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# Src family kinases and the MEK/ERK pathway in the regulation of myeloid differentiation and myeloid leukemogenesis

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## Introduction

The business of defending the body against invading microorganisms relies heavily on the action of monocytes/macrophages and granulocytes (also called neutrophils). These differentiated cell types are derived from myeloid stem and progenitor cells present in the bone marrow. In response to foreign microorganisms, the production of monocytes/macrophages and granulocytes is dramatically increased, allowing the host to fight infection. Once the infection has cleared, the production of these cells is curtailed and their circulating and tissue levels quickly return to normal. In particular, the levels of granulocytes drop quickly once they are no longer needed, due to the inherent short half-lives of these differentiated cells and their rapid death via apoptosis.

The physiological production of mature myeloid lineage cells from bone marrow progenitors has taken on special significance in the field of myeloid leukemias. Myeloid leukemias are characterized by genetic defects which serve to block the process of myeloid differentiation. The consequences of these differentiation blockades are twofold. First, the normal production of monocytes/macrophages and granulocytes is impaired. In cases of acute myeloid leukemias this can lead to high vulnerability to infections. Second, blockade of myeloid differentiation leads to aberrant accumulation of proliferative blast cells in the bone marrow. Eventually these proliferating and malignant blasts begin to crowd out normal marrow progenitor cells, further impairing effective immune responses, and manifesting full-blown leukemia (Sachs, 1980).

Molecular studies have identified a number of genetic defects that contribute to differentiation blockades in myeloid leukemias. Chromosomal translocations that give rise to fusion oncoproteins such as PML/RARα(de The et al., 1990; Kakizuka et al., 1991; Pandolfi et al., 1991), AML1/ETO (Erickson et al., 1992; Yuan et al., 2001) and CBFβ/MYH11 (Kogan et al., 1998), occur with high frequency in acute myeloid leukemia (AML). Often these fusion proteins incorporate portions of important transcription factors, such as RARα, and the resulting oncoprotein exhibits altered transcriptional activity relative to its wild-type counterpart (Melnick and Licht, 1999; Redner, 2002). Other types of AML express mutant forms of cytokine receptors, including FLT3/ITD (Gilliland and Griffin, 2002) or truncated G-CSF receptor (Dong et al., 1995). In addition, mutant forms of PU.1 and C/EBPα(Mueller et al., 2002; Snaddon et al., 2003), transcription factors that are known to be important for myeloid differentiation, are commonly observed in AML.

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While the biological impact of many AML-associated genetic defects have become apparent, namely, blockade of myeloid differentiation, the molecular mechanisms of these blockades remain incompletely understood. Attempts to understand the mechanisms of differentiation blockades in AML have been slowed due to only modest understanding of the molecular mechanisms that are responsible for normal myeloid differentiation. What has been established is that normal differentiation of myeloid progenitors into monocytes/macrophages or granulocytes is governed by the action of hematopoietic cytokines. For example, monocytic differentiation is driven, in part, by M-CSF, while granulocytic differentiation is driven, in part, by the action of G-CSF (Figure 1). These differentiation-inducing cytokines bind to cognate receptors on the cell surface, leading to the activation of a number of intracellular signaling pathways and molecules, including tyrosine and serine/threonine kinases. Eventually, these signaling pathways induce or activate key transcription factors, such as PU. 1 and C/EBPα, that serve to drive the process of myeloid differentiation. Recently, the development of unique animal models and application of highly specific pharmacologic inhibitors has begun to shed light on the importance and role of intracellular kinases in the regulation of myeloid differentiation. This review will focus on the involvement and role of Src family kinases (SFKs) and the MEK/ERK pathway in mediating normal myeloid differentiation. In addition, the consequences of aberrant SFK or MEK/ERK expression or activation towards the development and progression of leukemia will be discussed.

