells results in decreased apoptosis due to inhibition of caspase activity. Raf-1 has important roles in apoptosis as it phosphorylates and inactivates Bad (Wang et al., 1996). Raf-1 phosphorylates and co-immunoprecipitates with Bcl-2, as well as regulates Bag and Bad expression, in BCR/ABL-expressing cells (Salomoni et al., 1998). The ability of Raf proteins to phosphorylate MEK1 varies from B-Raf, Raf-1, A-Raf. The ability of Raf to abrogate cytokine dependency is inversely proportional to their MEK1 activity, with A-Raf, Raf-1, B-Raf (McCubrey et al., 1998; Hoyle et al., 2000). Stimulation of Raf activates MEK1 and ERK resulting in phosphorylation of transcription factors, proliferation, and inhibition of apoptosis (Steelman et al., 2004).

Raf-1 is also phosphorylated by Akt which has been associated with inhibition of Raf-1 activity (Wojknowski et al., 1997; Rommel et al., 1999). CAMP-dependent protein kinase (PKA) inhibits Raf-1 (Wu et al., 1993; Schramm et al., 1994; Dumaz et al., 2002). Protein kinase C isoforms (a, b and g) stimulates Raf-1 activity (Sozeri t al., 1992). Raf-1 has been postulated to have important roles in cell cycle progression, activation of the p53 and NF-kB transcription factors and the prevention of apoptosis (reviewed in Steelman et al., 2004)
Interactions between the Raf and PI3K/Akt pathways, or crosstalk, is an area of intense research Recently, it was demonstrated that it is more effective to inhibit the growth of Raf- and MEK1-transformed hematopoietic cells with inhibitors that target both the Raf/MEK/ERK and PI3K/Akt pathways (Navolanic et al., 2004).

MEK

MEK proteins are the primary downstream targets of Raf. The MEK family of genes consists of five genes: MEK1, MEK2, MEK3, MEK4 and MEK5. The structure of MEK consists of an amino-terminal negative regulatory domain and a carboxy-terminal MAP kinase-binding domain, which is necessary for binding and activation of ERKs (Huang et al., 1995; Tanoue et al., 2001; Crews et al., 1992). Deletion of the regulatory MEK1 domain results in constitutive MEK1 and ERK activation. Activated MEK1 could abrogate cytokine dependency of certain hematopoietic cells. Constitutive activity of MEK1 inhibits NF-kB transcription by negatively regulating p38MAPK activity (Carter et al., 2000).

ERK

The main physiological substrates of MEK are the members of the ERK (extracellular signal-regulated kinase) or MAPK (mitogen activated protein kinase) family of genes. The ERK family consists of four distinct groups of kinases: ERK, Jun amino terminal kinases (JNK1/2/3), p38MAPK (p38 a/b/g/d) and ERK5. In addition, there are ERK3, ERK4, ERK6, ERK7 and ERK8 kinases, which while related to ERK1 and ERK2 have different modes of activation, and their biochemical roles are not as well characterized. Downstream targets of ERK include the p90Rsk kinase and the CREB, c-Myc and other transcription factors. ERK and p90Rsk can enter the nucleus to phosphorylate transcription factors which can lead to their activation (reviewed in Steelman et al., 2004).

Nuclear factor kappa B (NFkB)

Cilloni et al. (2007) have presented a comprehensive review on NF-kB. NF-kB proteins are a small group of related and evolutionarily conserved proteins which in mammals consists of five members: Rel (c-Rel), RelA/p65, RelB, p50, and p52 (Ghosh et al., 1998; Hayden et al., 2004).In resting cells, NF-kB proteins arepredominantly cytoplasmic, associating with members of the inhibitory IkB family such as IkBa, IkBb and Ikbe (Ghosh et al., 1998).These interact with NF-kB through multiple ankyrin repeats and as a result inhibit its DNA binding activity. Two NF-kB activation pathways exist; the first is normally triggered in response to infections or exposure to pro-inflammatorycytokines that activate the IkB kinase (IKK) complex leading to phosphorylation-induced IkB degradation, the other pathway leads to selective activation of p52: RelB dimers. This pathway is triggered by certain membersof the tumor necrosis factor (TNF)cytokine family through selective activation of IKKa by the upstream kinase, NF-kappa B-inducing kinase (NIK).In response to many stimuli such as inflammatorycytokines, bacterial lipopolysaccharide, phorbol esters, viral infection or stress, IkB are phosphorylated on two critical serine residues (Senftleben et al., 2001).

