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C-Kit signal transduction and involvement in cancer

Review Article

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Abbreviations: acute myeloid leukemia (AML); basic Helix-Loop-Helix Leucine Zipper (bHLHZip); Bruton tyrosine kinase (Btk); diacylglycerol (DAG); epidermal growth factor (EGF); Extracellular Regulated Kinase (Erk); gastrointestinal stromal tumors (GIST); Hematopoietic stem cells (HSC); inositol-1, 4, 5-trisphosphate (IP_3); Interstitial cells of Cajal (ICC); Janus kinases (JAKs); mitogen activated protein kinase (MAPK); phorbol myristate acetate (PMA); phosphatidylinositol-3,4,5-trisphosphate (PIP_3); phosphoinositide 3'-kinase (PI3-kinase); phospholipase C- (PLC-); phospholipase D (PLD); phosphotyrosine binding (PTB); pleckstrin homology (PH); protein inhibitor of activated Stat3 (PIAS3); protein kinase C (PKC); protein tyrosine phosphatases (PTPs); small cell lung cancer (SCLC); Src family kinase (SFK); Src homology 2 (SH2); stem cell factor (SCF); suppressors of cytokine signaling (SOCS); Tec homology (TH); truncated form of c-Kit (tr-Kit)

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Summary

Receptor tyrosine kinases, such as c-Kit, are proteins whose function it is to transduce signals from the environment into the cell leading to complex behaviors such as proliferation, migration, survival and differentiation. Many of these behaviors are deregulated in cancer, which is characterized by uncontrolled proliferation, insensitivity towards death stimuli, migration of tumor cells away from the primary tumor site and in some cases also block of cellular differentiation leaving the cell in an immature proliferative state. To be able to target these processes it is vital to have a detailed understanding of the receptor function and the downstream pathways activated. In this article we will review the mechanisms by which c-Kit induces signal transduction as well as describing tumors in which c-Kit function is perturbed.

I. Introduction

A. c-Kit and Stem cell factor (SCF)

The viral oncogene v-Kit was identified in 1986 as the transforming gene of the Hardy-Zuckerman 4 feline sarcoma virus (Besmer et al, 1986) and shortly thereafter its cellular homolog, c-Kit, was cloned and sequenced (Yarden et al, 1987). Within a few years time, c-Kit was found to be allelic with the dominant white spotting locus (*W*) of mice (Chabot et al, 1988; Geissler et al, 1988). Numerous naturally occurring loss-of-function mutations in c-Kit have been found in both mice and humans. Complete loss of c-Kit expression leads to death *in utero* or perinatally, most likely due to severe anemia. Heterozygous animals display anemia, reduced fertility and defects in pigmentation. The severity of the phenotype has been found to inversely correlate with the tyrosine kinase activity of the receptor. Mutations in the so-called Steel (*Sl*) locus in mice, that encodes the ligand for c-Kit, stem cell factor (SCF), give rise to a phenotype very similar to mutations in c-Kit (Copeland et al, 1990; Williams et al, 1990). For a review on *W* and *Sl* mutations, see Lev et al, 1994.

B. c-Kit alternative splicing

Alternative mRNA splicing leads to the occurrence of four isoforms of c-Kit in humans and two in mice. In both mice and humans, two isoforms are characterized by the presence or absence of a tetrapeptide sequence (GNNK) in the extracellular part of the juxtamembrane region (Reith et al, 1991; Crosier et al, 1993; Zhu et al, 1994). This alternative splicing occurs due to alternate use of 5' splice donor sites (Hayashi et al, 1991). In addition, splice variants exist that differ in the presence or absence

of a single serine residue in the kinase insert region of human c-Kit, due to alternative splice acceptor site usage (Crosier et al, 1993). Furthermore, post meiotic germ cells of the testis have been found to express a shorter transcript of c-Kit. This transcript encodes a truncated version of c-Kit (tr-Kit) consisting only of the second part of the kinase domain. Thus, extracellular and transmembrane domains as well as the first part of the kinase domain are missing which results in a non-functional tyrosine kinase activity (Rossi et al, 1992). Nonetheless, tr-kit is able to signal. Microinjection of tr-kit into mouse eggs, triggers metaphase-to-anaphase transition by the sequential activation of the Src family kinase (SFK) Fyn and phospholipase C- 1 (PLC- 1), and their association with Sam68 (Paronetto et al, 2003). Interestingly, the truncated isoform of c-Kit has been found to be frequently expressed in prostate carcinoma, in particular in more advanced stages (Paronetto et al, 2004), where it induces activation of SFK's.

The two GNNK+ and GNNK- isoforms of c-Kit (also denoted Kit and KitA, respectively) are co-expressed in most tissues (Reith et al, 1991; Crosier et al, 1993; Zhu et al, 1994) with the GNNK- form predominating. Expression of the two isoforms has been studied in human acute myeloid leukemia (AML). It was shown that among various AML cell lines the ratio of the two isoforms varied from as low as 1.3 to as high as 12 (Piao et al, 1994). In contrast, the ratio in normal bone marrow was around 4.4-5.5. However, no correlation was found between the expression of either isoform and the response to therapy or other clinical parameters. Despite these findings, NIH3T3 cells expressing the different isoforms were shown to possess distinct transforming activity (Caruana et al, 1999). In the presence of the ligand SCF, the GNNK- form induced loss of contact inhibition, anchorage-independent growth and tumorigenicity, with no difference in ligand affinity between the two isoforms. Upon ligand stimulation, the GNNK- isoform was more highly tyrosine-phosphorylated, more rapidly internalized, and activated Extracellular Regulated Kinase (Erk) more strongly than the GNNK+ isoform. In a recent study, it was shown that the kinetics of phosphorylation of the adapter protein ShcA, previously demonstrated to be phosphorylated by SFKs downstream of c-Kit, was stronger and more rapid in the GNNK- form (Voytyuk et al, 2003). However, no functional evidence for a role of enhanced ShcA phosphorylation in mediating the increased activation of Erks has been reported. There is also a possibility that other docking proteins, such as Gab-1 or Gab-2, that are known to be phosphorylated by SFKs might contribute to the activation of Erk. Inhibition of SFKs by treatment with the selective inhibitor SU6656 altered the kinetics of activation of the GNNK- form of c-Kit so that they resembled those of the GNNK+ form. Thus, to summarize, a very minor difference in amino acid sequence in a region with no apparent enzymatic function or substrate binding ability, appears to lead to dramatic differences in signaling. Alternative splicing has been described in several tyrosine kinases and interestingly, several of these isoforms differ in the extracellular part of the juxtamembrane region, as is the case with c-Kit. Recently, Leibiger and colleagues described differences in the signal transduction pathways activated and the repertoire of gene expression induced upon stimulation of the two splice forms of the insulin receptor, INSR-A and INSR-B. The INSR-A isoform was shown to induce expression of the insulin gene, whereas the INSR-B isoform mediated induction of the glucokinase gene (Leibiger et al, 2001). Through differential use of exon 11, the two splice forms of the insulin receptor differ in only 12 amino acids in the C terminus of the a-subunit. Furthermore, splice variants involving deletion of parts of the juxtamembrane region of the extracellular domain have been described for both ErbB2 and ErbB4. In the case of ErbB2, a deletion of sixteen amino acids in the extracellular juxtamembrane region caused a stronger kinase activity and transforming ability of ErbB2 (Kwong and Hung, 1998).

