PERGAMON

IJBCB

The International Journal of Biochemistry & Cell Biology 31 (1999) 1053–1074

www.elsevier.com/locate/ijbcb

Review

Early signaling pathways activated by c-Kit in hematopoietic cells ☆

Diana Linnekin*

Basic Research Laboratory, Division of Basic Sciences, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD 21702-1201, USA

Received 20 January 1999; accepted 15 February 1999

Abstract

c-Kit is a receptor tyrosine kinase that binds stem cell factor (SCF). Structurally, c-Kit contains five immunoglobulin-like domains extracellularly and a catalytic domain divided into two regions by a 77 amino acid insert intracellularly. Studies in white spotting and steel mice have shown that functional SCF and c-Kit are critical in the survival and development of stem cells involved in hematopoiesis, pigmentation and reproduction. Mutations in c-Kit are associated with a variety of human diseases. Interaction of SCF with c-Kit rapidly induces receptor dimerization and increases in autophosphorylation activity. Downstream of c-Kit, multiple signal transduction components are activated, including phosphatidylinositol-3-kinase, Src family members, the JAK/STAT pathway and the Ras–Raf–MAP kinase cascade. Structure-function studies have begun to address the role of these signaling components in SCF-mediated responses. This review will focus on the biochemical mechanism of action of SCF in hematopoietic cells. Published by Elsevier Science Ltd.

Keywords: Stem cell factor; Signal transduction; Hematopoiesis; Growth factors; Cytokines

1. Introduction

Stem cell factor (SCF) is a growth factor critical in hematopoiesis as well as in the generation of melanocytes and germ cells, reviewed in references [1–8]. SCF also plays a role in development of the interstitial cells of Cajal in the intestine and in learning functions in the hippocampal region of the brain [9–11]. Occurring physiologically in either membrane-bound or soluble form, SCF promotes viability as well as proliferation and differentiation of hematopoietic progenitor cells. In addition, SCF is potently synergistic in combination with other growth factors such as Epo, IL-3 and GM–CSF. There are several excellent reviews of SCF biology in relation to hema-

☆ The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products or organizations imply endorsement by the US Government.

* Tel.: +1-301-846-5188; fax: +1-301-846-6641.

E-mail address: dlinnekin@mail.ncifcrf.gov (D. Linnekin)

1357-2725/99/$ - see front matter Published by Elsevier Science Ltd.
PII: S1357-2725(99)00078-3

topoiesis [2,4–6]. A comprehensive overview of SCF in relation to its various target tissues can be found in the review by Galli et al. [1]. The purpose of this review is to examine the early signaling pathways activated in response to SCF in hematopoietic cells.

SCF was cloned and characterized in 1990 [12–15]. The receptor for SCF is the product of the *Kit* proto-oncogene [16,17]. The *c-Kit* gene maps to the White spotting (*W*) locus in mice, while SCF is encoded by the Steel locus (*Sl*) [16–19]. The first descriptions of mutant *W* and *Sl* alleles were in 1927 and 1956, respectively [20]. Since that time, multiple alleles of both genes have been discovered and characterized [7,8,20]. While the absence of either SCF or c-Kit is lethal in utero, reductions in functional receptor, or ligand, results in aberrations in hematopoiesis, pigmentation and reproduction.

The *Kit* gene product has been associated with several forms of cancer. The *v-Kit* oncogene was originally identified as a component of Hardy–Zuckerman strain of feline sarcoma virus [21]. In humans, a series of gain-of-function mutations in the c-Kit juxtamembrane region have been found in gastrointestinal stromal cell tumors [22]. c-Kit is also aberrantly expressed in approximately 70% of all small cell carcinomas of the lung (SCCL), as well as in breast, cervical and ovarian tumors [23–27]. Coexpression of c-Kit and SCF in SCCL cells generates an autocrine loop that may play a role in the etiology of these cancers. A constitutively active form of human c-Kit (D816 V) has been found with high frequency in

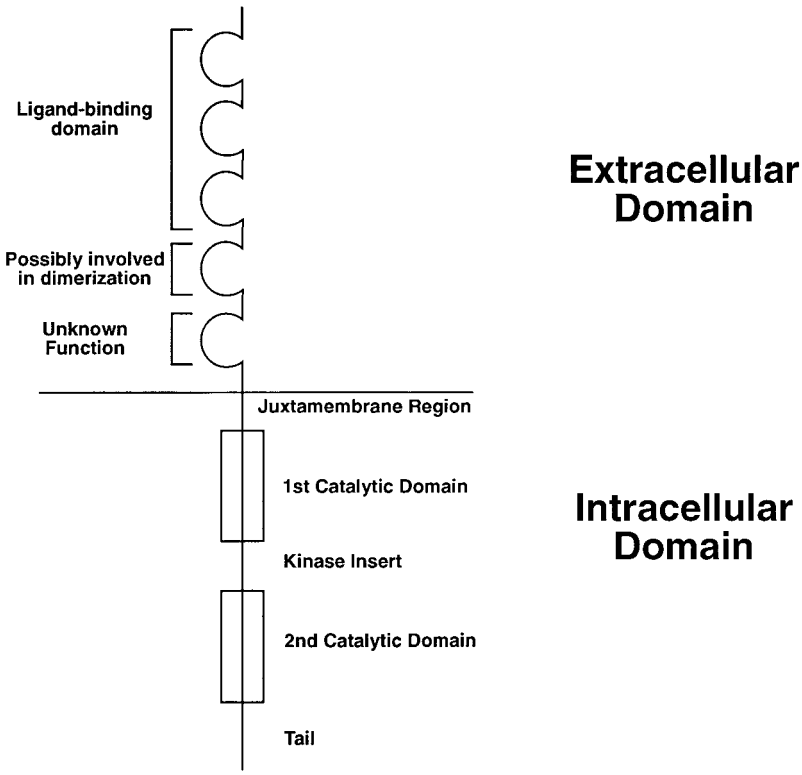

Fig. 1. Structural domains of c-Kit. c-Kit is divided into an intracellular and extracellular region. The intracellular region consists of five immunoglobulin-like domains. The first three are involved in binding SCF. The fourth may play a role in receptor dimerization and the function of the fifth immunoglobulin-like region is unknown. Intracellularly, the catalytic domain is divided into two by a kinase insert. The 30 amino acids distal to the transmembrane domain is the juxtamembrane region and the last 50 amino acids comprise the kinase tail.

patients with mastocytosis and associated hematological disorders [28]. Hematological disorders in these patients range from myelodysplasia to myeloproliferative disease. In addition, these individuals develop leukemia at high frequencies.

The important role of SCF in development of stem cells involved in hematopoiesis, pigmentation, intestinal function and reproduction, as well as its association with some forms of human disease, has lead to interest in understanding its mechanism of action. This review will focus on signaling mechanisms of SCF, specifically in hematopoietic cells.

2. SCF induces receptor dimerization

c-Kit is a receptor tyrosine kinase (RTK) closely related to the receptors for platelet-derived growth factor and colony-stimulating factor-1. Structurally, the c-Kit extracellular domain can be divided into five immunoglobulin-like regions (Fig. 1). The studies of Yarden and coworkers have shown that the first three immunoglobulin-like regions bind SCF, inducing homodimerization of the receptor [29–31]. Two models for ligand-induced dimerization of c-Kit have been suggested [32–39]. The first proposes that binding of dimerized SCF initiates receptor dimerization [33,34,37]. Consistent with this, the dimeric form of SCF is more biologically active than the monomeric form [32,33]. The second model for dimerization of c-Kit proposes that SCF binding of c-Kit monomer induces conformational changes resulting in interaction of the fourth immunoglobulin-like domain between two molecules of c-Kit [39]. While a soluble form of c-Kit containing only the first three immunoglobulin-like regions is capable of dimerizing, the fourth immunoglobulin-like domain is critical for dimerization of full length, membrane-associated c-Kit.

The majority of studies examining c-Kit dimerization have been performed with recombinant proteins in vitro or by affinity crosslinking of cell lines engineered to express c-Kit. Recently, Broudy et al. [40] used fluorescent resonance energy transfer (FRET) to detect SCF-induced c-Kit dimerization in both hematopoietic cell lines as well as the progeny of normal BFUe progenitor cells.

3. c-Kit autophosphorylation

The cDNA sequence of the c-Kit proto-oncogene predicted that the protein was a RTK [41,42]. Indeed, in vitro kinase assays of c-Kit immunoprecipitates found intrinsic tyrosine kinase activity [41,43]. The organization of the cytoplasmic domain of c-Kit is similar to that of the receptors for colony-stimulating factor-1 and platelet-derived growth factor. The catalytic domain is divided by a 77 amino acid insert (Fig. 1). The first catalytic domain contains the ATP binding region while the second catalytic domain has a number of possible autophosphorylation sites. SCF-induced dimerization of c-Kit is rapidly followed by increases in autophosphorylation activity [34–38]. While many of the early studies examining SCF-induced increases in c-Kit autophosphorylation were performed in fibroblast or endothelial cells transfected with c-Kit, identical results have been obtained in hematopoietic cell lines of multiple lineages [44–46].

SCF exists in both soluble and transmembrane forms in vivo. Though the soluble form has biological activity, mice expressing only soluble SCF (Steel-dickie, Sl^{d}) have abnormalities in hematopoiesis, pigmentation and reproduction [20]. Interestingly, these two forms of SCF have different effects on c-Kit kinase activity [47,48]. Increases in c-Kit autophosphorylation were maintained over 2 h after stimulation with membrane-bound SCF, while autophosphorylation in response to soluble SCF returned to basal levels in half that time. This may be due to the slower rate of c-Kit downregulation in response to membrane bound SCF [47]. Similar to soluble SCF, membrane bound SCF also dimerizes [48,49]. Although truncation of the cytoplasmic region of SCF does not impair ligand dimerization, the alteration of amino acids 238–273 in the cytoplasmic tail of SCF does reduce dimerization [49]. This mutation has been found in the Sl^{17H} allele and is associated with subtle defects in

hematopoiesis. Thus, dimerization of membrane bound SCF likely plays a role in maifntaining normal hematopoiesis in vivo. The signaling mechanisms mediating these events remain unexplored.

Studies of *c-Kit* gene products resulting from different alleles of the White spotting (*W*) locus have demonstrated the importance of c-Kit kinase activity in SCF signaling. The severity of the phenotype of each of these mutants is proportional to the degree of impairment in kinase activity. One mutant with a particularly severe phenotype and concomitant reduction in kinase activity is W^{42}. W^{42} results from substitution of aspartic acid 790 with asparagine of murine c-Kit and has a dramatic reduction in intrinsic kinase activity [50]. The molecular basis of the known *W* mutants and the corresponding phenotypes have previously been summarized [7,8,51,52].

The relationship of c-Kit kinase activity and cellular proliferation can further be illustrated by the factor-independent proliferation, as well as transforming abilities, of several gain-of-function mutations. The best characterized of these is the substitution of valine for aspartic acid 816 of human c-Kit (D816 V in human c-Kit and D814 V in murine c-Kit). This mutation has been identified in factor-independent mastocytoma lines derived from humans (HMC1), mice (P-815) and rats (RBL-2H3) [53,54]. Expression of D816 V c-Kit in factor-dependent cell lines results in factor-independent cell lines that produce tumors in mice [55–58]. This mutation is also found in most patients with mastocytosis with associated hematological disorders [28]. Studies of c-Kit activity in cells expressing the D816 V c-Kit mutant have found constitutive phosphorylation on tyrosine residues and increases in in vitro catalytic activity compared to wild type c-Kit [53–56,59]. Peptide mapping studies have suggested differences both in autophosphorylation sites and substrate specificity between wild type c-Kit and the D816 V c-Kit mutant [59]. In contrast to a number of other gain-of-function c-Kit mutants, activation of D816 V c-Kit occurs in the absence of receptor dimerization [55,60].

The c-Kit juxtamembrane region (amino acids 544–577) is the site of multiple mutations that constitutively activate c-Kit. Substitution of glycine for valine 559 of murine c-Kit results in constitutive dimerization and activation of c-Kit as well as increases in its oncogenic potential [55,57,60,61]. Deletion of seven amino acids in the juxtamembrane region also generates a constitutively active form of c-Kit that spontaneously dimerizes [61]. Patients with gastrointestinal stromal cell tumors have a series of mutations in the c-Kit juxtamembrane region that also leads to constitutive activation [22].