## Structure, function, and activation of Src family kinases

Src family kinases (SFKs) are intracellular tyrosine kinases that are activated upon engagement of growth factor or cytokine receptors, cellular attachment, or binding of antigen to antigen receptors. Nine different SFKs are known to exist, and include Src, Lyn, Fgr, Hck, Lck, Blk, Fyn, Yes, and Yrk (Thomas and Brugge, 1997). While Src, Fyn, and Yes are ubiquitously expressed, the expression of Lyn, Fgr, Hck, Blk, and Lck appears to be largely restricted to hematopoietic cells (Abram and Courtneidge, 2000; Corey and Anderson, 1999). Myeloid lineage cells predominantly express Lyn, Fgr, and Hck. Src was the first member of this family to be discovered and has represented the prototype for molecular and functional studies (Boggon and Eck, 2004; Martin, 1970). Additionally, Src is the SFK that has most frequently been associated with human cancers.

The overall structural organization of SFKs is conserved among the different family members and is reasonably represented by that of Src. Src consists of at least seven distinct functional domains (Figure 2). The N-terminus of Src begins with an SH4 domain, which contains sites for myristoylation and palmitoylation. SFKs are consistently modified by myristolyation, and occasionally by palmitoylation (Koegl et al. 1994;Resh, 1999). These modifications serve to anchor SFKs on the inners surface of the plasma membrane, bringing them into close proximity with activators such as cytokine receptors and substrates such as FAK. Following the SH4 domain is a unique region that differs among the different SFKs. The unique region is followed by an SH3 domain. The SH3 domain binds to proline-rich regions in substrate proteins, and, as described below, participates in intramolecular interactions that are important for autoinhibition of the enzyme (Gonfloni et al., 1997;Moarefi et al., 1997).The SH3 domain is followed by an SH2 domain which, like the SH2 domains in numerous other signaling proteins, recognizes and binds to phophorylated tyrosine residues. The Src SH2 domain also participates in autoinhibition of the enzyme through binding to phosphoTyr$^{527}$ present in the C-terminal region of the protein (Sicheri et al., 1997;Williams et al., 1997;Xu et al., 1997). Between the SH2 domain and the catalytic domain lies a region termed the SH2-kinase linker domain. The catalytic domain (also called the SH1 domain) of Src is a bi-lobed structure, where ATP nestles into the hinge region connecting the two lobes (Boggon and Eck, 2004). The C-terminal lobe of the catalytic domain contains an activation loop harboring Tyr$^{416}$; phosphorylation of this residue is important for activation of the enzyme (Roskoski, 2005;Smart et al., 1981). The

catalytic domain is followed by the C-terminal region containing the phosphoTyr$^{527}$ regulatory residue (Cooper et al., 1986;Roskoski, 2005).

In an unstimulated state, the activity of Src is autoinhibited via intramolecular interactions. Inactive Src lacks phosphorylation at Tyr$^{416}$, but is phosphorylated on Tyr$^{527}$ by c-Src kinase (CSK) or the related enzyme, CSK homologous kinase (CHK) (Davidson et al., 1997; Hamaguchi et al., 1996; Nada et al., 1991). The phosphoTyr$^{527}$ residue is bound via an intramolecular interaction with the SH2 domain of Src (Sicheri et al., 1997; Williams et al., 1997; Xu et al., 1997). Similarly, in the inactive Src enzyme, the SH3 domains binds to a proline-containing sequence in the SH2-kinase linker domain (Gonfloni et al., 1997; Moarefi et al., 1997). Together, these two intramolecular interactions serve to prevent phosphorylation of Tyr$^{416}$ in the enzyme activation loop (Xu et al., 1999). The importance of these intramolecular interactions has been clearly delineated by the determination of the crystal structures of Src and Hck in the autoinhibited state (Schindler et al., 1999; Sicheri et al., 1997; Xu et al., 1997). In addition, viral Src (v-Src) is known to be constitutively active and is missing the corresponding Tyr$^{527}$ regulatory residue (Cooper et al., 1986; Takeya and Hanafusa, 1983). Moreover, mutations in the SH3 or SH2 domains of Src that abrogate the key intramolecular interactions have been shown to result in enzyme activation (Boggon and Eck, 2004).