II. Signal transduction through c-Kit

Signaling downstream of c-Kit has been studied extensively in a variety of cell systems with mast cells, which express endogenous c-Kit, being the most commonly used model system. Several studies have used transient transfection systems that lead to strong overexpression, such as COS cells or HEK293. In particular in the early studies, investigators have used chimeras, *i. e.* the extracellular part of another receptor, *e. g.* the epidermal growth factor (EGF) receptor fused to the intracellular part of c-Kit. These differences in experimental setup make it difficult to directly compare the results obtained in different laboratories. Discrepancies in the literature will be discussed here. In this review the individual tyrosine phosphorylation sites of c-Kit are for simplicity, coherence and for the purpose of avoiding confusion numbered according to the human c-Kit sequence, even in cases where the murine c-Kit was studied.

A. Activation of c-Kit through ligand induced oligomerization

Binding of SCF to c-Kit leads to oligomerization of the receptors followed by activation of its intrinsic tyrosine kinase activity (Blume-Jensen et al, 1991). It is hypothesized that oligomerization is driven by the simultaneous binding of a dimeric SCF molecule to two receptor monomers (Philo et al, 1996; Lemmon et al, 1997). The activated receptor becomes autophosphorylated on a number of tyrosine residues (**Figure 1**). These tyrosine residues are mainly located outside the kinase domain, and serve as docking sites for signal transduction molecules containing Src homology 2 (SH2) or phosphotyrosine binding (PTB) domains (for review, see Pawson, 2004).

The c-Kit ligand, SCF, is expressed as a glycosylated transmembrane protein. Alternative splicing leads to two isoforms of SCF that differ in the absence or presence of a particular proteolytic cleavage site (Huang et al, 1992). The isoform containing the cleavage site undergoes proteolysis and becomes soluble upon release from the plasma membrane, whereas the isoform lacking the

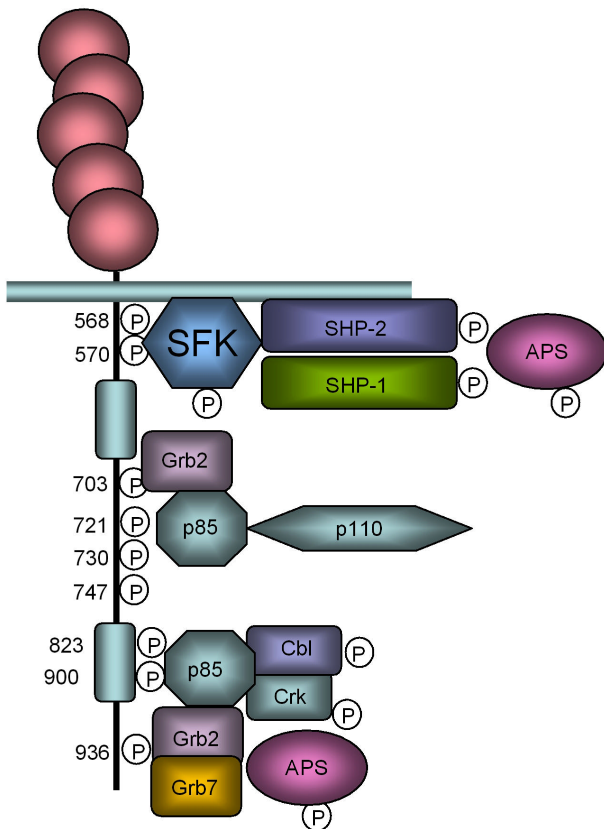

Figure 1. Signal transduction molecules binding to the activated c-Kit receptor. Upon ligand-binding, c-Kit dimerizes and its intrinsic tyrosine kinase activity isactivated, leading to phosphorylation of key residues. These residues constitute high affinity binding sites for signal transduction molecules. The numbers refer to tyrosineresidues phosporylated in c-Kit and the corresponding signal transduction molecule isdepicted.

cleavage site remains cell-associated. The two isoforms are not equivalent in their signaling capabilities. They each have specific abilities to transmit signals. Stimulation with the soluble isoform leads to a rapid and transient activation and autophosphorylation of c-Kit, followed by a fast degradation, whereas stimulation with the membrane-associated isoform leads to a more sustained activation of c-Kit (Miyazawa et al, 1995). Differences exist also in signaling downstream of c-Kit. The membrane-bound ligand induced a more persistent activation of Erk1/2 and p38 mitogen activated protein kinase (MAPK), when compared to the soluble ligand (Kapur et al, 2002). It is thought that the differences in signaling might in part arise from the fact that membrane anchoring of the ligand might prevent internalization of the receptor-ligand complex. Using immobilized agonistic anti-Kit monoclonal antibodies to induce receptor dimerization in the absence of internalization, Kurosawa et al. could mimic the action of membrane-bound SCF (Kurosawa et al, 1996).

B. Internalization and degradation of c-Kit

Ligand-induced down-regulation of RTKs is an important means of modulating signaling in the normal physiology of cell surface receptors. RTKs including c-Kit become ubiquitinated upon ligand-stimulation (Blume-Jensen et al, 1991). It was thought that RTKs become polyubiquitinated and degraded in the proteasomes, but recently it was shown that in the case of RTKs, monoubiqutination rather than polyubiqutination takes place (Haglund et al, 2003) and targets the receptors for internalization and degradation in the lysosomes. This is in contrast to polyubiquitination of cytosolic proteins that targets them for degradation in the proteasomes. Furthermore, other investigators have found that c-Kit is degraded both in the lysosomes and the proteasomes (Zeng et al, 2004).

Important components of the ubiquitination machinery are the ubiquitin E3 ligases that covalently attach ubiquitin moieties to target proteins. In RTK signaling, one of the important E3 ligases is the adapter protein Cbl, and its close family members Cbl-B and Cblc, which bind to activated receptors and other tyrosine phosphorylated proteins via its SH2 domain (Joazeiro et al, 1999). Phosphorylation by SFKs leads to activation of Cbl (Yokouchi et al, 2001). Cbl is able to bind to activated c-Kit through the adapter proteins CrkL (Wisniewski et al, 1996; Sattler et al, 1997) and APS (Wollberg et al, 2003) and is phosphorylated in response to SCF stimulation.

Several studies have shown that internalization of c-Kit is dependent on the activity of SFKs (Broudy et al, 1999; Jahn et al, 2002; Voytyuk et al, 2003). Given the role of monoubiquitination in internalization, it is likely that SFK-dependent activation of Cbl and subsequent monoubiquitination regulates internalization of c-Kit. Recently, Zeng et al, could demonstrate that degradation of c-Kit was partly mediated by the proteasomal and lysosomal pathway (Zeng et al, 2004).