Lastly, the *v-Kit* oncogene has constitutive kinase activity and is transforming [21]. Structurally, it lacks the extracellular region and the 50 amino acid carboxyl terminus of c-Kit [42]. In addition, tyrosine 569 and valine 570 are deleted, glycine is substituted for aspartic acid 761 and a five amino acid insertion is found in the carboxyl terminus [42,62]. As expected, v-Kit was phosphorylated on tyrosine residues in vitro [63]. In contrast, in vivo, v-Kit was phosphorylated primarily on serine and threonine residues [63].

4. Phosphatidylinositol-3-kinase and c-Kit signaling

SCF activates multiple signaling components, however, the best characterized of these with regards to structure-function relationships is phosphatidylinositol-3-kinase (PI3 K). PI3 K is a heterodimer composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The 85 kDa subunit (p85) contains several motifs implicated in protein–protein interactions. These motifs include two SH2 domains, an SH3 domain and a proline-rich domain. Increases in autophosphorylation activity of RTKs recruits p85 through the SH2 domain and localizes the 110 kDa catalytic subunit with potential substrates including phosphatidylinositol, phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate. Recently, phosphatidylinositol-3,4-bisphosphate, a product of PI3 K, was found

to play a role in activation of AKT, a serine–threonine kinase involved in cellular survival. Reviews of PI3 K can be found in references [64–66].

SCF induces the association of PI3 K with c-Kit as well as increases in tyrosine phosphorylation of the 85 kDa subunit. While initial studies used NIH3T3 fibroblasts transfected with a chimeric molecule containing the EGF receptor extracellular region and the c-Kit intracellular region, identical results were obtained with full length c-Kit transfected in fibroblastic cell lines as well as in hematopoietic cells [46,67,68]. Mutants lacking kinase activity do not activate PI3 K in response to SCF, while cells expressing a constitutively active c-Kit mutant have increased levels of PI3 K activity [46]. Thus, activation of PI3 K by c-Kit correlates directly with c-Kit catalytic activity.

Two proteins that associate with PI3 K after stimulation with SCF are Crkl and c-Cbl. [69,70]. Crkl is an adaptor protein that is phosphorylated on tyrosine residues in response to SCF [69,70]. In Mo7e cells, SCF induces the association of PI3 K and Crkl through the Crkl SH3 domain [70]. Stimulation with SCF also induces tyrosine phosphorylation of c-Cbl and association of c-Cbl with PI3 K [70,71]. It is likely that these proteins are involved in coupling and/or modulating SCF-induced responses mediated by PI3 K.

Studies from a number of laboratories found that PI3 K bound the kinase insert domain of c-Kit [46,72]. Tyrosine 719 of murine c-Kit (Y721 of human c-Kit) associates with the 85 kDa subunit of PI3 K and mutation to phenylalanine eliminates both the capacity of PI3 K to associate with c-Kit as well as the SCF-induced increases in PI3 K activity [73]. In contrast, the autophosphorylation activity of Y719F c-Kit is not impaired. Studies with Y719F c-Kit suggest that PI3 K has multiple roles in SCF-mediated responses and that these roles vary with cell lineage. Expression of Y719F c-Kit in murine mast cells derived from c-Kit-deficient W^{sh} mice, resulted in impaired adhesion, membrane ruffling and actin assembly in response to SCF [74,75]. Partial inhibition of SCF-mediated proliferation and survival were also observed [75]. Expression of Y719F c-Kit in the murine myeloid cell line DA-1 suggests that PI3 K also plays a role in cellular trafficking of c-Kit. Although mutation of tyrosine 719 did not alter SCF-induced internalization of c-Kit, the receptor remained localized near the plasma membrane and movement though endocytic pathways was impaired [76,77]. Internalization of wild-type c-Kit was not dependent on calcium, while ligand-induced internalization of the Y719F mutant was [76]. PI3 K may also play a role in differentiation mediated by c-Kit in the myeloid progenitor cell line FDC-P1 [78]. Lastly, expression of the Y721F human c-Kit mutant in fibroblastic cell lines reduced SCF-induced activation of AKT and partially impaired the anti-apoptotic capacity of SCF [79]. Thus, similar to its role in signaling pathways of other RTKs, PI3 K is clearly involved in multiple biological responses mediated by SCF.

5. The JAK/STAT pathway and c-Kit signaling

Members of the Janus family of protein tyrosine kinases (JAKs) are activated by ligands interacting with a variety of receptors lacking intrinsic kinase activity. Among these are hematopoietic growth factors that bind receptors in the cytokine receptor superfamily. These include the erythropoietin (Epo) receptor, the granulocyte–macrophage colony-stimulating factor (GM–CSF) receptor, the interleukin 3 (IL-3) receptor as well as numerous others. Reviews relating to activation of Janus kinases by the cytokine receptor superfamily can be found in references [80,81]. Recently, animals deficient for JAK1, JAK2 and JAK3 have been described, and the phenotypes of these animals illustrate the important role of this kinase family in the development of hematopoietic lineages [82–85]. Less clear is the relationship of Janus family kinases in responses mediated by RTKs, in particular c-Kit. Studies from two groups have shown that JAK2 associated with c-Kit and that SCF induced activation of JAK2 [86,87]. Of note was the rapid and transient increase in JAK2 phos-

phorylation observed by Weiler et al. [86]. Pretreatment of cells with the protein tyrosine phosphatase inhibitor sodium vanadate reduced the rate of JAK2 dephosphorylation [88]. One protein tyrosine phosphatase that plays a role in dephosphorylation of JAK2 after stimulation by Epo is SHP1 [91]. Because SCF induces association of SHP1 with c-Kit, SHP1 may mediate the rapid dephosphorylation of JAK2 after stimulation with SCF [89,90].

One pathway activated downstream of JAK family members are signal transducers and activators of transcription (STATs). This family of transcription factors associate with phosphorylated tyrosine residues on activated receptors, are both tyrosine and serine phosphorylated, form homo- and/or heterodimers, translocate to the nucleus, bind DNA and activate transcription of a variety of genes. To date, seven STAT family members have been identified. They are STATs 1–6 with STAT5 having an *a* and *b* isoform. Comprehensive reviews of this family of transcription factors have recently been published [92,93].

SCF induces association of STAT1 with c-Kit, increases in STAT1 tyrosine phosphorylation and increases in STAT1 DNA binding activity in hematopoietic cell lines and normal progenitor cells [94]. SCF also activates STAT5 and induces serine phosphorylation of STAT3 [95,96]. Broxmeyer and coworkers [96] have suggested that STAT3 may be involved in synergistic biological responses elicited by SCF in combination with other growth factors. In contrast to these findings, several investigators have reported that SCF had no effect on JAK or STAT family members [97–101]. It remains unclear if these differences relate to technical parameters or possibly to lineage specific differences in cell lines.

While studies with antisense oligonucleotides suggest that JAK2 is required for maximal proliferation in response to SCF, experimentation with JAK2-deficient mice will provide more definitive answers on the role of JAK2 in SCF-mediated responses [86]. These animals die in utero at a similar stage of embryonic development as c-Kit or SCF-deficient mice [20,83,84]. While progenitor cells expressing c-Kit were reported at higher frequencies in the fetal livers of JAK2-deficient mice (66%) than wild-type littermates (20%), SCF-induced colony formation of JAK2-deficient fetal liver cells was reduced compared to progenitors isolated from the fetal liver of control mice [83,84]. These studies suggest a role for JAK2 in optimal responses to SCF in normal hematopoietic progenitor cells.

6. Src family members and c-Kit signaling

Src family members are involved in a wide range of cellular functions including cell adhesion, cell motility, cell cycle progression, survival, differentiation, protein trafficking and cellular architecture, reviewed in [102–104]. They interact with one or more components of most known signaling pathways. With regard to signaling through RTKs, Src family members are activated in response to numerous RTK ligands including platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and colony-stimulating factor-1 (CSF-1) [105]. Little is known about Src family members in relation to SCF signaling. The Src family member Lyn is expressed at high levels in SCF-responsive cell lines and normal progenitor cells [106]. SCF induces increases in Lyn activity and association of Lyn with c-Kit. In vitro studies with GST fusion proteins have shown that Lyn binds tyrosine residues in the juxtamembrane region of c-Kit. Several lines of evidence suggest that Lyn plays a role in SCF-mediated proliferation [106]. Reduction in Lyn protein with antisense oligonucleotides inhibited SCF-induced proliferation. Similarly, PP1, a drug that inhibits Lyn activity but not c-Kit, also inhibited SCF-induced proliferation. It is probable that SCF activates other Src family members in cells lacking Lyn. The SH2 domain of c-Src coprecipitates c-Kit and treatment with the PKC inhibitor calphostin enhances this association [107]. Further, Fyn, another Src family member bound a phosphopeptide sequence corresponding to tyrosines 568 and 570 of human c-Kit [108].

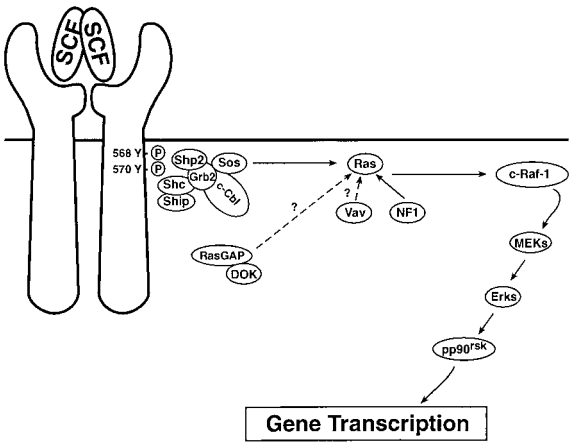

Fig. 2. SCF-induced activation of the Ras–Raf–MAP kinase cascade. Ligand-induced increases in tyrosine phosphorylation of c-Kit recruits Grb2 to the receptor complex. Grb2 may bind either directly to c-Kit or indirectly through interaction with Shc or SHP2. Because Grb2 and the nucleotide exchange factor Sos are constitutively associated, this translocation results in colocalization of Sos and Ras and subsequent activation of Ras. SCF-induced activation of Ras may also be modulated by RasGAP, Vav and NF1. Activation of Ras results in activation of the serine–threonine kinase c-Raf-1 which in turn activates the MAP kinase cascade.

One means of down-regulating the activity of Src family members is through phosphorylation of a highly conserved carboxyl-terminal tyrosine residue. Both Csk, a ubiquitously expressed tyrosine kinase, and Chk, a Csk-homologous kinase, phosphorylate Src family members at this site [109]. Chk is constitutively expressed in megakaryoctes, natural killer cells and the brain [110–113]. Chk expression is induced in activated T cells and in myeloid lineages treated with cytokines [114,115]. SCF induces increases in Chk expression that peak 8 h after stimulation and are maintained for 24 h [115]. In contrast, SCF has no effect on Csk expression [115]. Studies with phosphopeptides suggest that Chk associates with human c-Kit through phosphorylated tyrosines 568 and 570 [108]. This is of particular note since these tyrosines are in the juxtamembrane region of c-Kit and this region also associates with Lyn and Fyn [106,108]. Thus, colocalization of Chk and Src family members may be a mechanism to downregulate Src family activity after activation by SCF.

7. The Ras–Raf–MAP kinase cascade and c-Kit signaling

One signaling pathway activated in response to many growth factors is the Ras–Raf–MAP kinase cascade, reviewed in [116–118]. In brief, phosphorylated tyrosine residues on ligand-activated receptors recruit multiple SH2-containing proteins to the receptor complex and these proteins couple RTKs to activation of Ras. Included among these are Grb2, Shc, SHP2 and Grap. A Grb family member is constitutively associated with Sos, a guanine nucleotide exchange factor. Recruitment of Grb2 to the activated receptor complex, whether directly (through interaction with phosphorylated receptor) or indirectly (through interaction with receptor-associated Shc, SHP2 or other proteins) results in colocalization of Sos and Ras and subsequent increases in Ras activity. This in turn promotes the interaction of Ras with the serine–threonine kinase c-Raf-1 and activation of MEK, a MAP kinase. MEK phosphorylates MAP kinase and MAP kinase phosphorylates a number of substrates including $pp90^{rsk}$. In addition, this signaling pathway is regulated by numerous other proteins.