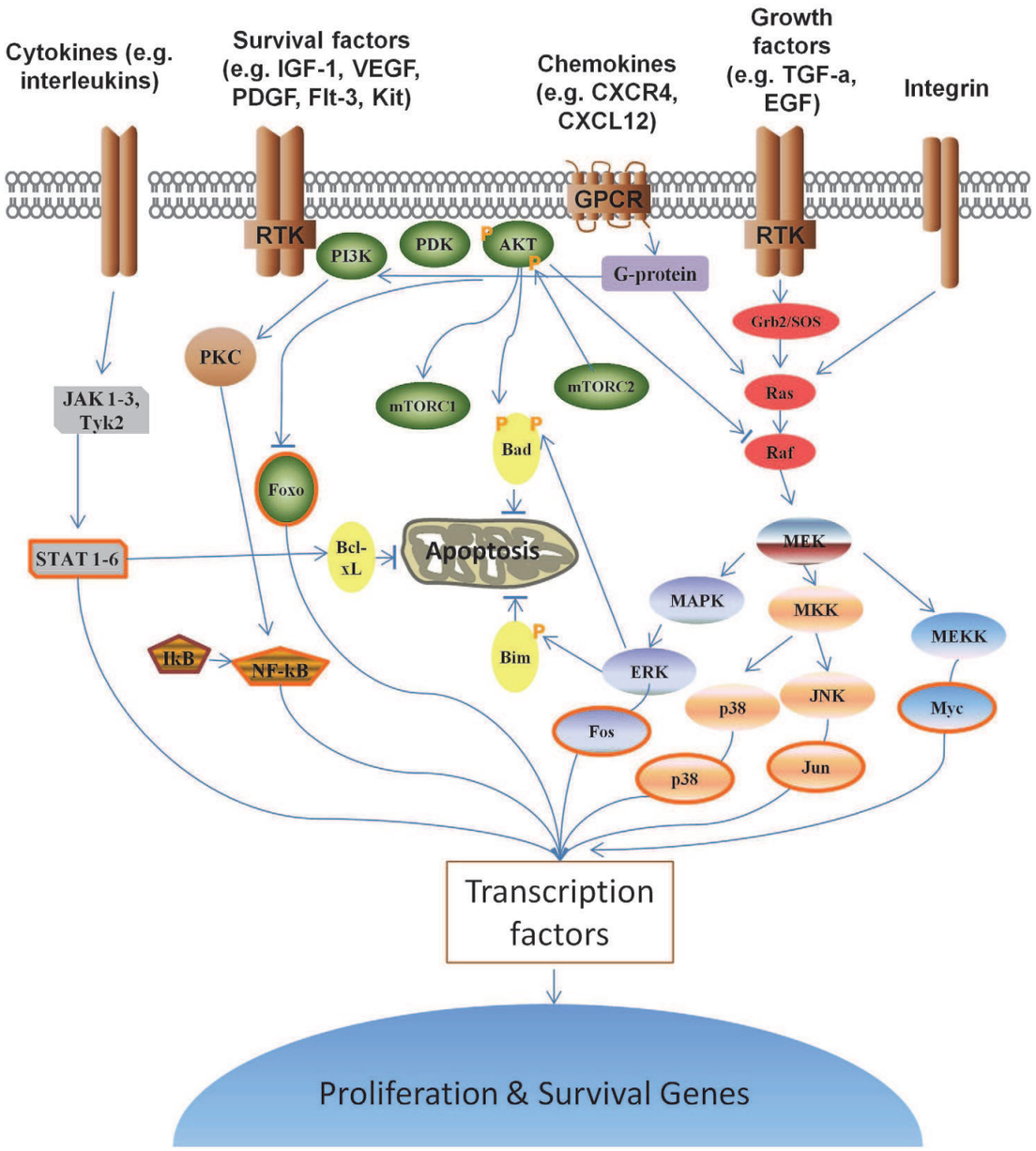

Fig. 1. Signal transduction pathways (refer text for details)