C. The Ras/Erk pathway

Numerous studies have demonstrated the critical importance of the Ras/Erk pathway in cell division, survival and transformation (Lewis et al, 1998). Ras is a small G-protein that can alternate between an active GTP-bound form and an inactive GDP-bound form. Although Ras can activate a number of signal transduction molecules such as PI3-kinase and Rac (Qiu et al, 1995; Rodriguez-Viciana et al, 1997), its role in the Ras/Erk cascade is the most well characterized. RTKs activate Ras through association with Sos, a guanine nucleotide exchange factor that facilitates exchange of GDP for GTP leading to activation of Ras (Buday and Downward, 1993). In the cell, Sos exists in a preformed complex with the adapter protein Grb2, which in turn associates via its SH2 domain to phosphorylated tyrosine residues within the consensus sequence pYXN. These tyrosine residues exist either in the receptor or in downstream signal transduction molecules such as the protein tyrosine phosphatase SHP-2, the adapter proteins ShcA (Lowenstein et al, 1992; Rozakis-Adcock et al, 1992; Li et al, 1994) Gab-1 or Gab-2 (Nishida et al, 1999). Thus, the Grb2-Sos complex is recruited to the vicinity of the plasma membrane, where it can act on Ras. Activated GTP-bound Ras has the ability to interact with the serine/threonine kinase Raf-1 leading to its activation. Raf-1 kinase phosphorylates and activates the dual-

specificity kinases Mek1 and Mek2 (Kyriakis et al, 1992). The serine/threonine kinases Erk1 and Erk2 are activated through phosphorylation by Mek1/2 (Crews and Erikson, 1992). Activated Erks dimerize and are translocated to the nucleus (Khokhlatchev et al, 1998) where transcription factors are phosphorylated. Phosphorylation of transcription factors, such as c-Fos, regulates their activity and thereby influences gene transcription (Murphy et al, 2002).

A number of studies have demonstrated the ability of SCF to activate the Ras-Erk pathway. The adapter protein Grb2 can directly associate with c-Kit (Tauchi et al, 1994a) through phosphorylated Y703 and Y936 in c-Kit (Thömmes et al, 1999). In addition, Grb2 can associate with SHP-2, ShcA, Gab-1 and Gab-2 following SCF stimulation (Nishida et al, 1999; Tauchi et al, 1994a,b). The adaptor protein Gab-2 can link to the Ras/Erk pathway through association with SHP-2 (Dorsey et al, 2002). Several studies have suggested an important role for SCF-induced activation of SFKs in the activation of the Ras/Erk cascade (Bondzi et al, 2000; Ueda et al, 2002; Kimura et al, 2004; Lennartsson et al, 1999;). However, others have shown no effect for SFK inhibition on the activity of Erk (Timokhina et al, 1998). In the same study, an independence of PI3-kinase for activation of Erk in mast cells was also demonstrated. However, under certain conditions activation of Erk has been implicated to be dependent on the activity of PI3-kinase. Recently, Wandzioch et al. showed that inhibition of PI3-kinase with the pharmacological agent LY294002 effectively inhibited Erk phosphorylation in a hematopoietic progenitor cell line (Wandzioch et al, 2004). Moreover, it has been demonstrated that GM-CSF and SCF synergistically activated the Ras/Erk pathway in Mo7e cells in a PI3-kinase dependent manner (Lennartsson et al, 2004). However, in these cells Erk phosphorylation by SCF or GM-CSF alone was independent on PI3-kinase.

D. PI3-kinase

PI3-kinase is a class of lipid kinases that phosphorylate the 3'-hydroxyl group of phosphoinositides. Although phosphatidylinositol can be phosphorylated by PI3-kinase *in vitro*, phosphatidylinositol-4,5-bisphosphate (PIP_2) is the physiologically relevant substrate (for review, see Foster et al, 2003). The resulting product, phosphatidylinositol-3,4,5-trisphosphate (PIP_3), is able to physically associate with proteins containing a pleckstrin homology (PH) domain, leading to their recruitment to plasma membrane where they can be activated.

A majority of the published work on PI3-kinase has been focused on the classical class I PI3-kinases. The regulatory p85 subunit of PI3-kinase contains two SH2 domains by which it binds to target proteins. Upon binding, conformational changes are induced and PI3-kinase enzyme is activated (Carpenter et al, 1993). The p110 subunit of PI3-kinase contains the enzymatic activity to phosphorylate phosphoinositides. The two subunits exist in the cell as a preformed complex. Interestingly, there is a stoichiometric excess of p85 subunit within the cell (Ueki et al, 2002), suggesting other functions of p85. It is well known that p85 also has the ability to associate with the adapter proteins such as CrkII, CrkL and Cbl, respectively (Hartley et al, 1995; Sattler et al, 1997).

The activation of PI3-kinase by c-Kit has been linked to mitogenesis, differentiation, adhesion, secretion, survival, and actin cytoskeletal reorganization (Serve et al, 1995; Vosseller et al, 1997; Blume-Jensen et al, 1998; Kubota et al, 1998; Timokhina et al, 1998). In c-Kit, Y721 was found to directly interact with PI3-kinase (Serve et al, 1995). c-Kit promotes survival via PI3-kinase dependent activation of Akt and phosphorylation of Bad, a pro-apoptotic molecule, at S136 *in vivo*. Furthermore, mutation of S741 and S746 in c-Kit, the two serine sites involved in negative regulation by PKC, led to increased mitogenic response and increased activation of PI3-kinase, as well as enhanced Akt activation, Bad phosphorylation and survival (Blume-Jensen et al, 1998). Recently Jin et al. showed that in cultured primordial follicles of the ovary, stimulation of c-Kit led to prevention of apoptosis through a PI3-kinase mediated pathway (Jin et al, 2005). SCF stimulation led to upregulation of the anti-apoptotic proteins Bcl-2 and Bcl-xL, while it induced a downregulation of the expression of the pro-apoptotic factor Bax. Inhibition of PI3-kinase reversed the regulation of Bcl-xL and Bcl-2, but not Bax.

An alternative pathway for c-Kit mediated survival is through Akt-mediated phosphorylation and inactivation of the forkhead transcription factor FoxO3a (Engström et al, 2003). Whether FoxO transcription factors or Bad are involved in the anti-apoptotic signaling induced by c-Kit seems to be cell type dependent.

A number of studies have implicated the importance of PI3-kinase activity in transforming ability of mutated c-Kit. Using immortalized murine progenitor cells transduced with the Y721F mutant D816V c-Kit, Chian et al. showed that transformation by this constitutively active form of c-Kit is dependent on PI3-kinase (Chian et al, 2001). In another study, mutants with the cytoplasmic tyrosines of c-Kit individually mutated to phenylalanine residues were used to assess the role of individual signal transduction pathways in D816V transformation (Hashimoto et al, 2003). Two mutants, Y721F and a deletion mutant in which the two most distal tyrosines in the carboxyterminal tail were mutated, severely impaired receptor activation. The deletion included Y936, that previously was shown to be a docking site for Grb2, Grb7 and APS (Thömmes et al, 1999; Wollberg et al, 2003). Interestingly, in contrast to their effect on transformation, these mutants showed no effect on normal ligand-induced activation of c-Kit.

The physiological role of c-Kit-mediated activation of PI3-kinase was demonstrated in two studies using transgenic mice expressing c-Kit with a Y721F mutation. Blume-Jensen et al. showed that c-Kit-induced activation of PI3-kinase was essential for male fertility, while in another study Kissel et al. also could demonstrate an effect on female fertility ((Blume-Jensen et al, 2000; Kissel et al, 2000). Thus, loss of c-Kit mediated PI3-kinase signaling may be compensated for in a number of physiological processes, but appears to be critical in gametogenesis. These findings assume that Y721 solely serves as the docking site for PI3-kinase and for no other signal

transduction molecule. This, however, remains to be proven. One of the PI3-kinase association sites present in the closely related PDGF -receptor, Y751, has also been known to bind to the adapter protein Nck (Nishimura et al, 1993). Thus, some of the found Y721F phenotypic outcomes might be due to blocking of additional, hitherto unknown interactions and signal transduction pathways.