Fig. 2 summarizes what is known about SCF-induced activation of the Ras–Raf–MAP kinase cascade. Ligand-induced autophosphorylation of c-Kit induces association of Grb2, Grap, SHP2 and Shc with c-Kit [119–123]. Shc coprecipitates with a phosphopeptide sequence corresponding to tyrosines 568 and 570 in the human c-Kit juxtamembrane region. While this interaction may be indirect, the binding of SHP2, Grb2 and Grap to c-Kit is likely direct [108,119]. After association with the activated c-Kit receptor complex, both SHP2 and Shc are phosphorylated on tyrosine residues and this facilitates association with Grb2 or Grap. SCF also induces association of a 145 kDa phosphotyrosylprotein with Shc [124]. This protein has been cloned and named SHIP [125–127]. Interestingly, SHIP is an SH2-containing protein with 5′ phosphatase activity specific for inositol 1,3,4,5 tetraphosphate (I1,3,4,5-P4) and phosphatidylinositol 3,4,5, trisphosphate (PI-3,4,5-P3). Based on the phenotype of SHIP- de-

ficient mice, it appears that SHIP is a negative regulator of hematopoiesis [128].

Another protein that regulates Ras activity in response to many growth factors is RasGAP, a GTPase activating protein. Although some investigators have reported small increases in tyrosine phosphorylation of RasGAP after stimulation with SCF, many have not [45,46,68,129]. More recently, in vitro studies have suggested that RasGAP is capable of interacting with c-Kit, however the affinity for c-Kit is lower than that of PI3 K and phospholipase Cγ (PLCγ) [130,131]. The 62 kDa GAP-associated protein, Dok, was recently cloned [132,133]. Dok is phosphorylated in response to SCF and is constitutively phosphorylated in hematopoietic cell lines transformed by $p210^{bcr-abl}$ [134]. Although the tyrosine phosphorylated fraction of this protein associates with RasGAP, it is unclear if this interaction is involved in regulation of Ras activity or other signaling pathways.

Studies with Nf1 deficient progenitor cells have shown that this GTPase activating protein is also involved in modulating Ras activation by SCF [135]. The dose response curve for SCF-induced colony formation of Nf1 deficient progenitor cells was shifted to the left as compared to progenitors that expressed Nf1. These observations correlated with increases in MAP kinase activity in Nf1-deficient progenitors. These findings are of particular note, given the high frequencies of leukemia in both humans and mice that express reduced levels of NF1.

Vav, a 95 kDa protein expressed specifically in hematopoietic cells, may have guanine nucleotide exchange factor activity. Similar to other growth factors, SCF induces tyrosine phosphorylation of Vav, however its role in SCF-induced activation of the Ras pathway as well as other GTPases remains to be determined [136,137].

Thus, SCF-induced activation of Ras is regulated through multiple mechanisms. An immediate consequence of Ras activation is interaction with c-Raf-1 and increases in c-Raf-1 catalytic activity. Numerous laboratories have found increases in c-Raf-1 activity in response to SCF and have determined that this serine–threonine kinase is critical in SCF-mediated responses [45,67,138–140]. SCF also induces increases in tyrosine phosphorylation and kinase activity of MEK1, MEK2 and the MAP kinases [99–101,138,141–143]. Downstream of the MAP kinases, the S6 kinase $pp90^{rsk}$ is activated [142]. In addition to activating the classical MAP kinase pathway, SCF also activates the JNK kinase, a MAP kinase activated in response to stress [144].

8. Negative regulators of SCF signaling

Several lines of evidence suggest that SHP1, an SH2-containing protein tyrosine phosphatase specific for hematopoietic cells, is a negative regulator of c-Kit signaling. SCF induces association of the SHP1 SH2 domain with phosphorylated tyrosine 569 of murine c-Kit [89,90]. Transfection of Y569F c-Kit into BaF3 cells resulted in increases in SCF-induced proliferation [90]. Genetic evidence also suggests that SHP1 is a negative regulator of c-Kit. Motheaten mice (*me*) and motheaten viable mice lack functional SHP1 and have hyperproliferative progenitor cells [145]. To determine if the *W* locus and the *me* locus were complementary, hetrozygotic W^v mice were crossed with hetrozygotic *me* mice and backcrossed to obtain all allelic combinations of these two genes [146,147]. Expression of the W^v c-Kit mutant reduced the severity of the *me* phenotype and reduction in functional SHP1 also improved the W^v phenotype [146,147]. While the in vivo mast cell deficiencies found in W^v mice were improved in W^v/me mice, the macrocytic anemia was not. In vitro, SCF-mediated survival of mast cells from W^v/me mice was improved as compared to W^v mice, however, the *me* locus had no effect on SCF-induced proliferation of mast cells from either normal or W^v mice. These findings suggest lineage specificity in the role of SHP1 in SCF-mediated responses in progenitor cells and mast cells.

SHP2, another SH2-containing protein tyrosine phosphatase has also been implicated as a negative regulator of c-Kit signaling. SHP2 associates with murine c-Kit through phosphorylated tyrosine residue 567 after stimulation with SCF [90]. Expression of Y567F c-Kit in BaF3

cells results in increased sensitivity to SCF and increases in intrinsic responsiveness. As discussed previously, SHP2 also couples c-Kit to the Ras–Raf–MAP kinase cascade [123]. Studies with mice chimeric for SHP2 expression demonstrated an important role for SHP2 in erythroid and myeloid development [148]. Thus, SHP2 is likely a positive regulator of early hematopoiesis and perhaps serves as a negative regulator in more differentiated cells.

Another signaling component that negatively regulates SCF-induced proliferation is protein kinase C (PKC). Treatment of cells with SCF induces both tyrosine and serine phosphorylation of c-Kit in vivo [149]. In vitro, c-Kit is a substrate for PKC alpha and PKC-mediated serine phosphorylation of c-Kit had no effect on ligand binding but did reduce kinase activity [149]. Inhibition of PKC activity increased association of PI3 K with c-Kit and correlated with increases in SCF-mediated proliferation and decreases in cell motility in response to SCF. Studies using GST fusion proteins containing the SH2 domains of the 85 kDa regulatory subunit of PI3 K, Grb2, PLCγ, and Src found that calphostin, a PKC inhibitor, enhanced association with c-Kit in vitro [107]. These findings suggest that PKC-mediated serine phosphorylation of c-Kit reduces the capacity of multiple SH2-containing signaling components to associate with c-Kit. In contrast, inhibition of PKC had little effect on SCF-induced activation of c-Raf-1 and Erk2. Mapping studies demonstrated that serines 741 and 746 are phosphorylated by PKC both in vivo and in vitro, while serines 821 and 959 are phosphorylated in vivo, but not directly by PKC [150]. Mutation of serines 741 and 746 increased both c-Kit kinase activity and c-Kit-associated PI3 K activity in response to SCF. This double mutation also resulted in enhanced activation of AKT as well as increases in SCF-mediated cell survival [79]. The PKC isoform(s) mediating these responses in vivo have not been identified. Because these studies were performed in either endothelial cells or fibroblasts transfected with c-Kit, it will be interesting to determine the roles of PKC family members in SCF-mediated responses in hematopoietic cells.

9. Other signaling components activated by SCF

A variety of other signaling components are activated in response to SCF, although the signaling pathways these proteins are involved with, and their role in SCF-mediated responses, remain to be defined. For example, PLCγ hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3). SCF induces weak association between the SH2 domain of PLCγ and tyrosine 936 of human c-Kit as well as small increases in tyrosine phosphorylation of PLCγ [46,67,68,107,131,138]. Although PLCγ is a substrate for c-Kit, it is unlikely that SCF activates the classical PLCγ pathway. SCF does not induce increases in IP3 levels and increases in DAG are likely mediated by phospholipase D [67,151,152].

Tec, a Src-like kinase related to Btk and Itk, is highly expressed in hematopoietic cells and constitutively associated with c-Kit [97]. Stimulation with SCF resulted in increases in tyrosine phosphorylation of Tec as well as increases in its catalytic activity. Recent evidence in other receptor models suggests that Tec binds phosphatidylinositol-3,4,5 trisphosphate and that this facilitates PLCγ generation of IP3 and DAG [153]. The role of Tec in modulation of SCF signaling pathways remains to be explored.

Lastly, a 200 kDa glycoprotein associated with c-Kit after stimulation with SCF [154]. This protein was rapidly phosphorylated on tyrosine residues after stimulation with SCF but not GM–CSF or other ligands interacting with cytokine receptor superfamily members. The identity of this protein remains to be determined.

10. C-Kit signaling and cellular matrix components

Hematopoietic progenitor cells interact with both stromal cells and components of the extracellular matrix. These events, in conjunction with signals mediated by soluble and membrane-bound growth factors, are important in the regulation of hematopoiesis. Interaction of differentiated progeny, such as mast cells, with components of the

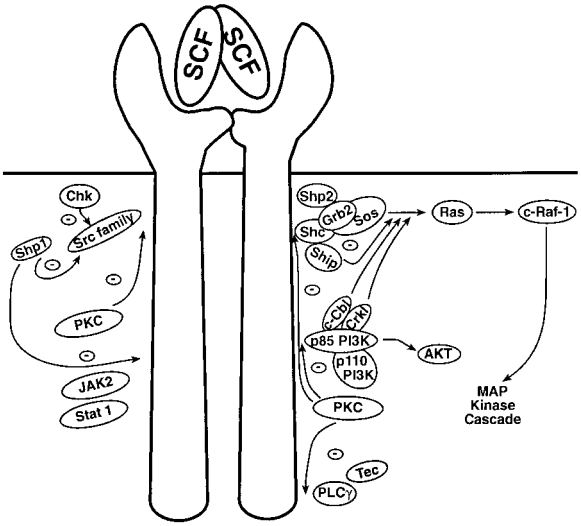

Fig. 3. Summary of the relationship between the multiple signaling pathways activated by SCF. SCF activates Src family members, PI3 K, the Ras–Raf–MAP kinase cascade and the JAK/STAT pathway. Interaction between different components of these pathways occurs at multiple points. With regard to negative regulators of these pathways, Src family members are negatively regulated by Chk and SHP1. PKC-mediated serine phosphorylation of c-Kit impairs interaction of the SH2 domain of Src, Grb2, PI3 K and PLCγ with c-Kit. Ship is a 5′ inositol phosphatase that may play a role in modulating activation of Ras as well as PLCγ and AKT.

extracellular matrix is also important in their functional responses. SCF-induced adhesion occurs through multiple mechanisms. One mechanism involves interaction of membrane-bound SCF expressed on stromal cells with c-Kit expressed on progenitor cells. The absence of c-Kit kinase activity may not impair adhesion mediated by this interaction [155,156]. SCF is also involved in cellular adhesion through fibronectin receptors. SCF-induced activation of PI3 K plays an important role in mast cell adhesion to fibronectin [74]. SCF also activates, or coactivates, kinases implicated in formation of focal adhesions. Stimulation of the megakaryblastic cell line CMK with SCF induced increases in phosphorylation of RAFTK (Related Adhesion Focal Tyrosine Kinase, also termed Pyk2 or CAK-B). This activation was dependent on PKC [157]. Stimulation of the erythroblastic cell line TF-1 with SCF had no effect on phosphorylation of FAK but did enhance integrin-mediated activation of FAK as well as phosphorylation of paxillin [158].

11. Integrated signaling mechanisms

Although delineation of linear signaling pathways is conceptually attractive, many of the signaling components activated by SCF play roles in multiple pathways. In most cases, SCF-mediated responses result from the input of multiple, interconnected signaling pathways (summarized in Fig. 3). For example, SHP2 is likely involved in activation of the Ras–Raf–MAP kinase cascade but may also negatively regulate other signaling pathways [90,123]. Phosphorylation of c-Kit by PKC isoforms decreases interaction of c-Kit with multiple SH2-containing proteins, including the 85 kDa regulatory subunit of PI3 K, Grb2, PLCγ, and Src. SCF induces association of PI3 K with both c-Cbl and Crkl [70]. Crkl is an adaptor protein that interacts with components of multiple signaling pathways [69]. c-Cbl also associates with Grb2 and may modulate Ras activation in response to SCF [159]. Mutation of the PI3 K binding site on c-Kit expressed in murine mast cells abrogates SCF-induced PI3 K activity and partially impairs activation of Ras and c-fos and junB induction by SCF [74]. Treatment of cells with SCF also induces association of SHIP and Shc. SHIP is a 5′ phosphatase recruited to signaling complexes through an SH2 domain [125,126]. Recent studies suggest that SHIP plays a role in the regulation of multiple signaling components, including PLCγ, Ras and AKT [127,153]. Thus, the biological outcome of SCF/c-Kit interaction depends on integrated signals resulting from the activation of multiple pathways.