Activation of Src requires dephosphorylation of Tyr$^{527}$ and phosphorylation of Tyr$^{416}$ in the activation loop (Roskoski, 2005). Both of these events are dependent on disruption of the interaction between the SH2 domain and phosphoTyr$^{527}$, as well as the interaction between the SH3 domain and the SH2-kinase linker domain. Disruption of the SH2/phosphoTyr$^{527}$ interaction can occur when the SH2 domain binds instead to phosphotyrosine residues on a different protein. It is interesting that the preferred binding substrate of the Src SH2 domain is phosphoTyr-Glu-Glu-Ile (Songyang et al., 1993), though the site surrounding Tyr$^{527}$ in Src contains a glycine residue instead of an isoleucine residue. This has suggested that proteins bearing the preferred sequence may be able to easily disrupt the Src SH2/phosphoTyr$^{527}$ intramolecular interaction. Similarly, the SH2-kinase linker region lacks the Pro-Xxx-Xxx-Pro sequence preferred by SH3 domains, indicating that proteins bearing this sequence may be likely to displace the intramolecular inhibitory interaction between the Src SH3 domain and the SH2- kinase linker region.

## Src family kinases in myeloid malignancies

Members of the SFK family have been implicated in both chronic and acute myeloid leukemias. In the case of CML, SFKs likely mediate survival and proliferative signals emanating from the BCR/ABL tyrosine kinase. Hck and Lyn physically associate with BCR/ABL, leading to their activation (Danhauser-Riedl et al., 1996; Klejman et al., 2002). Activation of Hck by BCR/ABL has been shown to lead to activation of STAT5, an important downstream mediator of BCR/ABL (Klejman et al., 2002). Experiments utilizing pharmacologic or dominant-negative inhibitors of SFKs have demonstrated important roles for these enzymes in facilitating BCR/ABL-dependent proliferation and cytokine independence (Klejman et al., 2002; Lionberger et al., 2000; Wilson et al., 2002). Moreover, overexpression or hyperactivation of SFKs has been observed in cases of CML that are resistant to the BCR/ABL inhibitor STI-571 (Kantarjian et al., 2007). These findings have suggested that targeting of the SFKs may have therapeutic benefit in the treatment of STI-571-resistant CML. Interestingly, structural studies have shown that Src and ABL bear striking similarities (Nagar et al., 2002; Nagar et al., 2003). It is not surprising then, that several small molecule inhibitors of SFKs also potently inhibit ABL and BCR/ABL (Kantarjian et al., 2007). In particular, dasatinib (BMS-354825; (Lombardo et al., 2004)) inhibits SFKs and BCR/ABL with $IC_{50}$ values in the low nanomolar range. Notably, dasatinib inhibits BCR/ABL-induced cell growth in STI-571 resistant cells, and exhibits anti-

tumor effects in murine models of STI-571-resistant disease (O'Hare et al., 2005; Shah et al., 2004). Treatment with dasatinib has demonstrated significant clinical benefit in several human trials of STI-571-resistant CML (Cortes et al., 2007; Guilhot et al., 2007; Hochhaus et al., 2007; Talpaz et al., 2006)

Overexpression and/or hyperactivation of SFKs have also been observed in acute leukemias. Lyn is overexpressed and hyperactivated in a majority of primary AML blasts, and pharmacologic inhibition of SFKs inhibits the growth of human leukemia cell lines and patient blasts (Roginskaya et al., 1999; Sakhinia et al., 2005). Recent evidence indicates that SFKs, particularly Lyn, may mediate the effects of FLT3/ITD in AML (Okamoto et al., 2007; Robinson et al., 2005). The FLT3/ITD mutation is found in approximately 30 percent of AML patients (Small, 2006). While stimulation of wild-type FLT3 receptor results in binding and activation of Lyn, the FLT3/ITD constitutively-active mutant binds Lyn with even greater affinity (Okamoto et al., 2007). Moreover, pharmacologic inhibition of SFKs, or siRNA-mediated downregulation of Lyn, suppresses cytokine-independent growth of FLT3/ITD-expressing cells (Okamoto et al., 2007; Robinson et al., 2005). Thus, while SFKs have been shown to play an important role in mediating the effects of BCR/ABL in CML, they also appear to play a critical role in mediating the effects of FLT3/ITD expression in AML.