Bone-marrow-derived mast cells from mice with a targeted deletion of the p85 subunit of PI3-kinase demonstrated a dramatically reduced SCF-mediated proliferative response compared to wild-type cells, further emphasizing the role of PI3-kinase in c-Kit signaling (Fukao et al, 2002). This effect paralleled a reduction of SCF-induced activation of JNK in the p85 -deficient mast cells. Interestingly, SCF-stimulated activation of Akt was only partially impaired in p85 -deficient bone marrow derived mast cells, and no effect on c-Kit mediated survival was observed. Therefore additional, PI3-kinase independent signal transduction pathways may contribute to c-Kit-mediated survival. In addition, SCF-mediated chemotaxis has also been demonstrated to be dependent on p85 (Tan et al, 2003).

It should be noted that discrepancies do exist in results obtained from p85 -deficient cells and Y721F mutant c-Kit. The reason for these differences could be that Y721 is able to dock to signal transduction molecules other than PI3-kinase, as mentioned above. Furthermore, p85 is known to bind to proteins other than the p110 subunit of PI3-kinase. In an elegant study, Ali and colleagues generated transgenic mice expressing a kinase inactive form of the p110 subunit of PI3-kinase (Ali et al, 2004). Thus, the lipid kinase activity of PI3-kinase was targeted, while other possible functions of the p85 subunits were intact. It was shown that the p110 isoform of PI3-kinase plays an essential role in allergic response. Inactivation of p110 in mast cells led to defective SCF-mediated *in vitro* proliferation, adhesion and migration, as well as impaired allergen-IgE-induced degranulation and cytokine release. Furthermore, mice carrying an inactivated version of p110 were protected against anaphylactic allergic responses.

Apart from the classical type I forms of PI3-kinase, the type II isoform PI3KC2 was shown to physically associate with activated c-Kit and mediate part of the SCF-dependent activation of Akt in small lung carcinoma cells (Arcaro et al, 2002). Interestingly, in contrast to the type I PI3-kinases, PI3KC2b association with c-Kit was ligand-independent and constitutive. However, ligand stimulation of c-Kit led to tyrosine phosphorylation of PI3KC2 . The site of interaction with c-Kit is not known, although given that the association to c-Kit seems constitutive, and that other receptors previously shown to interact with the classical isoforms of PI3-kinase did not interact with PI3KC2 , it appears likely that the classical PI3-kinase association site is not involved.

E. Phospholipase C-

Two isoforms of PLC- exists, PLC- 1 and PLC- 2, and they both consist of two SH2 domains, one SH3 domain, one PH domain and a catalytic domain. While - 1 is ubiquitously expressed, PLC- 2 is mainly expressed in the hematopoietic system (for review, see Carpenter and Ji, 1999). The substrate upon which PLC acts is the phosphoinositide PIP_2, whereby the second messengers DAG and inositol-1, 4, 5-trisphosphate (IP_3) are generated. DAG is an activator of the classical and novel forms of PKC, while IP_3 binds to specific calcium channels present on the endoplasmic reticulum, triggering release of Ca^{2+} from internal stores. The intracellular concentration of free Ca^{2+} regulates a number of cellular processes (for review, see Berridge et al, 2003).

Some studies have demonstrated association with and activation of PLC- by c-Kit, while others have failed to do so. It has been claimed that Y730 is the site of association of PLC- 1. Using overexpression of EGFR-c-Kit chimeras and PLC- 1 in HEK293 cells, Herbst et al, demonstrated ligand-induced tyrosine phosphorylation of PLC- 1, although weaker than that seen with the EGF receptor (Herbst et al, 1991). In a later study, the same authors saw no association between PLC- 1 and the EGFR-c-Kit chimera unless they overexpressed PLC- 1 (Herbst et al, 1995). However, those studies were performed using chimeric receptors with the extracellular domain of the EGF receptor fused to the intracellular part of c-Kit. In addition, association was only seen when the receptor was overexpressed together with overexpressed PLC- 1. In a recent study, Gommerman et al. studied the differential signaling of various c-Kit mutants induced by membrane-bound and soluble SCF, respectively, using retrovirally transduced 32D cells (Gommerman et al, 2000). Using soluble SCF, a weak SCF-stimulated tyrosine phosphorylation of PLC- 1 was shown in wild-type murine c-Kit expressing cells, but in cells expressing the Y730F mutant, no phosphorylation of PLC- 1 was seen. Furthermore, cells expressing the Y730F mutant c-Kit did not respond with calcium release following treatment with SCF. Interestingly, PLC- 1 phosphorylation was much stronger in bone-marrow-derived mast cells than in 32D infectants. Likewise, Trieselmann et al. showed that stimulation of mast cells by membrane-bound, but not soluble SCF, was dependent on PLC- activation (Trieselmann et al, 2003). They also demonstrated ligand-induced tyrosine phosphorylation of PLC- 2. Other studies have shown that activation of PLC- by c-Kit might be involved in the SCF-mediated protection against apoptosis induced by radiation and chemotherapy (Plo et al, 2001; Maddens et al, 2002). Also studying bone marrow mast cells, Huber et al. detected a robust and sustained SCF-stimulated tyrosine phosphorylation of PLC- 2 (Huber et al, 1998). Others, however, failed to detect c-Kit mediated activation of PLC- , but were able to detect SCF-dependent activation of phospholipase D (PLD; Koike et al, 1993). These data were further confirmed by Kozawa et al. who also could inhibit SCF-stimulated PLD activity with the PI3-kinase inhibitor LY294002 (Kozawa et al, 1997).

There are several plausible explanations to these discrepancies. It is possible that some of the differences in the findings as to whether PLC- is activated or not might arise from different expression levels of the two isoforms

of PLC- in different cell types, with the cell types expressing PLC- 2 showing stronger activation of PLC. Another possible explanation for differences in the data on activation of PLC- 1 might be the differential signaling abilities of alternative splice forms of c-Kit. It is known that the two alternative splice forms denoted $GNNK^+$ and $GNNK^-$ do signal at quantitatively and qualitatively different levels (Caruana et al, 1999; Voytyuk et al, 2003). Thus, expression of various splice forms of c-Kit might influence the outcome of studies on PLC- activation. A direct physical interaction between c-Kit and PLC- isoforms has not been demonstrated, except in cells overexpressing the receptor and PLC- 1. Thus, it is possible that activation of PLC- might be a result of activation of other tyrosine kinases downstream of c-Kit that might not necessarily require a direct physical association between PLC- and c-Kit. One such candidate kinase is the Src family of tyrosine kinases.