12. C-Kit structure-function summary

SCF activates multiple signal transduction components including PI3 K, Src family members, the Ras–Raf–MAP kinase cascade and the JAK/STAT pathway. Activation of many of

Table 1
Sites on c-Kit that associate with signaling components

Associated protein(s)	Tyrosine residue(s) (Human/Murine Sequence)	Biological function/cell line	Reference
Lyn	544–577 (human)	Proliferation/Mo7e	106
Fyn	Y568, Y570 (human)	?	108
Shc	Y568, Y570 (human)	?	108
Shp1	Y569 (murine)	Negative regulator of proliferation/BaF3	90
Shp2	Y567 (murine)	Negative regulator of proliferation/BaF3	90
Chk	Y568, Y570 (human)	?	108
p85 PI3 K	Y719 (murine)	Adhesion/mast cells	74
	Y719 (murine)	Partial effect on survival, proliferation/mast cells	74
	Y719 (murine)	c-Kit trafficking/DA1 cells	76
	Y721 (human)	Differentiation/FDC-P1 cells	78
	Y721 (human)	Survival/fibroblasts	79
?	Y821 (murine)	Survival and proliferation/mast cells	74
PLCgamma	Y936 (human)	?	131

these pathways is dependent on interaction of one or more upstream signaling components with specific sites on c-Kit. Table 1 is a summary of tyrosine residues on c-Kit that interact with components of these pathways. In addition, if known, the biological consequence of mutation of each site is also shown. Fig. 4 summarizes other regions of c-Kit known to be involved in its function.

Of particular note in Table 1 are the large numbers of proteins found associated with tyrosines 568 and 570 in the human c-Kit juxtamembrane region (amino acids 544–577). This region binds Src family members, Chk, Shc, SHP1 and SHP2 [90,106,108]. To date, only one group has published mutagenesis studies of these residues. Mutation of either tyrosine 567 or 569 of murine c-Kit (corresponding to tyrosines 568 and 570 in human c-Kit) to phenylalanine and expression in BaF3 cells resulted in increased proliferation in response to SCF as compared to wild-type c-Kit [90]. It will be important to determine if similar results are obtained when these mutants are expressed in other cell lines. The effects of mutating both of these tyrosines has not been examined.

Mutations in the juxtamembrane region have also been associated with constitutively active forms of c-Kit. Substitution of valine for glycine 560 activates human c-Kit [55,57,60,61]. Murine c-Kit lacking amino acids 573–579 is also constitutively active [61]. Gastrointestinal stromal cell tumors contain mutations in the juxtamembrane region that activate human c-Kit [22]. Thus, the juxtamembrane region is critical in regulation of c-Kit activity.

Another site on c-Kit that is well characterized in relation to SCF-mediated responses is tyrosine 721 in the kinase insert region of human c-Kit (719 of murine c-Kit). Extensive studies from Besmer and coworkers [73] have shown that the 85 kDa regulatory subunit of PI3 K binds this residue and that mutation abrogates SCF-induced activation of PI3 K. The absence of SCF-induced PI3 K activity dramatically impaired adhesion of mast cells to fibronectin as well as reduced SCF-mediated survival and proliferation [74]. In other cell lineages this region is implicated in trafficking of c-Kit as well as SCF-mediated survival, proliferation and differentiation [75–79].

Two serine residues in the kinase insert region (Serines 741 and 746 of human c-Kit) may also play a role in regulation of biological responses mediated by SCF. Mutation of these residues correlates with increases in SCF-mediated motility and decreases in proliferation in transfected endothelial cells [107].

Substitution of valine for glycine 816 in the second catalytic domain of human c-Kit results

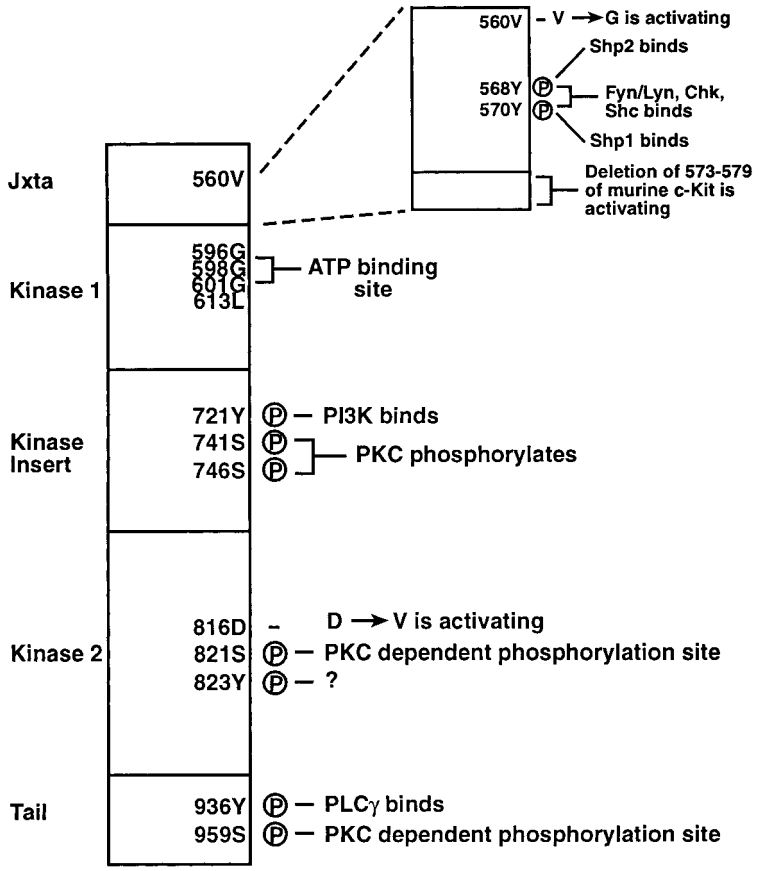

Fig. 4. Regions of human c-Kit involved in its function. The five intracellular regions of c-Kit are depicted in this cartoon. The juxtamembrane region binds multiple signaling components including SHP1, SHP2, Shc, Chk and Src family members. Several mutations have been identified within this region that result in constitutive activation of c-Kit. The first catalytic domain of c-Kit contains amino acid residues involved in binding ATP. The kinase insert contains the tyrosine that binds PI3 K as well as two serine residues phosphorylated by PKC. The second catalytic domain contains aspartic acid 816 (human c-Kit sequence). Substitution of this amino acid with valine results in a constititutively active form of c-Kit. The second catalytic domain also contains an important autophosphorylation site (tyrosine 823 of human c-Kit) as well as a serine residue that is phosphorylated in a PKC-dependent pathway. The carboxyl-tail of c-Kit has another serine residue that is phosphorylated in a PKC-dependent pathway as well as a tyrosine residue that interacts with PLCγ.

in constitutive activation [53–56,59]. Further, as discussed in previous sections, this mutation has been found in patients with a form of mastocytosis with associated hematological disorders [28]. Tyrosine 823 is another amino acid in the second catalytic domain of human c-Kit (821 of murine c-Kit) that is important for SCF-mediated survival and proliferation in mast cells [74]. The signaling component(s) that interact with this tyrosine has not yet been elucidated.

13. SCF-mediated synergy

A remarkable feature of SCF is its capacity to synergize with other hematopoietic growth factors. This is a critical because stem cells and multipotential progenitor cells respond optimally to growth factors in combination. The mechanisms mediating synergy are poorly understood. Studies from the laboratory of Keller and coworkers [160] demonstrated that synergistic responses of hematopoietic progenitor cells to combinations

of ligands binding members of the cytokine receptor superfamily correlated with the capacity of these growth factors to modulate receptor expression. In contrast, SCF synergizes with growth factors through a different mechanism. SCF does not alter the affinity, or numbers, of GM–CSF receptors in Mo7e cells, a human cell line that proliferates synergistically in response to GM–CSF in combination with SCF [138,161]. Similarly, SCF did not alter expression of IL-3 receptors in either human or murine cell lines reported to respond synergistically to this combination of growth factors [161,162]. Consistent with these findings, treatment of CD34 enriched human progenitor cells with SCF had no effect on expression of IL-6, IL-3, GM–CSF or Epo receptors [163]. Less is known about the effect of GM–CSF and IL-3 on SCF binding. However, neither IL-3 or GM–CSF effected c-Kit mRNA levels in Mo7e cells [164].

Although SCF does not modulate expression of growth factors receptors, there is evidence suggesting that c-Kit phosphorylates at least one cytokine receptor. SCF induces tyrosine phosphorylation of the Epo receptor in both HCD-57 cells, a murine erythroleukemia cell line, and purified human erythroid progenitors [100,143,165]. Conversely, Epo induces tyrosine phosphorylation of c-Kit in a human erythroleukemic cell line [40]. In HCD-57 cells, c-Kit and the Epo receptor have been reported to coimmunoprecipitate and in vitro studies with GST fusion proteins suggest this interaction is mediated through phosphorylated tyrosines residues in the extended box 2 region of the Epo receptor [165]. Lodish and coworkers [166] have suggested that an interaction between c-Kit and the Epo receptor occurs in normal eyrthroid progenitors and that this plays a role in the development of CFUe erythroid progenitors.

Another cytokine receptor phosphorylated in response to SCF is the beta chain of the IL-3 receptor. Treatment of B6SUtA1 cells with SCF induces serine and threonine phosphorylation of the B_{IL3} subunit of the IL-3 receptor [162]. Reduction in c-Kit activity with the kinase inhibitor genistein had no effect on the capacity of SCF to induce phosphorylation of B_{IL3}, while treatment with PKC inhibitors did. Since SCF has no effect on either the affinity or numbers of IL-3 receptors, SCF-induced activation of PKC may play a role in modulating one or more IL-3 signaling pathways. The absence of synergistic increases in tyrosine phosphorylation in response to SCF and IL-3 suggests the synergistic response to these factors may involve serine/threonine kinases.

Consistent with the above findings, many reports in the literature found no correlation between early increases in protein tyrosine phosphorylation and the synergistic interactions of SCF with GM–CSF or IL-3 [45,98,138]. SCF stimulated autophosphorylation of c-Kit, but GM–CSF in combination with SCF caused no additional increase in c-Kit autophosphorylation in Mo7e cells [45]. Recently, a new megakaryoblastic cell line was described that responded to GM–CSF and SCF synergistically [167]. In contrast to studies with Mo7e cells, GM–CSF and SCF reportedly did increase autophosphorylation of c-Kit as compared to SCF alone in these cells.

The effect of SCF in combination with GM–CSF or IL-3 on specific signaling components downstream of c-Kit has also been examined. In studies of Mo7e cells, no synergistic increase in tyrosine phosphorylation of RasGAP, c-Raf-1 or MAP kinase were observed [45,138]. Similarly, in FDCP-MixA4 cells and B6 M cells, SCF and IL-3 did not induce synergistic increases in tyrosine phosphorylation of c-Raf-1, Shc, JAK2, Erk1 or Erk2 [98,101]. In contrast, the combination of SCF and IL-3 did induce synergistic increases in the kinase activity of Erk1 in both of these cell lines [98,101]. In addition, SCF and IL-3 in combination did not synergistically induce increases in the mRNA levels of the serine/threonine kinase PIM1 in B6 M cells, although costimulation of Mo7e cells with SCF and gamma interferon did [168].

Recently, the signaling pathways of Epo in combination with SCF were examined in human erythroid progenitor cells. Similar to studies of SCF in combination with GM–CSF and IL-3, no synergistic increase in tyrosine phosphorylation of either c-Kit, or the Epo receptor, were observed [143]. In contrast, synergistic increases

in phosphorylation of MEK, Erk1 and Erk2 were found and this correlated with increases in MAP kinase activity [143]. Thus, in relation to early signaling events, synergistic activation of members of the MAP kinase family in response to SCF in combination with other growth factors has been the most consistent finding.

The effects of SCF in combination with GM–CSF and IL-3 on expression of transcription factors have also been examined. As described above, SCF activates both JAK2 and STAT1 in a variety of cell lines. One group reported that while SCF activated STAT5 in mast cells, it did not activate STAT5 in the mast cell line B6 M [95,98]. Studies with B6 M cells further suggest that SCF in combination with IL-3 does not increase the tyrosine phosphorylation or DNA binding activity of STAT5 above that observed with IL-3 [98]. One intriguing study found that SCF induced serine phosphorylation of STAT3 in Mo7e cells. Also, some increases in DNA binding activity of STAT3 were observed in extracts of cells stimulated with SCF in combination with IL-9 [96].

Induction of immediate early response genes have also been examined in response to SCF in combination with other growth factors. Synergistic induction of a number of early response genes was found in Mo7e cells treated with sub-optimal concentrations of SCF with either GM–CSF or IL-3 [169]. Included among these were c-fos, junB, egr-1 and c-myc. Thus, the synergistic activation of members of the MAP kinase pathway may be involved in increases in expression of some immediate early response genes. In contrast to these findings, treatment of B6 M cells with SCF and IL-3 in combination did not induce synergistic increases in expression of c-myc or c-fos [98].