## Src family kinases in myeloid differentiation and myelopoiesis

Evidence suggesting a role for SFKs in myeloid differentiation and myelopoiesis *in vivo* has come from several different angles. Early correlative studies noted an increase in the expression and activities of SFKs during myeloid differentiation. Specifically, Src was noted to be induced and activated during monocytic differentiation of HL60 and U937 cells (Barnekow and Gessler, 1986; Gee et al., 1986; Meier et al., 1992; Yu and Glazer, 1987). Lyn and Fgr were found to be induced and activated in HL60 cells undergoing either monocytic or granulocytic differentiation (Katagiri et al., 1991; Katagiri et al., 1996; Miyazaki et al., 1993; Notario et al., 1989). Induced expression of Fgr and Hck was also observed during myeloid differentiation of leukemic blasts (Willman et al., 1991), while Fgr was found to be upregulated during myeloid differentiation of normal hematopoietic progenitors (Link and Zutter, 1995; Willman et al., 1987). The induction of SFK activities during myeloid differentiation is consistent with their activation by differentiation-inducing cytokines. G-CSF promotes granulocytic differentiation, and ligand-activated G-CSF receptors bind and activate both Lyn and Hck (Corey et al., 1994; Ward et al., 1998).

The precise role that SFKs play during myeloid differentiation remains unclear. However, evidence has accumulated that SFKs play an important role in promoting cell survival during the differentiation process. Antisense-mediated downregulation of Fgr or Lyn stimulates cell death during retinoic acid-induced differentiation of HL60 cells (Katagiri et al., 1996). Lyn also appears to mediate G-CSF-induced activation of PI3-K and Akt (Zhu et al., 2006), cellular kinases that inhibit apoptotic cell death. As both monocytic and granulocytic differentiation require days to complete, mechanisms, possibly involving SFKs, clearly must be in place to sustain the survival of the nonproliferating, differentiating cells.

Insights regarding the roles of SFKs during myelopoiesis *in vivo* have come from gene knockout mice. Src knockout mice exhibit normal myelopoiesis, but are deficient in bone remodeling and develop osteopetrosis (Soriano et al., 1991). Mice that are deficient in Lyn, Fgr, and Hck display normal resting granulopoiesis (Fitzer-Attas et al., 2000; Meng and Lowell, 1997). However, *lyn*$^{-/-}$ mice have increased numbers of granulocytic progenitors, granulocytic-monocytic progenitors, and mutipotential progenitors (Harder et al., 2001; Mermel et al., 2006). In addition, Lyn-deficient mice develop monocyte/macrophage tumors (Harder et al., 2001). Collectively, these findings suggest an important role for Lyn in

negatively regulating progenitor pool expansion. A hyper-responsiveness to G-CSF is seen in myeloid progenitors from *hck*$^{-/-}$ mice, indicating a role for Hck in suppressing G-CSF-induced proliferation of granulocyte progenitors (Mermel et al., 2006). *hck*$^{-/-}$*fgr*$^{-/-}$ double knockout mice manifest impaired adhesion-dependent neutrophil functions, suggesting a role for SFKs in the cellular function of these highly differentiated cells (Lowell et al., 1996; Mocsai et al., 1999). Triple knockout mice that are deficient in Hck, Lyn, and Fgr exhibit elevated levels of bone marrow progenitors and enhanced neutrophil responsiveness to G-CSF (Mermel et al., 2006). Although many of the abnormalities observed in SFK knockout mice are subtle in nature, it should be appreciated that redundancy among the different SFKs may serve to mask additional roles in myeloid differentiation and myelopoiesis. None-the-less, clear evidence exists that certain SFKs act to inhibit progenitor pool expansion, and are important for the specialized function of fully differentiated myeloid cells.

## The MEK/ERK signaling pathway

The induction of myeloid differentiation by hematopoietic cytokines such as G-CSF (granulocytic differentiation) or M-CSF (monocytic differentiation) leads to the activation of corresponding cell surface receptors for these cytokines. Activation of the G-CSF or M-CSF receptors (G-CSFR or M-CSFR) results in the activation of a number of intracellular signaling pathways and molecules, including kinases and nonkinases, and ultimately results in the activation of specific transcription factors. It is currently understood that the activation of these transcription factors, and the subsequent modulation of target gene expression, drives the differentiation process.