F. The Src family of tyrosine kinases

The SFK is named after its prototypic family member c-Src, the cellular homolog of the transforming protein of Rous sarcoma virus, v-Src. Some members, like Src, Yes and Fyn, are ubiquitously expressed, while others, such as Lck, Hck, Fgr, Lyn and Blk, display a more restricted expression pattern and are mainly expressed in cells of the hematopoietic lineages (Abram and Courtneidge, 2000). They consist of an N-terminal sequence which directs myristoylation and in some cases palmitoylation. These lipid modifications serve to anchor the kinases to the plasma membrane. They also contain an SH3 domain, an SH2 domain and a tyrosine kinase domain. They have been implicated in a number of cellular functions, including survival, chemotaxis, adhesion, proliferation and protein trafficking.

Ligand-induced activation of c-Kit leads to a rapid increase in SFK activity (Linnekin et al, 1997; Krystal et al, 1998). A number of investigators have shown that SFKs associate primarily with phosphorylated Y568 in c-Kit, while Y570 contributes to the overall affinity of binding by acting as an acidic determinant (Price et al, 1997; Timokhina et al, 1998; Lennartsson et al, 1999). SCF-induced chemotaxis of Mo7e cells was dependent on SFK activity (Broudy et al, 1999). In another study, overexpression of a dominant negative form of Lyn in either primary hematopoietic progenitor cells or bone marrow derived mast cells led to inhibition of both SCF-mediated proliferation and chemotaxis (O'Laughlin-Bunner et al, 2001). In Mo7e cells, activation of the SFK Lyn was demonstrated to occur during the late G1 phase of SCF-stimulated cell cycle progression (Mou and Linnekin, 1999). Using an approach where 32D cells were transfected with chimeric c-Kit containing the extracellular domain of the M-CSF receptor, and by mutating seven tyrosine residues of the intracellular part of c-Kit, Hong et al. demonstrated a complete loss of mitogenic response of 32D cells (Hong et al, 2004). However, by adding back Y568 and Y570 to this mutant, the mitogenic response was restored, as well as survival and migration. Furthermore, restoration of the Src binding sites also lead to restored activation of the Ras/Erk pathway. This is in agreement with previous findings that SFKs play an important role in phosphorylating ShcA, thereby recruiting the Grb2-Sos complex, leading to activation of Ras (Lennartsson et al, 1999; Bondzi et al, 2000). In addition, SCF-induced activation of other signal transduction molecules such as Rac and JNK were shown to be restored by adding back Y568 and Y570.

The function of SFKs in a more physiological context was addressed by Agosti et al, (2004) who generated transgenic mice carrying c-Kit with a Y568F mutation. They found that mutation of Y568, the primary binding site of SFKs in c-Kit, led to a block in pro T cell and pro B cell development, in contrast to the Y721F mutant (affecting PI3-kinase activation) that had no effect on hematopoiesis. These data suggest that SFKs mediate a critical signal for lymphocyte development. However, one of the difficulties in interpreting these data lies in the probability that additional signal transduction molecules apart from SFKs might be involved. For example, the protein tyrosine phosphatase SHP-2 (Kozlowski et al, 1998), the tyrosine kinase CHK (Jhun et al, 1995) and the adaptor protein APS (Wollberg et al, 2003) have also been shown to bind to phosphorylated Y568.

Using a similar approach, Kimura et al. generated mice carrying the Y568F and Y570 of c-Kit. These tyrosine residues were shown to be crucial for the function of c-Kit in melanogenesis and mast cell development. In contrast, they were dispensable for the normal development of erythroid cells, interstitital cells of Cajal and germ cells. They also displayed splenomegaly, dysregulation of B-cell and megakaryocyte development (Kimura et al, 2004). However, since Y568 has been demonstrated to bind to the protein tyrosine phosphatase SHP-2 (Kozlowski et al, 1998) and the adapter protein APS (Wollberg et al, 2003), and Y570 has been shown to be associating with the protein tyrosine phosphatase SHP-1 (Kozlowski et al, 1998), the phenotype of the Y568F/Y570F mice might not necessarily solely reflect a function of the SFK, but also the function of other signal transduction molecules.

G. Other tyrosine kinases: Tec, CHK, Fer and Fes

Tec belongs to a family of tyrosine kinases that also includes the Bruton tyrosine kinase (Btk), Bmx, Itsk/Tsk, and Rlk/Txk (Smith et al, 2001). They each contain a pleckstrin homology (PH) domain, and a Tec homology (TH) domain in the amino-terminus followed by SH3, SH2 and tyrosine kinase domains. In contrast to the SFKs, they lack membrane targeting myristoylation site, but are recruited to the plasma membrane through the PH domain interacting with membrane-bound PIP_3. Activation of Tec family kinases is thought to be mediated by members of the Src family.

Tec is phosphorylated on tyrosine residues and activated upon ligand stimulation of c-Kit (Tang et al, 1994). More recent studies have shown that Tec forms multiprotein complexes with Lyn and the adapter protein Dok-1 (van Dijk et al, 2000; Liang et al, 2002). Phosphorylation of Tec and Dok-1 was dependent on their recruitment to the plasma membrane through activation of

PI3-kinase (van Dijk et al, 2000). Both Lyn and Tec were capable of phosphorylating Dok-1, but using cells derived from animals with a targeted deletion of Lyn (Liang et al, 2002) it was shown that Lyn was required for SCF-dependent phosphorylation of Dok-1.

CHK (for Csk Homologous Kinase, also known as MATK) displays an about 50 % sequence identity with Csk, and like Csk it phosphorylates and inactivates SFKs. Similarly to SFKs, CHK has been shown to associate to the phosphorylated Y568 in the juxtamembrane region of ligand-stimulated c-Kit (Jhun et al, 1995).

The cytoplasmic tyrosine kinases Fer and Fes belong to a separate family of tyrosine kinases. They both contain an SH2 domain immediately aminoterminal to the kinase domain. Following SCF-stimulation Fer associates with c-Kit and becomes phosphorylated on tyrosine residues (Kim and Wong, 1995). Using mast cells derived from transgenic mice carrying a kinase inactivating mutation of Fer, Craig and Greer found a requirement for Fer kinase activity for sustained p38 kinase activation and maximal chemotactic response to SCF (Craig and Greer, 2002). Fes has been found to bind to c-Kit (Masuhara et al, 2000), although its role in c-Kit signaling remains to be shown.

H. The JAK/STAT pathway

The Janus kinases (JAKs) are a family of closely related cytoplasmic tyrosine kinases that are activated through ligand stimulation of cytokine receptors or RTKs. Downstream targets of the JAKs are the signal transducers and activators of transcription (STAT) that are phosphorylated by JAKs and thereby activated. STAT proteins are a class of transcription factors with DNA binding domains, an SH2 domain and a carboxy-terminal transactivating domain. Upon tyrosine phosphorylation, STATs dimerize through interaction between the phosphorylated tyrosine residues and the SH2 domain of STAT, leading to dimerization. STATs can form both homodimers of identical STATs or heterodimers between different members of the STAT family. The dimerized STATs translocate to the nucleus, where they regulate expression of responsive genes (for review, see Kerr et al, 2003). The JAK/STAT pathway is activated following SCF stimulation. c-Kit stimulates rapid and transient tyrosine phosphorylation of JAK2 (Brizzi et al, 1994). JAK2 was found to be constitutively associated with c-Kit, with increased association after ligand stimulation of c-Kit (Weiler et al, 1996). A role of JAK2 in c-Kit mediated signaling was suggested by the finding that treatment of cells with JAK2 antisense oligonucleotides resulted in a marked decrease in SCF-induced proliferation. In addition, SCF-induced growth of fetal liver cells from mice carrying a targeted deletion of JAK2 was shown to be reduced compared to cells from wild-type mice (Radosevic et al, 2004). Furthermore, JAK2 was also required for differentiation of the Kit+ progenitor cells into mast cells.