In addition to possible synergistic increases in some early response genes, one group has reported activation of signaling components key to the G_1/S transition of the cell cycle that correlates with the capacity of SCF to synergize with growth factors. Enhanced phosphorylation of the Rb gene product was reported in response to SCF in combination with GM–CSF as compared to either factor alone [170]. Alterations in cellular levels of the cyclin-dependent kinase inhibitors Cip-1 and Kip-1 also correlated with synergistic interactions of GM–CSF and SCF [170]. Further, synergistic increases in expression of the 68 kDa calmodulin binding protein (CaM-BP68), were observed in response to SCF in combination with IL-3 [171]. CaM-BP68 is involved in the G_1/S transition of the cell cycle. Thus, SCF in combination with other growth factors may induce synergistic increases in the expression or activity of signaling components associated with DNA synthesis and subsequent cell cycle progression.

14. Conclusions

In the nine years since SCF was identified as the c-Kit ligand, a remarkable amount has been learned about its mechanism of action. SCF activates multiple signaling pathways and these pathways lead to a variety of biological responses. While many of the early studies focused on signaling pathways activated by SCF in fibroblasts transfected with c-Kit, subsequent work in hematopoietic cell lines generally supports these findings. Understanding the role of these signaling pathways in SCF-mediated responses remains a more difficult task. In particular, cellular lineage is clearly critical in determining the biological outcome of different signaling pathways. Studies in hematopoietic cell lines are certainly one means to address these questions, however it is important to interpret these studies in the context of the lineage and differentiation state of the cell line. In addition, the means through which the cells were immortalized is also an important consideration. Elegant studies reconstituting mast cells from *W* mice with different c-Kit mutants have likely provided the most accurate information on the structure-function relationships of c-Kit in mast cells. It will be important to develop similar models to examine these questions in progenitor cells. Lastly, the mechanisms mediating the capacity of SCF to synergistically stimulate progenitor cells in combination with other growth factors remains an important question in the field. To date, progress in this area

has been hampered by the few numbers of cell lines available that reproducibly respond synergistically to combinations of growth factors. In addition, obtaining sufficient numbers of normal hematopoietic progenitor cells to perform signaling experiments is difficult. However challenging, insight into mechanisms of synergy could be an important key to understanding regulation of quiescent stem cells and provide insight into a fundamental question in hematopoiesis. Answers to these questions may also provide the means to mobilize these cells for use in treating disease with approaches such as gene therapy or transplantation.

Acknowledgements

The author would like to thank Dr Doug Lowy, Dr Virginia Broudy and Ms Bridget O'Laughlin for their critical review of this manuscript. The efforts of Ms Karen Canon in preparation of the references were also greatly appreciated.

References

[1] S.J. Galli, K.M. Zsebo, E.N. Geissler, The kit ligand, stem cell factor, Advances in Immunology 55 (1994) 1–97.
[2] V.C. Broudy, Stem cell factor and hematopoiesis, Blood 90 (1997) 1345–1364.
[3] O.N. Witte, Steel locus defines new multipotent growth factor, Cell 63 (1990) 5–6.
[4] I.K. McNiece, R.A. Briddell, Stem cell factor, Journal of Leukocyte Biology 57 (1995) 14–22.
[5] P. Valent, The riddle of the mast cell: kit (CD117)-ligand as the missing link?, Immunology Today 15 (1994) 111–114.
[6] I.K. McNiece, K.M. Zsebo, The role of stem cell factor in the hematopoietic system, Cancer Investigation 11 (1993) 724–729.
[7] K. Morrison-Graham, Y. Takahashi, Steel factor and c-Kit receptor: from mutants to a growth factor system, BioEssays 15 (1993) 77–83.
[8] R.A. Fleischman, From white spots to stem cells: the role of the Kit receptor in mammalian development, Trends in Genetics 9 (1993) 285–290.
[9] J.D. Huizinga, L. Thuneberg, M. Klüppel, J. Malysz, H.B. Mikkelsen, A. Bernstein, *W/kit* gene required for interstitial cells of Cajal and for intestinal pacemaker activity, Nature 373 (1995) 347–349.
[10] L. Puddington, S. Olson, L. Lefrancois, Interactions between stem cell factor and c-kit are required for intestinal immune system homeostasis, Immunity 1 (1994) 733–739.
[11] B. Motro, J.M. Wojtowicz, A. Bernstein, D. van der Kooy, Steel mutant mice are deficient in hippocampal learning but not long-term potentiation, Proceedings of the National Academy of Sciences USA 93 (1996) 1808–1813.
[12] J.G. Flanagan, P. Leder, The *kit* ligand: a cell surface molecule altered in steel mutant fibroblasts, Cell 63 (1990) 185–194.
[13] K.M. Zsebo, J. Wypych, I.K. McNiece, H.S. Lu, K.A. Smith, S.B. Karkare, R.K. Sachdev, V.N. Yuschenkoff, N.C. Birkett, L.R. Williams, V.N. Satyagal, W. Tung, R.A. Bosselman, R.A. Mendiaz, K.E. Langley, Identification, purification, and biological characterization of hematopoietic stem cell factor from buffalo rat liver-conditioned medium, Cell 63 (1990) 195–201.
[14] F.H. Martin, S.V. Suggs, K.E. Langley, H.S. Lu, J. Ting, K.H. Okino, C.F. Morris, I.K. McNiece, F.W. Jacobsen, E.A. Mendiaz, N.C. Birkett, K.A. Smith, M.J. Johnson, V.P. Parker, J.C. Flores, A.C. Patel, E.F. Fisher, H.O. Erjavec, C.J. Herrera, J. Wypych, R.K. Sachdev, J.A. Pope, I. Leslie, D. Wen, C-H. Lin, R.L. Cupples, K.M. Zsebo, Primary structure and functional expression of rat and human stem cell factor DNAs, Cell 63 (1990) 203–211.
[15] D.M. Anderson, S.D. Lyman, A. Baird, J.M. Wignall, J. Eisenman, C. Rauch, C.J. March, H.S. Boswell, S.D. Gimpel, D. Cosman, D.E. Williams, Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms, Cell 63 (1990) 235–243.
[16] K.M. Zsebo, D.A. Williams, E.N. Geissler, V.C. Broudy, F.H. Martin, H.L. Atkins, R-Y. Hsu, N.C. Birkett, K.H. Okino, D.C. Murdock, F.W. Jacobsen, K.E. Langley, K.A. Smith, T. Takeishi, B.M. Cattanach, S.J. Galli, S.V. Suggs, Stem cell factor is encoded at the *SI* locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor, Cell 63 (1990) 213–224.
[17] E. Huang, K. Nocka, D.R. Beier, T-Y. Chu, J. Buck, H-W. Lahm, D. Wellner, P. Leder, P. Besmer, The hematopoietic growth factor KL is encoded by the *SI* locus and is the ligand of the *c-kit* receptor, the gene product of the *W* locus, Cell 63 (1990) 225–233.
[18] B. Chabot, D.A. Stephenson, V.M. Chapman, P. Besmer, A. Bernstein, The proto-oncogene *c-kit* encoding a transmembrane tyrosine kinase receptor maps to the mouse *W* locus, Nature 335 (1988) 88–89.
[19] E.N. Geissler, M.A. Ryan, D.E. Housman, The dominant-white spotting (*W*) locus of the mouse encodes the *c-kit* proto-oncogene, Cell 55 (1988) 185–192.
[20] E.S. Russell, Hereditary anemias of the mouse: a

review for geneticists, Advances in Genetics 20 (1979) 357–459.

[21] P. Besmer, J.E. Murphy, P.C. George, F. Qiu, P.J. Bergold, L. Lederman, H.W. Snyder Jr, D. Brodeur, E.E. Zuckerman, W.D. Hardy, A new acute transforming feline retrovirus and relationship of its oncogene v-*kit* with the protein kinase gene family, Nature 320 (1986) 415–421.

[22] S. Hirota, K. Isozaki, Y. Moriyama, K. Hashimoto, T. Nishida, S. Ishiguro, K. Kawano, M. Hanada, A. Kurata, M. Takeda, G.M. Tunio, Y. Matsuzawa, Y. Kanakura, Y. Shinomura, Y. Kitamura, Gain-of-function mutations of c-*kit* in human gastrointestinal stromal tumors, Science 279 (1998) 577–580.

[23] A.M. Turner, K.M. Zsebo, F. Martin, F.W. Jacobsen, L.G. Bennett, V.C. Broudy, Nonhematopoietic tumor cell lines express stem cell factor and display c-*kit* receptors, Blood 80 (1992) 347–381.

[24] R. Matsuda, T. Takahashi, S. Nakamura, Y. Sekido, K. Nishida, M. Seto, T. Seito, T. Sugiura, Y. Ariyoshi, T. Takahashi, R. Ueda, Expression of the c-*kit* protein in human solid tumors and in corresponding fetal and adult normal tissues, American Journal of Pathology 142 (1993) 339–346.

[25] M. Inoue, S. Kyo, M. Fujita, T. Enomoto, G. Kondoh, Coexpression of the c-*kit* receptor and the stem cell factor in gynecological tumors, Cancer Research 54 (1994) 3049–3053.

[26] S.J. Hines, C. Organ, M.J. Kornstein, G.W. Krystal, Coexpression of the c-*kit* and stem cell factor genes in breast carcinomas, Cell Growth and Differentiation 6 (1995) 769–779.

[27] G.W. Krystal, S.J. Hines, C.P. Organ, Autocrine growth of small cell lung cancer mediated by coexpression of c-*kit* and stem cell factor, Cancer Research 56 (1996) 370–376.

[28] H. Nagata, A.S. Worobec, C.K. Oh, B.A. Chowdhury, S. Tannenbaum, Y. Suzuki, D.D. Metcalfe, Identification of a point mutation in the catalytic domain of the protooncogene c-*kit* in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder, Proceedings of the National Academy of Sciences USA 92 (1995) 10,560–10,564.

[29] J.M. Blechman, S. Lev, M.F. Brizzi, O. Leitner, L. Pegoraro, D. Givol, Y. Yarden, Soluble c-kit proteins and antireceptor monoclonal antibodies confine the binding site of the stem cell factor, Journal of Biological Chemistry 268 (1993) 4399–4406.

[30] S. Lev, J. Blechman, S-I. Nishikawa, D. Givol, Y. Yarden, Interspecies molecular chimeras of kit help define the binding site of the stem cell factor, Molecular and Cellular Biology 13 (1993) 2224–2234.

[31] J.M. Blechman, S. Lev, D. Givol, Y. Yarden, Structure-function analyses of the kit receptor for the steel factor, Stem Cells 11 (1993) 12–21.

[32] T. Arakawa, D.A. Yphantis, J.W. Lary, L.O. Narhi, H.S. Lu, S.J. Prestrelski, C.L. Clogston, K.M. Zsebo, E.A. Mendiaz, J. Wypych, Glycosylated and unglycosylated recombinant-derived human stem cell factors are dimeric and have extensive regular secondary structure, Journal of Biological Chemistry 266 (1991) 18,942–18,948.

[33] Y-R. Hsu, G-M. Wu, E.A. Mendiaz, R. Syed, J. Wypych, R. Toso, M.B. Mann, T.C. Boone, L.O. Narhi, H.S. Lu, K.E. Langley, The majority of stem cell factor exists as monomer under physiological conditions, Journal of Biological Chemistry 272 (1997) 6406–6415.

[34] J.S. Philo, J. Wen, J. Wypych, M.G. Schwartz, E.A. Mendiaz, K.E. Langley, Human stem cell factor dimer forms a complex with two molecules of the extracellular domain of its receptor, kit, Journal of Biological Chemistry 271 (1996) 6895–6902.

[35] S. Lev, Y. Yarden, D. Givol, A recombinant ectodomain of the receptor for the stem cell factor (SCF) retains ligand-induced receptor dimerization and antagonizes SCF-stimulated cellular responses, Journal of Biological Chemistry 267 (1992) 10,866–10,873.

[36] S. Lev, Y. Yarden, D. Givol, Dimerization and activation of the kit receptor by monovalent and bivalent binding of the stem cell factor, Journal of Biological Chemistry 267 (1992) 15,970–15,977.

[37] M.A. Lemmon, D. Pinchasi, M. Zhou, I. Lax, J. Schlessinger, Kit receptor dimerization is driven by bivalent binding of stem cell factor, Journal of Biological Chemistry 272 (1997) 6311–6317.