While the M-CSFR is a ligand activated tyrosine kinase (Sherr and Rettenmier, 1986; Sherr et al., 1988), the G-CSFR does not contain intrinsic kinase activity. Rather, ligand binding induces G-CSFR dimerization, followed by activation of receptor-associated kinases, including members of the JAK kinase family (Ihle and Kerr, 1995). In either case, activation of the G-CSFR or the M-CSFR has been shown to result in the rapid phosphorylation and activation STAT3, STAT5, PI-3K, and PLC-γ(de Koning et al., 1998; Hunter and Avalos, 1998; Liu et al., 1998; Tian et al., 1996). Ras also becomes activated, and this leads to the recruitment and activation of the Raf kinase family, comprised of Raf-1, A-Raf, and B-Raf (de Koning et al., 1998; McCubrey et al., 2006). Raf enzymes phosphorylate a number of proteins, but key among these substrates are the kinases MEK-1 and MEK-2. All three Raf enzymes phosphorylate MEK-1 and MEK-2 on two closely spaced serine residues ($Ser^{217}$ and $Ser^{221}$ in human MEK-1), which results in activation of the MEK-1/-2 enzymes (Alessi et al., 1994; Dent et al., 1992; Kyriakis et al., 1992; Papin et al., 1995). MEK-1 and -2 are dual specificity kinases, and are somewhat remarkable in that their only known targets are the kinases ERK-1 and ERK-2 (also known at MAP kinase-1 and MAP kinase-2). MEK-1/-2 phosphorylate ERK-1 and -2 on threonine and tyrosine residues ($Thr^{202}$ and $Tyr^{204}$ in ERK-1; $Thr^{185}$ and $Tyr^{187}$ in ERK-2), leading to the activation of the ERK-1/-2 enzymes (Chang and Karin, 2001; Crews et al., 1992; Payne et al., 1991). Activated ERK-1 and -2 have been shown to phosphorylate a number of different substrates, including $p90^{RSK}$ and the transcription factors Elk-1, AP-1, and c-Myc (Deng et al., 2000; Hill et al., 1993; Karin, 1995; Lazar et al., 1995; Seth et al., 1992).

The ability of the MEK/ERK signaling pathway to promote cellular proliferation has been well-established (Cowley et al., 1994; Hoshino et al., 1999; Mansour et al., 1994; McCubrey et al., 2007a; Pages et al., 1993). The impact of MEK/ERK activation on proliferation appears to stem, at least in part, from ERK-mediated phosphorylation of AP-1, a transcription factor that drives expression of the *cyclin D1* gene (Treinies et al., 1999). ERK-1 and -2 also phosphorylate carbamoyl phosphate synthetase II, a key enzyme in pyrimidine biosynthesis (Graves et al., 2000).

Activation of the MEK/ERK pathway also supports cellular survival, by inhibiting apoptosis (Ballif and Blenis, 2001; Bonni et al., 1999; Deng et al., 2000; Hoshino et al., 1999). Indeed, the anti-apoptotic action of BCR/ABL has been shown to be dependent on downstream activation of the MEK/ERK pathway (Jin et al., 2006; Kang et al., 2000; Nawata et al., 2003; Yu et al., 2002b). Moreover, forced expression of constitutively-active MEK-1 enzyme potently inhibits cytokine withdrawal-induced apoptosis (Blalock et al., 2000). These effects on cell survival may be due to ERK-1/-2- mediated phosphorylation of the apoptosis regulatory proteins Bcl-2 and Bim (Deng et al., 2000; Ley et al., 2003). In addition, ERK-1/-2 also influence the cellular activities of caspase-9, Bad, Mcl-1, and survivin (McCubrey et al., 2007b).

In addition to effects on cell survival and proliferation, recent evidence indicates that MEK/ERK activation can promote cellular differentiation (Miranda and Johnson, 2007). These findings, and the role that cellular context plays in the ability of the MEK/ERK pathway to stimulate differentiation, are discussed below.