This modification triggers IkB ubiquitination and destruction via the 26Sproteasome degradation machinery. As a consequence, NF-kBis freed to enter the nucleus and regulate transcription of over 150 genes encoding cell adhesion molecules, cytokines, growth factors, components of the immune systems and anti-apoptotic genes such as FLIP (FLICE inhibitory protein), cIAPs (inhibitor of apoptosis), Bcl-2 and Bcl-xL (Aggarwall, 2004). It is also implicated in the regulation of cell proliferation by controlling D-type cyclins (Takebayashi et al., 2003).

The three main signaling pathways are kept in check by naturally occurring inhibitors or tumor suppressor proteins. For example, the JAK/STAT pathway has the SOCS/CIS family of proteins, which serve to limit its effects by a negative feedback pathway. The Raf/MEK/ERK pathway can be negatively regulated by the PI3K/Akt cascade as well as the MKP1 phosphatase, which inactivates phosphorylated ERK. The PI3K/Akt pathway has the PTEN and SHIP phosphatases, which serve to fine-tune its antiapoptotic effects (reviewed in Steelman et al., 2004).

4. Aberrant STP and drug resistance in AML

Genetic events that give rise to leukemic transformation occur through activation of components of receptor tyrosine kinase (RTK) signaling pathways (Liu and Zhou, 2004). These include fusion proteins or gene mutations such as seen with activated TEL-JAK, STAT5A and BCR-ABL. Transforming activity of oncogenic PTK is mediated by parallel activation of several downstream signaling pathways. Final downstream mediators of this complex signaling network are phosphoproteins that translocate to the nucleus and act as transcriptional regulators activating a distinct group of target genes. The oncogenic activity of a given PTK is mediated by several signaling pathways including JAK/STAT, Ras/MAPK, PI3K/AKT, or NF-kB.

Oncogenic activity severs dependence of transformed cell on external stimulation for survival. TEL-JAK fusion proteins contain the oligomerization domain of TEL and the tyrosine kinase domains of JAK1, JAK2, JAK3, or TYK2. These efficiently substitute for the survival and mitogenic signals controlled by IL-3, without concomitant activation of the IL-3 receptor. STAT5 are constitutively active in TEL-JAK2- and TEL-JAK1-expressing cells (Lacronique et al., 2000). The BCR-ABL oncogene produces an activated tyrosine kinase fusion protein and gain independence from IL-3 for cell growth (Mandanas et al., 1992). Activated forms of Ras, Raf, MEK, PI3K and Akt however, show significant differences in the ability to abrogate cytokine dependence (Steelman et al., 2004).

TEL/JAK2 isoforms, depending on the location of the breakpoints in the JAK2 gene, have been described in acute lymphoblastic leukemia of the B-cell type and atypical CML (Lacronique et al., 1997). Somatically acquired JAK2 mutation (V617F) was detected in 472/944 (50%) of patients with Ph-negative chronic myeloproliferative disorders [including polycythemia vera (PV), idiopathic myelofibrosis (IMF) and essential thrombocytosis (ET)] with predominance in PV (66%) followed by IMF (42%) and ET (26%) (Jones et al., 2005). Recent investigation of novel mutations in JAK2 revealed a higher incidence, ~99% and 55% in PV and ET, respectively (Tefferi, 2010).