Ligand-stimulated activation of c-Kit leads to physical association with and activation of STAT1 STAT3, STAT5A and STAT5B (Brizzi et al, 1999; Deberry et al, 1997; Ning et al, 2001; Ryan et al, 1997). It has been shown that STAT3 activation is required for the constitutively active D816H mutant of c-Kit in order to be tumorigenic (Ning et al, 2001).

However, as is the case with the activation of PLC- , there are discrepancies in the literature. Some investigators have failed to detect activation of the JAK/STAT pathway by c-Kit (Jacobs-Helber et al, 1997; O'Farrell et al, 1996; Pearson et al, 1998). A possible explanation is cell type specific signaling or differences in the experimental setup as well as the very rapid and transient JAK2 activation by SCF.

I. Adapter proteins

Adapter proteins are proteins with several domains that specify protein-protein interactions. The multiple domains enable them to interact with several proteins simultaneously. The ability of linking proteins together through specific and many times regulated protein-protein interactions, enables signaling to be sequentially and spatially regulated (for review, see Pawson and Scott, 1997).

Grb2 was originally identified as a protein interacting with the phosphorylated EGF receptor (Lowenstein et al, 1992) and found to mediate activation of the Ras/Erk pathway by RTKs. Grb2 is a ubiquitously expressed protein containing one SH2 domain and two SH3 domains. Tyrosine phosphorylated c-Kit has been shown to associate with Grb2 (see above under C "The Ras/Erk pathway") through Y703 and Y936 of c-Kit (Thömmes et al, 1999).

The adapter protein Gads (also denoted Mona, Grap2, GrpL or Grf40) is closely related to Grb2 and expressed in hematopoietic cells (for review, see Liu et al, 2001) and has been shown to interact with c-Kit in a manner similar to Grb2 (Liu and McGlade, 1998). However, it is not known whether Gads interact with the same tyrosine residues as Grb2. Another member of the same family of adapter proteins with a very similar structure, Grap, also interacts with c-Kit (Feng et al, 1996).

ShcA is a ubiquitously expressed adapter protein that contains one SH2 domain and a PTB domain, that both enable ShcA to interact with phosphorylated proteins (for review, see Ravichandran, 2001). Phosphorylation of ShcA by RTKs, directly or indirectly via SFKs, leads to the creation of high affinity binding sites for Grb2, which in turn leads to activation of the Ras/Erk pathway. *In vitro* data suggest that ShcA interacts with the juxtamembrane domain of c-Kit (Price et al, 1997).

The adapter protein Grb7 belongs to a family of closely related proteins consisting of Grb7, Grb10 and Grb14 that each exist as several alternatively spliced variants (for review, see Han et al, 2001). Grb7 contains an SH2 and a so-called GM region (for Grb and Mig) which includes a PH domain and shows sequence homology with the C. elegans protein Mig-10, that has been implicated in embryonic migration. Grb7 interacts with activated c-Kit through Y936 in the carboxyterminal tail of the receptor (Thömmes et al, 1999). However, the exact role of Grb7 in c-Kit signaling remains to be elucidated. Grb10 was identified in a yeast two-hybrid screen using the D816V constitutively active mutant of c-

Kit as a bait (Jahn et al, 2002). The interaction between Grb10 and c-Kit was shown to be mediated through its SH2 domain, while the PH domain mediates interaction with the serine/threonine kinase Akt. It was further demonstrated that Grb10 and c-Kit are able to activate Akt in a synergistic manner.

The adaptor protein Lnk belongs, together with APS and SH2-B, to a family of closely related adapter proteins. All three proteins share a common domain structure in that they contain a conserved amino-terminal domain that includes a proline-rich stretch, a PH domain and an SH2 domain. They all contain a conserved tyrosine residue in their carboxy-termini that is presumed to be a phosphorylation site mediating interaction with Cbl (Iseki et al, 2000). Using transgenic mice lacking the expression of Lnk, it was shown that B cell precursor cells were hypersensitive to SCF stimulation (Takaki et al, 2000), leading to proportional accumulation of B cell precursors in the bone marrow and B cells in the spleen of transgenic mice. Thus, these data suggest that Lnk has a negative regulatory role in B cell production.

APS was originally identified in a yeast two-hybrid screen using constitutively active c-Kit as bait (Yokouchi et al, 1997). When APS is phosphorylated in its carboxyterminal tail, it physically associates with Cbl (Wakioka et al, 1999; Yokouchi et al, 1999). Being a ubiquitin E3-ligase, Cbl is able to monoubiquitinate activated RTKs, leading to their internalization and degradation in the lysosomes (Haglund et al, 2003). The primary associations sites for APS in c-Kit have been shown to be phosphorylated Y568 and Y936 (Wollberg et al, 2003). Mutation of both Y568 and Y936 was necessary to completely block binding of APS to c-Kit. Recently it was shown that APS exists as a dimer (Hu et al, 2003), which might explain why both sites are needed for full binding of APS to c-Kit. Interestingly, in the viral form of kit, v-Kit, both Y568 and Y936 are missing (Herbst et al, 1995). Thus, it has been speculated that loss of binding of APS, or maybe SH2-B or Lnk, in v-Kit could possibly lead to reduced ubiquitination and prolonged receptor signaling, that could possibly contribute to transformation. A number of transforming mutants of RTKs have been shown to lack association sites for Cbl, leading to reduced ubiqutination and stabilization of active receptors (Peschard and Park, 2003).

However, the physiology of mice with a targeted deletion of APS does not support a major role for APS in c-Kit signaling. The effects are mainly related to the immune system (Iseki et al, 2004), although mast cells derived from APS knock-out animals show a markedly augmented degranulation in response to c-Kit stimulation, as well as lower levels of F-actin (Kubo-Akashi et al, 2004). In contrast, targeted deletion of either Lnk or SH2-B did not lead to any marked effect on mast cell behavior.

Dok-1 is an adapter protein of 62 kDa originally identified as a tyrosine-phosphorylated protein associated with p120-RasGAP in fibroblasts transfected with v-Src (Ellis et al, 1990). It contains a phosphotyrosine binding (PTB) domain and a PH domain. Cells from Dok-1 knockout mice hyperproliferate in response to stimulation with a number of growth factors and cytokines, suggesting a role of Dok-1 as a negative regulator of cell proliferation (Yamanashi et al, 2000). Dok-1 was found to associate with activated c-Kit in chronic myelogenous leukemia progenitor cells (Carpino et al, 1997).

The Gab/Dos family of adaptor proteins are a family of scaffolding adaptors with similar overall structural organization (reviewed in Gu and Neel, 2003), containing an N-terminal PH domain, proline-rich motifs that can interact with SH3 domains and multiple tyrosine phosphorylation sites that can serve as docking sites for SH2 domain containing proteins. Both Gab-1 and Gab-2 are phosphorylated in response to SCF-stimulation (Nishida et al, 1999). Gab-2 is required for mast cell development and c-Kit signaling (Nishida et al, 2002), while Gab-1 does not seem to be essential for c-Kit signaling. Bone marrow mast cells derived from Gab-2-deficient mice grew poorly in response to SCF and activation of both Erk and Akt were impaired.