## Activation and importance of the MEK/ERK pathway in myeloid malignancies

The importance of the MEK/ERK pathway in promoting proliferation and survival is underscored by the prevalence of MEK/ERK hyperactivation in myeloid malignancies. Sustained hyperactivation of the MEK/ERK pathway has been reported in a majority of acute and chronic myeloid leukemias (Kim et al., 1999; Kornblau et al., 2006; Milella et al., 2001; Platanias, 2003; Ricciardi et al., 2005; Staber et al., 2004; Towatari et al., 1997). Pharmacologic inhibition of the MEK/ERK pathway inhibits proliferation and induces apoptosis in primary AML blasts (Lunghi et al., 2003). Moreover, in AML cells undergoing retinoid-induced differentiation, inhibition of MEK/ERK signaling converts the differentiation response to an apoptotic response (Milella et al., 2007). In cell line models, forced expression of a constitutively-active MEK-1 enzyme protects against cytokine withdrawal- and chemotherapy-induced apoptosis (Blalock et al., 2000; McCubrey et al., 2007b).

The MEK/ERK pathway likely plays an important role in mediating the effects of BCR/ABL in CML and FLT3/ITD in AML. The BCR/ABL tyrosine kinase activates the MEK/ERK pathway, leading to induction of proliferation and suppression of apoptosis (Cortez et al., 1997; Jin et al., 2006). The anti-apoptotic effects of BCR/ABL have been shown to mediated, in part, via MEK-1-mediated activation of NF-*k*B (Nawata et al., 2003). The FLT3/ITD mutant cytokine receptor commonly expressed in AML also activates the MEK/ERK signaling pathway (Stirewalt and Radich, 2003). In this case, activated ERKs may phosphorylate C/EBPαon serine 21, inhibiting the activity of this key transcription factor and, thereby, contributing to the differentiation blockade in FLT3/ITD-expressing AML (Radomska et al., 2006; Ross et al., 2004).

The central importance of the MEK/ERK pathway in sustaining cellular proliferation and survival in a large percentage of myeloid leukemias suggests that molecular targeting of this pathway may enhance the efficacy of anti-leukemic agents. Indeed, pharmacologic inhibition of the MEK/ERK pathway synergizes or enhances the anti-leukemic activities of a variety of agents. Specifically, MEK/ERK inhibition in AML cells has manifested synergistic (or enhancement) induction of apoptosis when combined with paclitaxel (Yu et al., 2001), TNFα (Nakada et al., 2001), the Bcl-2 inhibitor HA14-1 (Milella et al., 2002), UCN-01 (Yu et al., 2002a), radiation (Shonai et al., 2002), STI-571 (Yu et al., 2002b), lovastatin (Wu et al., 2004), and histone deacetylase inhibitors (Yu et al., 2005). The remarkable successes of these *in vitro* studies has heightened enthusiasm for clinical trials evaluating MEK/ERK inhibitors in combination with standard anti-leukemia agents

## Role of the MEK/ERK pathway in myeloid differentiation

As described above, an extensive literature supports an important role for the MEK/ERK pathway in promoting the proliferation and survival of myeloid leukemia cells. The sheer volume and clarity of this data has somewhat overshadowed recent findings linking MEK/ERK activation to induction of myeloid differentiation. Effects on differentiation have largely gone unnoticed since most studies investigating this pathway in myeloid lineage cells have been performed in differentiation-incompetent leukemic cells (Miranda and Johnson, 2007). However, studies using differentiation-competent myeloid cell lines, as well as normal myeloid progenitors, have now clearly demonstrated an important role for MEK/ERK activation in myeloid differentiation (Miranda and Johnson, 2007).