Flt-3 mediates its proliferative and antiapoptotic effects through several signaling pathways including the STAT5, Ras/MAPK and PI3K/AKT pathways. Overexpression of Flt-3 was detected in 73% of AML and 78% of ALL patients (Nakao et al., 1996). Flt-3 *length mutations* (internal tandem duplications (ITD) in the juxtamembrane domain) (Nakao et al., 1996), is observed in more than 20% of adult and more than 10% of pediatric AML patients harbor an Flt-3-ITD (reviewed in Testa et al., 2007). In general, patients with mutant FLT3 show higher cell counts and decreased overall survival. Absence of the wild-type allele in patients with Flt-3-ITD predicted poor prognosis in 82 adult *de novo* AML cases with otherwise normal cytogenetics who received uniform high-dose therapy. Of the the 23 (28%) patients with Flt3-ITD, disease-free survival (DFS) was inferior (P = 0.03), yet overall survival (OS) was not different (P = 0.14) (Whitman et al., 2001). In cytogenetic normal AML patients aged > 60

years treated on Cancer and Leukemia Group B frontline trials, FLT3-ITD remained associated with shorter disease-free survival (P < .001; hazard ratio 2.10) and overall survival (P < .001; hazard Ratio 1.97) in multivariable analyses (Whitman et al., 2010). Flt3 kinase domain point mutants is mutated in about 35% of AML (Stirewalt et al., 2003). In a study of 481 patients, FLT3 mutation did not have an impact on event-free survival (EFS) in patients with CBF-AML (*P* = .84) and poor-risk AML (*P* = .37). However, while event-free survival was worse in the FLT3-internal tandem duplication (ITD) group (20 weeks vs 41 weeks; P < .00,001) this was not observed for the FLT3-tyrosine kinase domain (TKD) point mutation group (61 weeks vs 41 weeks; P = .15) (Santos et al., 2011).

The profiles of signal transduction that correlated with poor response to chemotherapy showed potentiated STAT5 and STAT3 phosphorylations as well as attenuated STAT1 phosphorylation following cytokine stimulation (Irish et al., 2004)

Ras mutations are frequently observed in certain hematopoietic malignancies including myelodysplastic syndromes, juvenile myelomonocytic leukemia and acute myeloid leukemia (Bartram et al., 1988; Flotho et al., 1999; Stirewalt et al., 2001). lt has been shown to activate both the Raf/MEK/ERK and the PI3K/Akt pathways. Thus, mutations at Ras should theoretically activate both pathways simultaneously. Consequence of this activation may be the increased expression of growth factors that can potentially further activate this cascade by an autocrine loop. Many cytokine and growth factor gene promoters contain binding sites for transcription factors (Ets, Elk, Jun, Fos, CREB) whose activities are often activated by the Raf/MEK/ERK cascade (reviewed in Steelman et al., 2004).

There is increasing evidence that activation of the PI3K/AKT signaling pathway leading to downstream inactivation of Foxo transcription factors, activation of the mammalian target of rapamycin (mTOR), or induction of Skp2 (leading to degradation of the cell cycle inhibitor p27), plays a central role in transformation by several mutated PTK such as BCR/ABL, mutated FLT3 or KIT (Scheijen et al., 2004; Andreu et al., 2005). Emerging evidence suggests that activation of NF-kB involves crosstalk between the PI3K and Ras/MAPK pathways (Gelfanov et al., 2001; Kirchner et al., 2003). Several NF-kB target genes, such as cIAP1/cIAP2, Bcl-xL, or Mcl-1, are well-known inhibitors of apoptosis that may co-mediate the antiapoptotic effect of a constitutively activated PTK (Aichberger et al., 2005).

Expression of transcription factor fusions like AML1/ETO and PML/RARa in leukemic cells leads to induction of several genes associated with WNT signaling (Muller-Tidow et al., 2004). WNT signaling activation was found in a significant fraction of leukemic blasts from patients with AML-M0 (Zheng et al., 2004).