The Crk family of adapter proteins consists of one SH2 domain, as well as one or two SH3 domains. The family consists of four members: CrkI and CrkII (alternative splice forms of the same gene), CrkL (Feller, 2001) and the recently discovered CrkIII (Prosser et al, 2003). Ligand stimulation of c-Kit leads to phosphorylation of CrkL (Sattler et al, 1997), which indirectly associates with c-Kit through the p85 subunit of PI3-kinase. In addition, CrkL mediates interaction with Cbl, which thus likely contributes to c-Kit ubiquitination and degradation. The closely related protein CrkII was also shown to be phosphorylated in response to SCF stimulation and interact with c-Kit also indirectly via the p85 subunit of PI3-kinase (Lennartsson et al, 2003). This interaction was dependent on phosphorylation of Y900 in the second part of the kinase domain, which is not an autophosphorylation site but is phosphorylated through the action of SFKs.

J. Protein tyrosine phosphatases

The two closely related protein tyrosine phosphatases (PTPs) SHP-1 and SHP-2 constitute a family of proteins consisting of two amino-terminal SH2 domains, a PTP domain and a carboxyterminal tail (Neel et al, 2003). A number of SHP binding proteins have been reported including activated cytokine receptors and RTKs, as well as scaffolding adaptors, such as the Gab proteins. Activation of SHPs occurs through binding of the SH2 domains to tyrosine phosphorylated peptides, in particular biphosphorylated. Binding of the phosphopeptide leads to opening up of the phosphatase structure. SHP-1 associates with phosphorylated Y570 in c-Kit (Kozlowski et al, 1998) and is involved in negative regulation of c-Kit signaling (see also "Negative regulation of c-Kit signaling"). In contrast, SHP-2 is, despite the fact that it is a phosphatase, a positive regulator of signaling. SHP-2 physically interacts with SCF-stimulated c-Kit and becomes phosphorylated on tyrosine residues (Tauchi et al, 1994). The site of interaction was shown to be Y568 in the juxtamembrane region of c-Kit (Kozlowski et al, 1998), which also constitutes the docking site for a number of other signal transduction molecules, such as SFKs, CHK and APS. In most RTK signaling, SHP-2

activation is required for full activation of the Ras/Erk pathway, *e. g.* the PDGF -receptor (Rönnstrand et al, 1999). SHP-2 also plays an important role in mediating embryonic stem cell differentiation and hematopoiesis (Chan et al, 2003).

The tyrosine phosphatase PTP-RO is despite its lack of SH2 domains still able to associate with the c-Kit receptor (Taniguchi et al, 1999). Furthermore, PTP-RO becomes phosphorylated on tyrosine residues after SCF stimulation. By use of antisense oligonucleotides the function of PTP-RO could be inhibited, which led to significantly inhibited proliferation of Mo7e cells (Taniguchi et al, 1999).

Similar to PTP-RO, the protein tyrsoine phosphatase PTP-PEST was shown to bind to c-Kit in a ligand-dependent manner (Markova et al, 2003). However, the mode of interaction, the site of interaction and its role in c-Kit signaling remains to be elucidated.

K. Transcription factors

A number of genes are induced upon SCF-stimulation of cells. One is the Mitf protein, which is a member of the MYC superfamily of transcription factors (Hodgkinson et al, 1993; Hughes et al, 1993; Boissy and Nordlund, 1997) and is closely related to three other basic Helix-Loop-Helix Leucine Zipper (bHLHZip) transcription factors, Tfe3, Tfeb and TfeC. The phenotype of Mitf mutant mice is strikingly similar to that of mice with loss-of-function mutations of c-Kit or its ligand (spotted fur color, mast cell deficiency; reviewed in Boissy and Nordlund, 1997). This suggested a functional link between the Mitf transcription factor and c-Kit and its ligand. This has been proven by a number of investigators. *In vitro* experiments have demonstrated that the activity of the Mitf transcription factor is regulated by signaling through the c-Kit receptor tyrosine kinase. Ultimately, this signaling results in effects on the activation potential and/or stability of the Mitf protein (Hemesath et al, 1998; Wu et al, 2000; Weilbaecher et al, 2001). SCF stimulation of c-Kit results in Erk2-dependent phosphorylation of S73 of Mitf, which regulates its transcriptional activity. Furthermore, Price et al. have shown that only the phosphorylated version of Mitf can interact with the p300 co-activator protein (Price et al, 1998). Phosphorylation of S409 by the p90/Rsk kinase, downstream of Erk, is a second phosphorylation event that has been shown to link c-Kit and Mitf. This phosphorylation has been shown to affect the protein stability such that Mitf protein phosphorylated on S409 is degraded more rapidly than a mutant S409A Mitf protein. The increased degradation was shown to correlate with increased polyubiquitination of the protein that targets it for proteosome-dependent degradation (Wu et al, 2000). Recently, a functional cross talk between Mitf, protein inhibitor of activated Stat3 (PIAS3) and Stat3 was demonstrated (Sonnenblick et al, 2004). PIAS proteins are a class of proteins that act as inhibitors of Stat activity (Wormald and Hilton, 2004). Upon SCF-stimulation, PIAS3 is translocated from Mitf to the activated Stat3 due to phosphorylation of Mitf at S409. It is sugggested that such a mechanism leads to a fine-tuning of the regulation of transcriptions factors downstream of c-Kit.

Another transcription factor involved in c-Kit signaling is Slug, a member of the Snail family of zinc finger transcription factors. Mice with a targeted deletion of Slug show pigment deficiency, gonadal defects, and impairment of hematopoiesis, very much reminiscent of the phenotype of loss-of-function mutations in c-Kit (Perez-Losada et al, 2002). It was demonstrated that cells from Slug knockout animals despite the expression of c-Kit were defective in SCF-induced migration, suggesting a role for Slug downstream of c-Kit. It was recently shown that Slug function in c-Kit mediated radioprotection (Perez-Losada et al, 2003). Furthermore, in malignant mesothelioma, which is often resistant to conventional chemotherapy, it was established that c-Kit-dependent up-regulation of Slug conferred drug resistance (Catalano et al, 2004). In contrast, down-regulation of Slug using RNAi made the cells susceptible to apoptosis induced by chemotherapeutic drugs. Thus, Slug appears to have a central position in SCF-mediated protection from apoptosis induced by chemotherapy. Therefore development of therapeutic agents that target Slug or Slug regulated genes may provide ways to sensitize tumors for conventional chemotherapy.

L. Other signal trandsduction molecules

SWAP-70 is an unusual protein that was originally found as a part of a multiprotein DNA recombination complex in activated B cells (Borggrefe et al, 1998). It was later shown to also be expressed in mast cells. It carries a PH domain, that is required for membrane localization, three nuclear localization motifs and a nuclear export signal, and a Dbl domain. Recently, it was shown that mast cells derived from mice carrying a targeted deletion of SWAP-70 display impaired responses to c-Kit activation (Sivalenka and Jessberger, 2004). SCF-induced migration was deficient both in *in vitro* and *in vivo* assays. The reduced chemotaxis could be explained by aberrant polymerization of F-actin and impaired Rac activation. Furthermore, both SCF-induced calcium fluxes and Akt activation were impaired in SWAP-70$^{-/-}$ mast cells.