Experiments employing pharmacologic inhibitors of the MEK enzymes have shown that the MEK/ERK pathway is important for phorbol 12-myristate 13-acetate (PMA)-induced differentiation of K562 (Herrera et al., 1998; Shelly et al., 1998), TF1a (Hu et al., 2000), and U937 cells (Miranda et al., 2002). Similarly, in HL60 cells, activation of the MEK/ERK pathway is required for PMA- or 1,25-dihydroxyvitamin D3- induced monocytic differentiation and ATRA-induced granulocytic differentiation (Miranda et al., 2003; Miranda et al., 2002; Wang and Studzinski, 2001; Yen et al., 1998). Induction of monocytic or granulocytic differentiation by chemical stimuli results in rapid and prolonged activation of the MEK/ERK pathway, and prolonged, versus transient, activation appears necessary for fostering differentiation (Hu et al., 2000; Miranda et al., 2002). The induction of monocytic differentiation by IL-6 or M-CSF, and the induction of granulocytic differentiation by G-CSF, also is accompanied by rapid and sustained activation of the MEK/ERK pathway (Gobert Gosse et al., 2005; Miranda et al., 2005). Moreover, the requirement for sustained activation of this pathway during cytokine-induced myeloid differentiation has been demonstrated using both cell line models and bone marrow-derived normal myeloid progenitors (Miranda et al., 2005). Inhibition of cytokine-induced differentiation following pharmacologic inhibition of the MEK/ERK pathway is associated with inhibition of the STAT3 and PU.1 transcription factors, which may account for the blockade in differentiation (Miranda et al., 2005). Additional studies have shown that the MEK/ERK pathway may regulate the phosphorylation and/or localization of C/EBPα(Radomska et al., 2006; Ross et al., 2004), C/EBPβ (Marcinkowska et al., 2006), and AML1 (Tanaka et al., 1996), transcription factors that are known to be important for myeloid differentiation. Lastly, recent studies indicate significant crosstalk between cytokine-induced differentiation pathways and ATRA-induced differentiation pathways. The abilities of G-CSF and GM-CSF to potentiate ATRA-induced differentiation of AML cell lines and primary AML cells is strictly dependent on the MEK/ERK pathway (Glasow et al., 2005).

## Summary

The production of mature monocytes/macrophages and granulocytes via myeloid differentiation is a central component of the host defense mechanism against invading microorganisms. However, in myeloid leukemias genetic changes lead to blockade of myeloid differentiation. When this happens, immune responses can become severely impaired and the accumulation of proliferative blasts causes bone marrow crowding and onset of symptomatic leukemia. There is considerable hope that molecular targeting of specific signaling pathways and proteins will prove to be a viable strategy for restoring differentiation potential in myeloid leukemias. Indeed, in acute promyelocytic leukemia, treatment with ATRA can overcome the differentiation blockade and is an effective curative approach. To devise strategies and reagents that can be used to induce differentiation in other myeloid leukemias, it is important to gain an understanding of the molecular pathways that drive the normal differentiation process.

Emerging evidence implicates both Src family kinases and the MEK/ERK pathway in regulating myeloid differentiation.

It is interesting that Src family kinases appear to be negative regulators of myelopoiesis, while the MEK/ERK pathway is an important positive regulator of both monocytic and granulocytic differentiation. This suggests that pharmacologic inhibitors of SFKs may be of value in restoring or enhancing myeloid differentiation. In this regard, the SFK inhibitor dasatinib has recently been approved by the FDA for use in imatinib-resistant CML. Evaluation of dasatinib, alone or in combination with differentiation inducers, in the treatment of differentiation-defective AML seems warranted.

The important role that the MEK/ERK pathway plays in promoting myeloid differentiation appears to conflict with observations that the MEK/ERK pathway is hyperactivated or overexpressed in a majority of primary AMLs. Moreover, pharmacologic inhibition of the MEK/ERK pathway in AML results in the induction of apoptosis, indicating that MEK/ERK activation is important for the survival of AML cells. Collectively, these data suggest that the MEK/ERK pathway may play more than one role in myeloid lineage cells, depending on the cellular context. In normal myeloid cells, activation of the MEK/ERK pathway is important for promoting differentiation. However, when differentiation becomes blocked, as is the case in most AMLs, MEK/ERK activation can no longer drive differentiation, and instead begins to support cellular survival or proliferation. Thus, therapeutic strategies aimed at provoking myeloid differentiation in AML by stimulating the MEK/ERK pathway are unlikely to be successful unless the differentiation blockade is simultaneously relieved.