[38] P. Blume-Jensen, L. Claesson-Welsh, A. Siegbahn, K.M. Zsebo, B. Westermark, C-H. Heldin, Activation of the human c-*kit* product by ligand-induced dimerization mediates circular actin reorganization and chemotaxis, EMBO Journal 10 (1991) 4121–4128.

[39] J.M. Blechman, S. Lev, J. Barg, M. Eisenstein, B. Vaks, Z. Vogel, D. Givol, Y. Yarden, The fourth immunoglobulin domain of the stem cell factor receptor couples ligand binding to signal transduction, Cell 80 (1995) 103–113.

[40] V.C. Broudy, N.L. Lin, H-J. Bühring, N. Komatsu, T.J. Kavanagh, Analysis of *c-kit* receptor dimerization by fluorescence resonance energy transfer, Blood 91 (1998) 898–906.

[41] Y. Yarden, W-J. Kuang, T. Yang-Feng, L. Coussens, S. Munemitsu, T.J. Dull, E. Chen, J. Schlessinger, U. Francke, A. Ullrich, Human proto-oncogene c-*kit*: a new cell surface receptor tyrosine kinase for an unidentified ligand, EMBO Journal 6 (1987) 3341–3351.

[42] F. Qiu, P. Ray, K. Brown, P.E. Barker, S. Jhanwar, F.H. Ruddle, P. Besmer, Primary structure of c-*kit*: relationship with the CSF-1/PDGF receptor kinase family — oncogenic activation of v-*kit* involves deletion of extracellular domain and C terminus, EMBO Journal 7 (1988) 1003–1011.

[43] S. Majumder, K. Brown, F-H. Qiu, P. Besmer, c-*kit* protein, a transmembrane kinase: identification in tis-

sues and characterization, Molecular and Cellular Biology 8 (1988) 4896–4903.

[44] A. Kuriu, H. Ikeda, Y. Kanakura, J.D. Griffin, B. Druker, H. Yagura, H. Kitayama, J. Ishikawa, T. Nishiura, Y. Kanayama, T. Yonezawa, S. Tarui, Proliferation of human myeloid leukemia cell line associated with the tyrosine-phosphorylation and activation of the proto-oncogene c-*kit* product, Blood 78 (1991) 2834–2840.

[45] K. Miyazawa, P.C. Hendrie, C. Mantel, K. Wood, L.K. Ashman, H.E. Broxmeyer, Comparative analysis of signaling pathways between mast cell growth factor (*c-kit* ligand) and granulocyte–macrophage colony-stimulating factor in a human factor-dependent myeloid cell line involves phosphorylation of Raf-1, GTPase-activating protein and mitogen-activated protein kinase, Experimental Hematology 19 (1991) 1110–1123.

[46] R. Rottapel, M. Reedijk, D.E. Williams, S.D. Lyman, D.M. Anderson, T. Pawson, A. Bernstein, The *Steel/W* transduction pathway: kit autophosphorylation and its association with a unique subset of cytoplasmic signaling proteins is induced by the steel factor, Mol. Cell. Biol. 11 (1991) 3043–3051.

[47] K. Miyazawa, D.A. Williams, A. Gotoh, J. Nishimaki, H.E. Broxmeyer, K. Toyama, Membrane-bound steel factor induces more persistent tyrosine kinase activation and longer life span of c-*kit* gene-encoded protein than its soluble form, Blood 85 (1995) 641–649.

[48] R. Kapur, M. Majumdar, X. Xiao, M. McAndrews-Hill, K. Schindler, D.A. Williams, Signaling through the interaction of membrane-restricted stem cell factor and c-*kit* receptor tyrosine kinase: genetic evidence for a differential role in erythropoiesis, Blood 91 (1998) 879–889.

[49] Y. Tajima, E.J. Huang, K. Vosseller, M. Ono, M.A.S. Moore, P. Besmer, Role of dimerization of the membrane-associated growth factor kit ligand in juxtacrine signaling: the SI^{17H} mutation affects dimerization and stability–phenotypes in hematopoiesis, Journal of Experimental Medicine 187 (1998) 1451–1461.

[50] J.C. Tan, K. Nocka, P. Ray, P. Traktman, P. Besmer, The dominant W^{42} *spotting* phenotype results from a missense mutation in the c-*kit* receptor kinase, Science 277 (1990) 209–212.

[51] K. Nocka, J.C. Tan, E. Chiu, T.Y. Chu, P. Ray, P. Traktman, P. Besmer, Molecular bases of dominant negative and loss of function mutations at the murine c-*kit*/white spotting locus: W^{37}, W^{V}, W^{41} and *W*, EMBO Journal 9 (1990) 1805–1813.

[52] A.D. Reith, R. Rottapel, E. Giddens, C. Brady, L. Forrester, A. Bernstein, *W* mutant mice with mild or severe developmental defects contain distinct point mutations in the kinase domain of the c-*kit* receptor, Genes and Development 4 (1990) 390–400.

[53] T. Furitsu, T. Tsujimura, T. Tono, H. Ikeda, H. Kitayama, U. Koshimizu, H. Sugahara, J.H. Butterfield, L.K. Ashman, Y. Kanayama, Y. Matsuzawa, Y. Kitamura, Y. Kanakura, Identification of mutations in the coding sequence of the proto-oncogene c-*kit* in a human mast cell leukemia cell line causing ligand-independent activation of c-*kit* product, Journal of Clinical Investigation 92 (1993) 1736–1744.

[54] T. Tsujimura, T. Furitsu, M. Morimoto, K. Isozaki, S. Nomura, Y. Matsuzawa, Y. Kitamura, Y. Kanakura, Ligand-independent activation of c-*kit* receptor tyrosine kinase in a murine mastocytoma cell line P-815 generated by a point mutation, Blood 83 (1994) 2619–2626.

[55] H. Kitayama, Y. Kanakura, T. Furitsu, T. Tsujimura, K. Oritani, H. Ikeda, H. Sugahara, H. Mitsui, Y. Kanayama, Y. Kitamura, Y. Matsuzawa, Constitutively activating mutations of c-*kit* receptor tyrosine kinase confer factor-independent growth and tumorigenicity of factor-dependent hematopoietic cell lines, Blood 85 (1995) 790–798.

[56] X. Piao, A. Bernstein, A point mutation in the catalytic domain of c-*kit* induces growth factor independence, tumorigenicity, and differentiation of mast cells, Blood 87 (1996) 3117–3123.

[57] H. Kitayama, T. Tsujimura, I. Matsumura, K. Oritani, H. Ikeda, J. Ishikawa, M. Okabe, M. Suzuki, K. Yamamura, Y. Matsuzawa, Y. Kitamura, Y. Kanakura, Neoplastic transformation of normal hematopoietic cells by constitutively activating mutations of *c-kit* receptor tyrosine kinase, Blood 88 (1996) 995–1004.

[58] P. Ferrao, T.J. Gonda, L.K. Ashman, Expression of constitutively activated human c-kit in myb transformed early myeloid cells leads to factor independence, histiocytic differentiation, and tumorigenicity, Blood 90 (1997) 4539–4552.

[59] X. Piao, R. Paulson, P. van der Geer, T. Pawson, A. Bernstein, Oncogenic mutation in the Kit receptor tyrosine kinase alters substrate specificity and induces degradation of the protein tyrosine phosphatase SHP-1, Proceedings of the National Academy of Sciences USA 93 (1996) 14,665–14,669.

[60] T. Tsujimura, Y. Kanakura, Y. Kitamura, Mechanisms of constitutive activation of c-*kit* receptor tyrosine kinase, Leukemia 11 (1997) 396–398.

[61] T. Tsujimura, M. Morimoto, K. Hashimoto, Y. Moriyama, H. Kitayama, Y. Matsuzawa, Y. Kitamura, Y. Kanakura, Constitutive activation of c-*kit* in FMA3 murine mastocytoma cells caused by deletion of seven amino acids at the juxtamembrane domain, Blood 87 (1996) 273–283.

[62] R. Herbst, S. Munemitsu, A. Ullrich, Oncogenic activation of v-*kit* involves deletion of a putative tyrosine-substrate interaction site, Oncogene 10 (1995) 369–379.

[63] S. Majumder, P. Ray, P. Besmer, Tyrosine protein kinase activity of the HZ4-feline sarcoma virus $P80^{gag\text{–}kit}$-transforming protein, Oncogene Research 5 (1990) 329–335.

[64] A. Toker, L.C. Cantley, Signalling through the lipid

products of phosphoinositide-3-OH kinase, Nature 387 (1997) 673–675.
[65] R.P. Shepherd, D.J. Withers, K. Siddle, Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling, Biochemical Journal 333 (1998) 471–490.
[66] V. Duronio, M.P. Scheid, S. Ettinger, Downstream signalling events regulated by phosphatidylinositol 3-kinase activity, Cell Signalling 10 (1998) 233–239.
[67] S. Lev, D. Givol, Y. Yarden, A specific combination of substrates is involved in signal transduction by the *kit*-encoded receptor, EMBO Journal 10 (1991) 647–654.
[68] A.D. Reith, C. Ellis, S.D. Lyman, D.M. Anderson, D.E. Williams, A. Bernstein, T. Pawson, Signal transduction by normal isoforms and *W* mutant variants of the kit receptor tyrosine kinase, EMBO Journal 10 (1991) 2451–2459.
[69] M. Sattler, R. Salgia, Role of the adapter protein CRKL in signal transduction of normal hematopoietic and BCR/ABL-transformed cells, Leukemia 12 (1998) 637–644.
[70] M. Sattler, R. Salgia, G. Shrikhande, S. Verma, E. Pisick, K.V.S. Prasad, J.D. Griffin, Steel factor induces tyrosine phosphorylation of CRKL and binding of CRKL to a complex containing c-Kit, phosphatidylinositol 3-kinase, and $p120^{CBL}$, Journal of Biological Chemistry 272 (1997) 10,248–10,253.
[71] D. Wisniewski, A. Strife, B. Clarkson, c-*kit* ligand stimulates tyrosine phosphorylation of the c-Cbl protein in human hematopoietic cells, Leukemia 10 (1996) 1436–1442.
[72] S. Lev, D. Givol, Y. Yarden, Interkinase domain of kit contains the binding site for phosphatidylinositol 3′ kinase, Proceedings of the National Academy of Sciences USA 89 (1992) 678–682.
[73] H. Serve, Y-C. Hsu, P. Besmer, Tyrosine residue 719 of the c-kit receptor is essential for binding of the P85 subunit of phosphatidylinositol (PI) 3-kinase and for c-*kit*-associated PI 3-kinase activity in COS-1 cells, Journal of Biological Chemistry 269 (1994) 6026–6030.
[74] H. Serve, N.S. Yee, G. Stella, L. Sepp-Lorenzino, J.C. Tan, P. Besmer, Differential roles of PI3-kinase and Kit tyrosine 821 in Kit receptor-mediated proliferation, survival and cell adhesion in mast cells, EMBO Journal 14 (1995) 473–483.
[75] K. Vosseller, G. Stella, N.S. Yee, P. Besmer, c-Kit receptor signaling through its phosphatidylinositide-3′-kinase-binding site and protein kinase C: role in mast cell enhancement of degranulation, adhesion, and membrane ruffling, Molecular Biology of the Cell 8 (1997) 909–922.
[76] J.L. Gommerman, R. Rottapel, S.A. Berger, Phosphatidylinositol 3-kinase and Ca^{2+} influx dependence for ligand-stimulated internalization of the c-Kit receptor, Journal of Biological Chemistry 272 (1997) 30,519–30,525.
[77] Y. Nelson, C-W. Hsiau, H. Serve, K. Vosseler, P. Besmer, Mechanism of down-regulation of c-kit receptor, Journal of Biological Chemistry 269 (1994) 31,991–31,998.
[78] Y. Kubota, T. Angelotti, G. Niederfellner, R. Herbst, A. Ullrich, Activation of phosphatidylinositol 3-kinase is necessary for differentiation of FDC-P1 cells following stimulation of type III receptor tyrosine kinases, Cell Growth and Differentiation 9 (1998) 247–256.
[79] P. Blume-Jensen, R. Janknecht, T. Hunter, The Kit receptor promotes cell survival via activation of PI 3-kinase and subsequent Akt-mediated phosphorylation of Bad on Ser136, Current Biology 8 (1998) 779–782.
[80] A.F. Wilks, A.G. Harpur, Cytokine signal transduction and the JAK family of protein tyrosine kinases, BioEssays 16 (1994) 313–319.
[81] J.N. Ihle, The *Janus* protein tyrosine kinases in hematopoietic cytokine signaling, Seminars in Immunology 7 (1995) 247–254.
[82] S.J. Rodig, M.A. Meraz, J.M. White, P.A. Lampe, J.K. Riley, C.D. Arthur, K.L. King, K.C.F. Sheehan, L. Yin, D. Pennica, E.M. Johnson Jr, R.D. Schreiber, Disruption of the *Jak1* gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses, Cell 93 (1998) 373–383.
[83] H. Neubauer, A. Cumano, M. Müller, H. Wu, U. Huffstadt, K. Pfeffer, Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis, Cell 93 (1998) 397–409.
[84] E. Parganas, D. Wang, D. Stravopodis, D.J. Topham, J-C. Marine, S. Teglund, E.F. Vanin, S. Bodner, O.R. Colamonici, J.M. van Deursen, G. Grosveld, J.N. Ihle, Jak2 is essential for signaling through a variety of cytokine receptors, Cell 93 (1998) 385–395.
[85] D.C. Thomis, C.B. Gurniak, E. Tivol, A.H. Sharpe, L.J. Berg, Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3, Science 270 (1995) 794–797.
[86] S.R. Weiler, S. Mou, C.S. DeBerry, J.R. Keller, F.W. Ruscetti, D.K. Ferris, D.L. Longo, D. Linnekin, JAK2 is associated with the c-kit proto-oncogene product and is phosphorylated in response to stem cell factor, Blood 87 (1996) 3688–3693.
[87] M.F. Brizzi, M.G. Zini, M.G. Aronica, J.M. Blechman, Y. Yarden, L. Pegoraro, Convergence of signaling by interleukin-3, granulocyte–macrophage colony-stimulating factor, and mast cell growth factor on JAK2 tyrosine kinase, Journal of Biological Chemistry 269 (1994) 31,680–31,684.
[88] D. Linnekin, S. Mou, C.S. Deberry, S.R. Weiler, J.R. Keller, F.W. Ruscetti, D.L. Longo, Stem Cell Factor, the JAK–STAT pathway and Signal Transduction, Leukemia and Lymphoma 27 (1997) 439–444.
[89] T. Yi, J.N. Ihle, Association of hematopoietic cell phosphatase with c-Kit after stimulation with c-Kit ligand, Molecular and Cellular Biology 13 (1993) 3350–3358.
[90] M. Kozlowski, L. Larose, F. Lee, D.M. Le, R.