Other causes of PI3K/Akt/mTOR activation in AML may be the result of several factors, including low levels of PP2A, autocrine/paracrine secretion of growth factors such as IGF-1 and VEGF (reviewed in Martelli et al., 2010). Interactions between leukemic cells and bone marrow stromal cells through CXCR4 (a GPCR) which is abundantly expressed on leukemic cell surface where it is up-regulated by hypoxic conditions and its physiological ligand, (Fierro et al., 2009; Fiegl et al., 2009) CXCL12, produced by stromal cells, (Fiegl et al., 2009; Ayala et al., 2009) could result in PI3K/Akt/mTOR activation (Zeng et al., 2009). Furthermore, interactions between β1 integrins on AML cells and stromal fibronectin could lead to pathway activation, (Matsunaga et al., 2003; Matsunaga et al., 2008) possibly through up-regulation of integrin-linked kinase 1 (ILK1) which is involved in Akt phosphorylation on Ser473 in a PI3K-dependent manner in AML cells (Tabe et al., 2007).

PI3K/Akt/ mTOR pathway influences proliferation, survival, and drug resistance of AML cells.

From 50% to 80% of patients with AML display Akt phosphorylated on either Thr308 or Ser473 (or both) (Xu et al., 2003; Min et al., 2003). Univariate analysis of 146 AML patients revealed those with low levels of pAKT had somewhat better CR rates (60% versus 50%; *P*=0.21), longer median CR durations (71 versus 32 weeks; *P*=0.13), and statistically significant longer median survival times (59 versus 30 weeks; *P*=0.02) compared with those with high levels of pAKT. In another study, single analysis of Akt phosphorylated at threonine 308 (Thr308) and serine 473 (Ser473) showed AktThr308(high) patients had significantly shorter overall survival (11 vs 47 months; P=0.01), event-free survival (9 vs 26 months; P=0.005) and relapse-free survival (10 months vs not reached; P=0.02) than Thr308(low) patients. This was not observed for Akt Ser473 (Gallay et al., 2009). Poor prognosis of AML patients with elevated PI3K/Akt/mTOR signaling could be also related to the fact that this pathway controls the expression of the membrane ATP binding cassette (ABC) transporter, multidrug resistance-associated protein 1, associated with a lower survival rate (Tazzari et al., 2007; Schaich et al., 2005). Nevertheless, a more recent report has highlighted that constitutive activation of PI3K/Akt/mTOR signaling could be a favourable prognostic factor in de novo cases of AML. One hypothesis for the lower relapse rate in patients with enhanced PI3K/Akt/mTOR signaling is that it could drive immature leukemic cells (LSCs and CFU-L) into S phase, thus rendering them more susceptible to polychemotherapy (Tamburini et al., 2005)

The AKT pathway was among the signaling cascades whose simultaneous activation with other pathways, such as PKCα and ERK, was found to confer a poor prognosis in AML (Altman et al., 2011). Eventhough often mutated in human cancer, MMAC1/PTEN gene are infrequent as genetic aberrations in myeloid leukaemia (Aggerholm et al., 2000)

NF-kB has been found to be activated in CD34+/CD38– blast cells derived from patients with *de novo* AML (Guzman et al., 2001; Baumgartner et al., 2002). Leukemic stem cells residing in this population are quiescent or slowly cycling and therefore less sensitive to chemotherapy. They are therefore likely to be responsible for disease relapse and represent the target for future innovative therapies (Bonnett et al.,1997; Lowenberg et al., 1999; Jordan, 2002). Activation of NF-kB in leukemia patients has been well documented though NF-kB activation is not uniform among AML patients. Forty percent of AML patients evaluated presented with increased NF-kB DNA binding activity. These patients are characterized by increased white cell counts at diagnosis and increased blast percentages in the bone marrow suggesting a link between NF-kB and cell proliferation. In particular, cyclin D1, whose expression is regulated by NF-kB. Alternatively, NF-kB action could be due to the induction of genes coding for AML growth factors such as GM-CSF or granulocyte colony-stimulating factor (G-CSF) (Cilloni et al., 2007).