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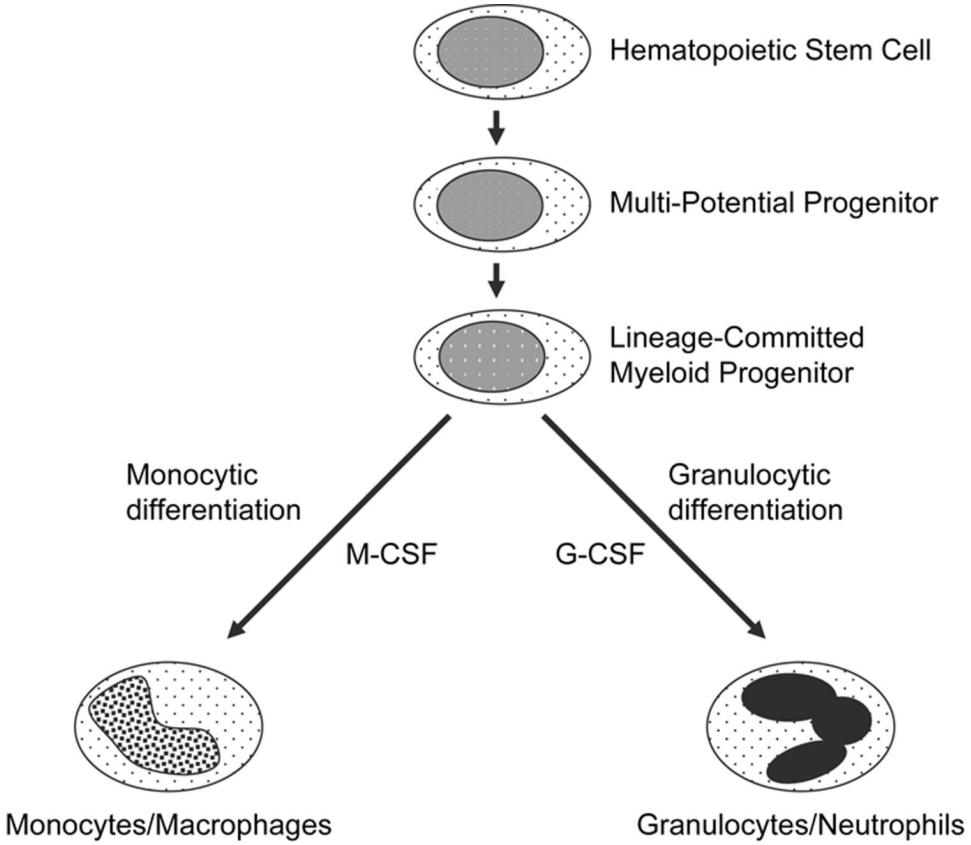


**Fig. 1.**
Schematic of myeloid differentiation. G-CSF promotes granulocytic differentiation, while M-CSF promotes monocytic differentiation.

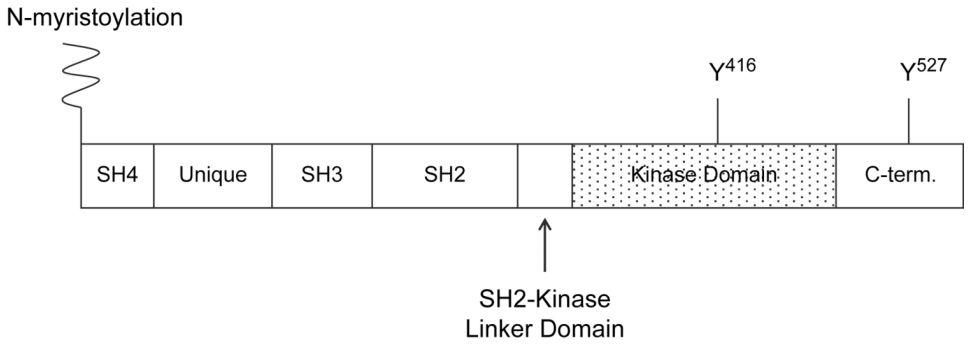


**Fig. 2.**
Schematic of Src structure.