Rottapel, K.A. Siminovitch, SHP-1 binds and negatively modulates the c-Kit receptor by interaction with tyrosine 569 in the c-Kit juxtamembrane domain, Molecular and Cellular Biology 18 (1998) 2089–2099.

[91] U. Klingmuller, U. Lorenz, L.C. Cantley, B.G. Neel, H.F. Lodish, Specific recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of JAK2 and termination of proliferative signals, Cell 80 (1995) 729–738.

[92] J.N. Ihle, Cytokine receptor signalling, Nature 377 (1993) 591–599.

[93] J.N. Ihle, STATs: signal transducers and activators of transcription, Cell 84 (1996) 331–334.

[94] C. DeBerry, S. Mou, D. Linnekin, STAT1 associates with c-kit and is activated in response to stem cell factor, Biochemical Journal 327 (1997) 73–80.

[95] J.J. Ryan, H. Huang, L.J. McReynolds, C. Shelburne, J. Hu-Li, T.F. Huff, W.E. Paul, Stem cell factor activates STAT-5 DNA binding in IL-3-derived bone marrow mast cells, Experimental Hematology 25 (1997) 357–362.

[96] A. Gotoh, H. Takahira, C. Mantel, S. Litz-Jackson, H.S. Boswell, H.E. Broxmeyer, Steel factor induces serine phosphorylation of STAT3 in human growth factor-dependent myeloid cell lines, Blood 88 (1996) 138–145.

[97] B. Tang, H. Mano, T. Yi, J.N. Ihle, Tec kinase associates with c-kit and is tyrosine phosphorylated and activated following stem cell factor binding, Molecular and Cellular Biology 14 (1994) 8432–8437.

[98] A-M. O'Farrell, M. Ichihara, A.L-F. Mui, A. Miyajima, Signaling pathways activated in a unique mast cell line where interleukin-3 supports survival and stem cell factor is required for a proliferative response, Blood 87 (1996) 3655–3665.

[99] B. Joneja, H-C. Chen, D. Seshasayee, A.L. Wrentmore, D.M. Wojchowski, Mechanisms of stem cell factor and erythropoietin proliferative co-signaling in FDC2-ER cells, Blood 90 (1997) 3533–3545.

[100] S.M. Jacobs-Helber, K. Penta, Z. Sun, A. Lawson, S.T. Sawyer, Distinct signaling from stem cell factor and erythropoietin in HCD57 cells, Journal of Biological Chemistry 272 (1997) 6850–6853.

[101] M.A. Pearson, A-M. O'Farrell, T.M. Dexter, A.D. Whetton, P.J. Owen-Lynch, C.M. Heyworth, Investigation of the molecular mechanisms underlying growth factor synergy: the role of ERK 2 activation in synergy, Growth Factors 15 (1998) 293–306.

[102] M.T. Brown, J.A. Cooper, Regulation, substrates and functions of src, Biochimica et Biophysica Acta 1287 (1996) 121–149.

[103] S.M. Thomas, J.S. Brugge, Cellular functions regulated by src family kinases, Annual Review of Cell and Developmental Biology 13 (1997) 513–609.

[104] J.T. Parsons, S.J. Parsons, Src family protein tyrosine kinases: cooperating with growth factor and adhesion signaling pathways, Current Options in Cell Biology 9 (1997) 187–192.

[105] T. Erpel, S.A. Courtneidge, Src family protein tyrosine kinases and cellular signal transduction pathways, Current Opinions in Cell Biology 7 (1995) 176–182.

[106] D. Linnekin, C.S. DeBerry, S. Mou, Lyn associates with the juxtamembrane region of c-Kit and is activated by stem cell factor in hematopoietic cell lines and normal progenitor cells, Journal of Biological Chemistry 272 (1997) 27,450–27,455.

[107] P. Blume-Jensen, L. Rönnstrand, I. Gout, M.D. Waterfield, C-H. Heldin, Modulation of Kit/stem cell factor receptor-induced signaling by protein kinase C, Journal of Biological Chemistry 269 (1994) 21,793–21,802.

[108] D.J. Price, B. Rivnay, Y. Fu, S. Jiang, S. Avraham, H. Avraham, Direct association of Csk homologous kinase (CHK) with the diphosphorylated site $Tyr^{568/570}$ of the activated c-KIT, Journal of Biological Chemistry 272 (1997) 5915–5920.

[109] L.M. Chow, A. Veillette, The Src and Csk families of tyrosine protein kinases in hemopoietic cells, Seminars in Immunology 7 (1995) 207–226.

[110] D.W. McVicar, B.K. Lal, M. Kawamura, Y.Q. Chen, K.Y. Zhang, J.E. Staples, R.J. Ortaldo, J.J. O'Shea, Molecular cloning of Lsk, a carboxyl-terminal Src kinase (Csk) related gene, expresed in leukocytes, Oncogene 9 (1994) 2037–2044.

[111] B.D. Bennet, S. Cowley, S. Jiang, R. London, B. Deng, J. Grabarek, J.E. Groopman, D.V. Goeddel, H. Avraham, Identification and characterization of a novel tyrosine kinase from megakaryocytes, Journal of Biological Chemistry 269 (1994) 1068–1074.

[112] S. Sakano, A. Iwama, J. Inazawa, T. Ariyama, M. Ohno, T. Suda, Molecular cloning of a novel non-receptor tyrosine kinase, HYL (hematopoietic consensus tyrosine-lacking kinase), Oncogene 9 (1994) 1155–1161.

[113] L.M. Chow, C. Jarvis, Q. Hu, S.H. Nye, F.G. Gervais, A. Veillette, L.A. Matis, Ntk: a Csk-related protein-tyrosine kinase expressed in brain and T lymphocytes, Proceedings of the National Academy of Sciences USA 91 (1994) 4975–4979.

[114] T. Musso, L. Varesio, X. Zhang, T.K. Rowe, P. Ferrara, J.R. Ortaldo, J.J. O'Shea, D.W. McVicar, IL-4 and IL-13 induce Lsk, a Csk-like tyrosine kinase, in human monocytes, Journal of Experimental Medicine 180 (1994) 2383–2388.

[115] S. Grgurevich, D. Linnekin, T. Musso, X. Zhang, W. Modi, L. Varesio, F.W. Ruscetti, J.R. Ortaldo, D.W. McVicar, The Csk-like proteins Lsk, Hyl, and Matk represent the same *C*sk *h*omologous *k*inase (Chk) and are regulated by stem cell factor in the megakaryoblastic cell line MO7e, Growth Factors 14 (1997) 103–115.

[116] H.D. Madhani, G.R. Fink, The riddle of MAP kinase signaling specificity, Trends in Genetics 14 (1998) 151–155.

[117] S.L. Pelech, J.S. Sanghera, MAP kinases: charting the regulatory pathways, Science 275 (1992) 1355–1356.
[118] J. Blenis, Signal transduction via the MAP kinases: proceed at your own RSK, Proceedings of the National Academy of Sciences USA 90 (1993) 5889–5892.
[119] T. Tauchi, H.S. Boswell, D. Leibowitz, H.E. Broxmeyer, Coupling between p210bcr-abl and Shc and Grb2 adaptor proteins in hematopoietic cells permits growth factor receptor-independent link to Ras activation pathway, Journal of Experimental Medicine 179 (1994) 167–175.
[120] R.L. Cutler, L. Liu, J.E. Damen, G. Krystal, Multiple cytokines induce the tyrosine phosphorylation of Shc and its association with Grb2 in hemopoietic cells, Journal of Biological Chemistry 268 (1993) 21,463–21,465.
[121] G-S. Feng, Y-B. Ouyang, D-P. Hu, Z-Q. Shi, R. Gentz, J. Ni, Grap is a novel SH3-SH2-SH3 adaptor protein that couples tyrosine kinases to the ras pathway, Journal of Biological Chemistry 271 (1996) 12,129–12,132.
[122] T. Matsuguchi, R. Salgia, M. Hallek, M. Eder, B. Druker, T.J. Ernst, J.D. Griffin, Shc phosphorylation in myeloid cells is regulated by granulocyte macrophage colony-stimulating factor, interleukin-3, and steel factor and is constitutively increased by $p210^{BCR/ABL}$, Journal of Biological Chemistry 269 (1994) 5016–5021.
[123] T. Tauchi, G-S. Feng, M.S. Marshall, R. Shen, C. Mantel, T. Pawson, H.E. Broxmeyer, The ubiquitously expressed Syp phosphatase interacts with c-*kit* and Grb2 in hematopoietic cells, Journal of Biological Chemistry 269 (1994) 25,206–25,211.
[124] L. Liu, J.E. Damen, R.L. Cutler, G. Krystal, Multiple cytokines stimulate the binding of a common 145-kilodalton protein to Shc at the Grb2 recognition site of Shc, Molecular and Cellular Biology 14 (1994) 6926–6935.
[125] J.E. Damen, L. Liu, P. Rosten, R.K. Humphries, A.B. Jefferson, P.W. Majerus, G. Krystal, The 145-kDa protein induced to associate with Shc by multiple cytokines is an inositol tetraphosphate and phosphatidylinositol 3,4,5-trisphosphate 5-phosphatase, Proceedings of the National Academy of Sciences USA 93 (1996) 1689–1693.
[126] M.N. Lioubin, P.A. Algate, S. Tsai, K. Carlberg, A. Aebersold, L.R. Rohrschneider, p150Ship, a signal transduction molecule with inositol polyphosphate-5-phosphatase activity, Genes and Development 10 (1996) 1084–1095.
[127] L. Liu, J.E. Damen, M. Ware, M. Hughes, G. Krystal, SHIP, a new player in cytokine-induced signalling, Leukemia 11 (1997) 181–184.
[128] C.D. Helgason, J.E. Damen, P. Rosten, R. Grewal, P. Sorensen, S.M. Chappel, A. Borowski, F. Jirik, G. Krystal, R.K. Humphries, Targeted disruption of *SHIP* leads to hemopoietic perturbations, lung pathology, and a shortened life span, Genes and Development 12 (1998) 1610–1620.
[129] V. Duronio, M.J. Welham, S. Abraham, P. Dryden, J.W. Schrader, $p21^{ras}$ activation via hemopoietin receptors and c-kit requires tyrosine kinase activity but not tyrosine phosphorylation of $p21^{ras}$ GTPase-activating protein, Proceedings of the National Academy of Sciences USA 89 (1992) 1587–1591.
[130] B.H. Jhun, B. Rivnay, D. Price, H. Avraham, The MATK tyrosine kinase interacts in a specific and SH2-dependent manner with c-Kit, Journal of Biological Chemistry 270 (1995) 9661–9666.
[131] R. Herbst, M.S. Shearman, B. Jallal, J. Schlessinger, A. Ullrich, Formation of signal transfer complexes between stem cell and platelet-derived growth factor receptors and SH2 domain proteins in vitro, Biochemistry 34 (1995) 5971–5979.
[132] N. Carpino, D. Wisniewski, A. Strife, D. Marshak, R. Kobayashi, B. Stillman, B. Clarkson, $p62^{dok}$: a constitutively tyrosine-phosphorylated, GAP-associated protein in chronic myelogenous leukemia progenitor cells, Cell 88 (1997) 197–204.
[133] Y. Yamanashi, D. Baltimore, Identification of the Abl- and rasGAP-associated 62 kDa protein as a docking protein, Dok, Cell 88 (1997) 205–211.
[134] D. Wisniewski, A. Strife, E. Berman, B. Clarkson, *c-kit* ligand stimulates tyrosine phosphorylation of a similar pattern of phosphotyrosyl proteins in primary primitive normal hematopoietic progenitors that are constitutively phosphorylated in comparable primitive progenitors in chronic phase chronic myelogenous leukemia, Leukemia 10 (1996) 229–237.
[135] B.Y-Y. Zhang, T.A. Vik, J.W. Ryder, E.F. Srour, T. Jacks, K. Shannon, D.W. Clapp, *Nf1* regulates hematopoietic progenitor cell growth and Ras signaling in response to multiple cytokines, Journal of Experimental Medicine 187 (1998) 1893–1902.
[136] T. Matsuguchi, R.C. Inhorn, N. Carlesso, G. Xu, B. Druker, J.D. Griffin, Tyrosine phosphorylation of $p95^{Vav}$ in myeloid cells is regulated by GM–CSF, IL-3 and Steel factor and is constitutively increased by $p210^{BCR/ABL}$, EMBO J. 14 (1995) 257–265.
[137] M. Alai, A.L-F. Mui, R.L. Cutler, X.R. Bustelo, M. Barbacid, G. Krystal, Steel factor stimulates the tyrosine phosphorylation of the proto-oncogene product, $p95^{vav}$, in human hemopoietic cells, Journal of Biological Chemistry 267 (1992) 18,021–18,025.
[138] M. Hallek, B. Druker, E.M. Lepisto, K.W. Wood, T.J. Ernst, J.D. Griffin, Granulocyte–macrophage colony-stimulating factor and steel factor induce phosphorylation of both unique and overlapping signal transduction intermediates in a human factor-dependent hematopoietic cell line, Journal of Cellular Physiology 153 (1992) 176–186.
[139] U. Brennscheidt, D. Riedel, W. Kölch, R. Bonifer, M.A. Brach, A. Ahlers, R.H. Mertelsmann, F.