The majority of LSCs are quiescent and insensitive to traditional chemotherapeutic drugs. This latter feature explains, at least in part, the difficulties in eradicating this cell population by conventional polychemotherapy. Thus, novel therapeutic strategies for AML eradication should also target LSCs (Misaghian et al., 2009). In AML, aberrant activation of several signal transduction pathways strongly enhances the proliferation and survival of both LSCs and CFU-Ls (McCubrey et al., 2008; Steelman et al., 2008). Therefore, these signaling networks are attractive targets for the development of innovative therapeutic strategies in AML (Scholl et al., 2008).

5. Conclusion

Expression of STP proteins is heterogenous and of prognostic value in AML (Kornblau et al., 2009). These signaling pathways in AML may in the future help rationally select targeted therapies in individual patients (Foran, 2010). While current classification schemes have prognostic relevance they generally do not alter therapeutic recommendations. As knowledge of mutated genes in cancersim proves, our ability to treat patients afflicted with certain diseases will increase substantially. The genetic mutation may affect multiple signal transduction pathways. Targeting multiple pathways may be more efficacious as this approach may suppress or eliminate tumor growth at lower concentrations of the drugs than that required to inhibit growth by targeting a single pathway (Steelmanet al., 2004).

The heterogeneity in AML continues to elude the best methods to characterize them. Genome and proteome-wide analysis has further revealed complexity in the makeup of the leukemic cell. The rapid advancement in targeted therapies implied the urgent need for alternative therapy and the readiness of the community to embrace it. Nevertheless so far, combinatorial medicine still holds out as the best option for successful treatment. If targeted therapies remain the way forward it will eventually bank deeply on the ability to identify molecular signatures in the individual leading to the establishment of personalized medicine.

Novel array technologies enabled the analysis of numerous features at the level of DNA for gene copy number variation, mutations, methylation in addition to mRNA transcription and regulatory microRNA. Emerging technologies to assess protein expression and phosphorylation levels within cells e.g. cytokine and chemokine arrays to assess external forces acting on leukemic cells and phosphoproteins in apoptosis, cell-cycle, and signal-transduction pathways, are highly needed. Protein expression and posttranslational modifications, either alone or in concert with other profiling approaches, could provide independent or complementary information not captured by transcriptional profiles. Protein signature groups, with prognostic information distinct from cytogenetics may reveal underlying similarities indistinguishable by cytogenetics (Kornblau et al., 2009).

Quantitative flow cytometry appears well suited for identifying predictive markers in AML patients because it offers obvious advantages over other techniques (western blot, for example), including rapidness, a much lower number of cells required to perform the assay, and the possibility of identifying different subclones in the leukemic population by coimmunostaining with multiple antibodies to surface antigens (Martelli et al., 2010). The mechanisms in leukemogenesis, drug resistance and relapse remain an area of much research. From cell biology to cytogenetics to molecular defects to signaling pathways, all have contributed to a better understanding of the cancer. New knowledge in epigenetics and microRNA remain to be elucidated.

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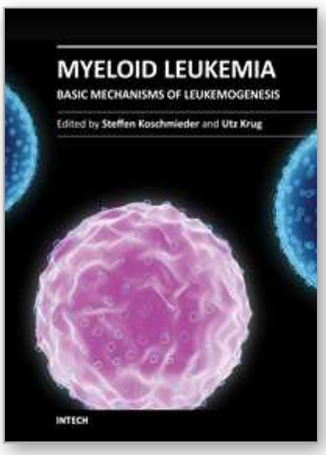

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The current book comprises a series of chapters from experts in the field of myeloid cell biology and myeloid leukemia pathogenesis. It is meant to provide reviews about current knowledge in the area of basic science of acute (AML) and chronic myeloid leukemia (CML) as well as original publications covering specific aspects of these important diseases. Covering the specifics of leukemia biology and pathogenesis by authors from different parts of the World, including America, Europe, Africa, and Asia, this book provides a colorful view on research activities in this field around the globe.

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