Herrmann, Raf-1 is a necessary component of the mitogenic response of the human megakaryoblastic leukemia cell line MO7 to human stem cell factor, granulocyte–macrophage colony-stimulating factor, interleukin 3, and interleukin 9[1], Cell Growth and Differentiation 5 (1994) 367–372.

[140] K.W. Muszynski, F.W. Ruscetti, G. Heidecker, U. Rapp, J. Troppmair, J.M. Gooya, J.R. Keller, Raf-1 protein is required for growth factor-induced proliferation of hematopoietic cells, Journal of Experimental Medicine 181 (1995) 2189–2199.

[141] K. Okuda, J.S. Sanghera, S.L. Pelech, Y. Kanakura, M. Hallek, J.D. Griffin, B.J. Druker, Granulocyte–macrophage colony-stimulating factor, interleukin-3, and Steel factor induce rapid tyrosine phosphorylation of p42 and p44 MAP kinase, Blood 79 (1992) 2880–2887.

[142] M. Tsai, R-H. Chen, S-Y. Tam, J. Blenis, S.J. Galli, Activation of MAP kinases, $pp90^{rsk}$ and pp70-S6 kinases in mouse mast cells by signaling through the c-*kit* receptor tyrosine kinase or Fc-RI: rapamycin inhibits activation of pp70-S6 kinase and proliferation in mouse mast cells, European Journal of Immunolology 23 (1993) 3286–3291.

[143] X. Sui, S.B. Krantz, M. You, Z. Zhao, Synergistic activation of MAP kinase (ERK1/2) by erythropoietin and stem cell factor is essential for expanded erythropoiesis, Blood 92 (1998) 1142–1149.

[144] I.N. Foltz, J.W. Schrader, Activation of the stress-activated protein kinases by multiple hematopoietic growth factors with the exception of interleukin-4, Blood 89 (1997) 3092–3096.

[145] L.D. Shultz, P.A. Schweitzer, T.V. Rajan, T. Yi, J.N. Ihle, R.J. Matthews, M.L. Thomas, D.R. Beier, Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene, Cell 73 (1993) 1445–1454.

[146] R.F. Paulson, S. Vesely, K.A. Siminovitch, A. Bernstein, Signalling by the *W/Kit* receptor tyrosine kinase is negatively regulated in vivo by the protein tyrosine phosphatase SHP1, Nature Genetics 13 (1996) 309–315.

[147] U. Lorenz, A.D. Bergemann, H.N. Steinberg, J.G. Flanagan, X. Li, S.J. Galli, B.G. Neel, Genetic analysis reveals cell type-specific regulation of receptor tyrosine kinase c-Kit by the protein tyrosine phosphatase SHP1, Journal of Experimental Medicine 184 (1996) 1111–1126.

[148] C-K. Qu, W-M. Yu, B. Azzarelli, S. Cooper, H.E. Broxmeyer, G-S. Feng, Biased suppression of hematopoiesis and multiple developmental defects in chimeric mice containing SHP-2 mutant cells, Molecular and Cellular Biology 18 (1998) 6075–6082.

[149] P. Blume-Jensen, A. Siegbahn, S. Stabel, C-H. Heldin, L. Rönnstrand, Increased Kit/SCF receptor induced mitogenicity but abolished cell motility after inhibition of protein kinase C, EMBO Journal 12 (1993) 4199–4209.

[150] P. Blume-Jensen, C. Wernstedt, C-H. Heldin, L. Rönnstrand, Identification of the major phosphorylation sites for protein kinase C in Kit/steam cell factor receptor in vitro and in intact cells, Journal of Biological Chemistry 270 (1995) 14,192–14,200.

[151] T. Koike, K. Hirai, Y. Morita, Y. Nozawa, Stem cell factor-induced signal transduction in rat mast cells, Journal of Immunology 151 (1993) 359–366.

[152] O. Kozawa, P. Blume-Jensen, C-H. Heldin, L. Rönnstrand, Involvement of phosphatidylinositol 3′-kinase in stem-cell-factor-induced phospholipase D activation and arachidonic acid release, European Journal of Biochemistry 248 (1997) 149–155.

[153] A.M. Scharenberg, J-P. Kinet, Ptdlns-3,4,5-P3: a regulatory nexus between tyrosine kinases and sustained calcium signals, Cell 94 (1998) 5–8.

[154] D. Linnekin, J.R. Keller, D.K. Ferris, S.M. Mou, V. Broudy, D.L. Longo, Stem cell factor induces phosphorylation of a 200 kDa protein which associates with c-kit, Growth Factors 12 (1995) 57–67.

[155] T. Kinashi, J.A. Escobedo, L.T. Williams, K. Takatsu, T.A. Springer, Receptor tyrosine kinase stimulates cell-matrix adhesion by phosphatidylinositol 3 kinase and phospholipase C-γ1 pathways, Blood 86 (1995) 2086–2090.

[156] T. Kinashi, T.A. Springer, Steel factor and c-*kit* regulate cell-matrix adhesion, Blood 83 (1994) 1033–1038.

[157] H. Takahira, A. Gotoh, A. Ritchie, H.E. Broxmeyer, Steel factor enhances integrin-mediated tyrosine phosphorylation of focal adhesion kinase ($pp125^{FAK}$) and paxillin, Blood 89 (1997) 1574–1584.

[158] D. Hiregowdara, H. Avraham, Y. Fu, R. London, S. Avraham, Tyrosine phosphorylation of the related adhesion focal tyrosine kinase in megakaryocytes upon stem cell factor and phorbol myristate acetate stimulation and its association with paxillin, J. Biol. Chem. 272 (1997) 10,804–10,810.

[159] M.F. Brizzi, P. Dentelli, L. Lanfrancone, A. Rosso, P.G. Pelicci, L. Pegoraro, Discrete protein interactions with the Grb2/c-Cbl complex in SCF- and TPO-mediated myeloid cell proliferation, Oncogene 13 (1996) 2067–2076.

[160] S.E.W. Jacobsen, F.W. Ruscetti, C.M. Dubois, J. Wine, J.R. Keller, Induction of colony-stimulating factor receptor expression on hematopoietic progenitor cells: proposed mechanism for growth factor synergism, Blood 80 (1992) 678–687.

[161] P.C. Hendrie, K. Miyazawa, Y-C. Yang, C.D. Langefeld, H.E. Broxmeyer, Mast cell growth factor (*c-kit* ligand) enhances cytokinase stimulation of proliferation of the human factor-dependent cell line, M07e, Experimental Hematology 19 (1991) 1031–1037.

[162] L. Liu, R.L. Cutler, A.L-F. Mui, G. Krystal, Steel factor stimulates the serine/threonine phosphorylation of

the interleukin-3 receptor, Journal of Biological Chemistry 269 (1994) 16,774–16,779.

[163] U. Testa, E. Pelosi, M. Gabbianelli, C. Fossati, S. Campisi, G. Isacchi, C. Peschle, Cascade transactivation of growth factor receptors in early human hematopoiesis, Blood 81 (1993) 1442–1456.

[164] Z-B. Hu, W. Ma, C.C. Uphoff, H. Quentmeier, H.G. Drexler, c-*kit* expression in human megakaryoblastic leukemia cell lines, Blood 83 (1994) 2133–2144.

[165] H. Wu, U. Klingmüller, P. Besmer, H.F. Lodish, Interaction of the erythropoietin and stem-cell-factor receptors, Nature 377 (1995) 242–246.

[166] H. Wu, U. Klingmüller, A. Acurio, J.G. Hsiao, H.F. Lodish, Functional interaction of erythropoietin and stem cell factor receptors is essential for erythroid colony formation, Proc. Natl. Acad. Sci. USA 94 (1997) 1806–1810.

[167] T. Kamijo, K. Koike, K. Takeuchi, T. Higuchi, N. Sawai, T. Kikuchi, H. Tsumura, H. Akiyama, T. Koike, E. Ishii, A. Komiyama, Analysis of synergism between stem cell factor and granulocyte–macrophage colony-stimulating factor on human megakaryoblastic cells: an increase in tyrosine phosphorylation of 145 kDa subunit of c-kit in two-factor combination, Leukemia Research 21 (1997) 1097–1106.

[168] M.T. Yip-Schneider, M. Horie, H.E. Broxmeyer, Transcriptional induction of *pim*-1 protein kinase gene expression by interferon γ and posttranscriptional effects on costimulation with steel factor, Blood 85 (1995) 3494–3502.

[169] M. Horie, H.E. Broxmeyer, Involvement of immediate-early gene expression in the synergistic effects of steel factor in combination with granulocyte–macrophage colony-stimulating factor or interleukin-3 on proliferation of a human factor-dependent cell line, Journal of Biological Chemistry 268 (1993) 968–973.

[170] C. Mantel, Z. Luo, J. Canfield, S. Braun, C. Deng, H.E. Broxmeyer, Involvement of $p21^{cip-1}$ and $p27^{kip-1}$ in the molecular mechanisms of steel factor-induced proliferative synergy in vitro and of $p21^{cip-1}$ in the maintenance of stem/progenitor cells in vivo, Blood 88 (1996) 3710–3719.

[171] G.P.V. Reddy, P.J. Quesenberry, Stem cell factor enhances interleukin-3-dependent induction of 68-kD calmodulin-binding protein and thymidine kinase activity in NFS-60 cells, Blood 87 (1996) 3195–3202.