M. Negative regulation of c-Kit signaling

In a number of cases, receptor tyrosine kinases have been shown to be regulated by serine/threonine kinases, such as protein kinase C (PKC), including the Met receptor (Gandino et al, 1994), the EGF receptor (Morrison et al, 1993) and the insulin receptor (Bollag et al, 1986). Blume-Jensen et al. could demonstrate that also the tyrosine kinase activity of c-Kit can be modulated through phosphorylation by PKC (Blume-Jensen et al, 1994). Down-modulation of c-Kit activity by PKC occurs through dual mechanisms. Activated PKC phosphorylates S741 and S746 in the kinase insert region of c-Kit, which leads to inhibition of kinase activity (Blume-Jensen et al, 1994; Blume-Jensen et al, 1995). Conversely, treatment of cells with the PKC inhibitor calphostin C, resulted in enhanced kinase activity of c-Kit and, furthermore, selectively increased activation of PI3-kinase (Blume-

Jensen et al, 1994). Mutation of S741 and S746 to alanine residues, resulted in a gain of function and markedly increased tyrosine kinase activity of c-Kit (Blume-Jensen et al, 1998). In addition, treatment of cells with phorbol myristate acetate (PMA), an activator of PKC, results in proteolytic release of the ligand-binding domain of c-Kit, that leads to decreased responsiveness to SCF stimulation (Yee et al, 1993, 1994). Stimulation of c-Kit with soluble SCF results in phosphoinositide 3'-kinase (PI3-kinase) dependent activation of phospholipase D (Kozawa et al, 1997), leading to release of phosphatidic acid, which can be dephosphorylated to yield diacylglycerol (DAG), an activator of PKC.

The protein tyrosine phosphatase SHP-1 interacts with Y570 of c-Kit and negatively regulates c-Kit signaling (Yi and Ihle, 1993; Kozlowski et al, 1998). SHP-1 consists of two SH2 domains and a carboxyterminal protein tyrosine phosphatase domain. The motheaten (*me*) mice express a loss-of-function mutation in SHP-1 and show a hyperproliferative phenotype of their hematopoietic progenitor cells (Shultz et al, 1993). However, loss of SHP-1 function did not affect SCF-induced proliferation of bone-marrow derived mast cells, suggesting that the role of SHP-1 might to some extent be cell-type specific (Lorenz et al, 1996).

The suppressors of cytokine signaling (SOCS) are a family of proteins that were originally cloned based on their ability to suppress cytokine signaling (for review, see (Wormald and Hilton, 2004)). They have a central SH2 domain flanked by an N-terminal domain of variable length and a C-terminal domain of 40 amino acids denoted the SOCS box. In a yeast two-hybrid screen using c-Kit as a bait, SOCS-1 was identified as an interactor with c-Kit (De Sepulveda et al, 1999). Its expression is induced upon stimulation of mast cells with SCF, and it associates with c-Kit via its SH2 domain. In contrast to its function in cytokine signaling, SOCS-1 selectively suppressed c-Kit-stimulated mitogenesis, while not affecting survival signals. The mechanism does not involve inactivation of the tyrosine kinase activity of c-Kit, but rather binding of Grb2 to SOCS-1 via its SH3 domain. SOCS-1 in its turn binds to Vav (De Sepulveda et al, 1999). Interestingly, targeted deletion of SOCS-1 did not lead to enhanced c-Kit signaling in bone marrow derived mast cells, as one might have expected, but rather a reduced proliferative response to SCF-stimulation (Ilangumaran et al, 2003). Furthermore, deletion of SOCS-1 led to increased levels of proteases, leading to degradation of signal transduction molecules.

III. c-Kit in homeostasis

There exist a large number of natural loss-of-function mutations in the *W* and *Sl* loci in mice, encoding for c-Kit and SCF respectively. The consequence of these mutations range from minor defects in catalytic activity to complete loss of c-Kit kinase activity as well as reduced to complete loss of expression of both c-Kit and SCF (Lev et al, 1994). These mutations have enabled scientists to appreciate the diverse effect c-Kit signaling has during mouse development and adult life. Detailed analysis of this extensive panel of mouse mutants has suggested critical functions of this receptor/ligand pair in the hematopoietic system, fertility, pigmentation, gut movement as well as in the nervous system (Russell, 1979; Keshet et al, 1991; Lev et al, 1994). Below is a short summary of the major *in vivo* functions of c-Kit signaling.

A. Hematopoiesis

Hematopoietic stem cells (HSC) have the ability to self-renew as well as differentiate into all hematopoietic cell lineages. HSC often divide asymmetrically, *i.e.* the daughter cells become a new HSC whereas the other starts to differentiate and lose the ability to self-renew. During the process of lineage commitment the ability to self-renew decreases but the proliferative activity increases leading to an expansion of cell number. In general, c-Kit is expressed on primitive hematopoietic cells such as stem- and progenitor cells and is lost during differentiation (Ogawa et al, 1991; Okada et al, 1991; Broudy, 1997; Lyman and Jacobsen, 1998). In fact, less than 0.1% of peripheral blood cells express c-Kit suggesting a minor role for c-Kit in differentiated hematopoietic cells (Ashman et al, 1991; Papayannopoulou et al, 1991; Broudy et al, 1992). Primitive hematopoietic cells depend on SCF for growth and survival, often in synergy with other growth factors and cytokines. An important exception among the hematopoietic cells are mast cells that retain a high c-Kit expression even as fully differentiated cells and depend on c-Kit signal transduction for their survival, growth and function (Oliveira and Lukacs, 2003). As a single factor SCF can induce differentiation of primitive hematopoietic cells from fetal mouse livers into mast cells (Radosevic et al, 2004). Hematopoietic defects in *W* and *Sl* mice include the development of erythrocytes, megakaryocytes and mast cells (Lev et al, 1994; Russell, 1979). *W/W* mice can be rescued by transplantation of wild-type HSC indicating that the lethality of these mice is due to anemia. A recent study demonstrated that transgenic expression of Epo can rescue *W/W* mice and thus support hematopoiesis in the absence of c-Kit (Waskow et al, 2004). This indicates that signals emanating from c-Kit that support hematopoiesis are not unique and can be replaced, at least in a mouse model, by signals from other receptor/ligand pairs.

Among the lymphoid lineages, c-Kit plays a role for the maintenance of the immune system in adult animals (Waskow et al, 2002). Thus, treatment with c-Kit inhibitors for an extended time period might affect the immune system. Indeed, in older mice, treatment with the kinase inhibitor STI-571 (Gleevec, imatinib mesylate) leads to a defect in development of pro-B and pro-T-cells (Agosti et al, 2004). These results are important to consider before initiating a long-term STI-571 treatment in patients.

B. Fertility

The fertility deficiency in *W* and *Sl* mice is probably linked to the loss of the normal ability of c-Kit to protect germ cells from apoptosis and to induce their migration and proliferation (Loveland and Schlatt, 1997). One important pathway for the ability of c-Kit to inhibit apoptosis is the PI3-kinase/Akt pathway (Blume-Jensen et

al, 1998). Indeed, knock-in studies using a mutant c-Kit receptor (c-Kit^{Y719F}) unable to activate PI3-kinase resulted in sterile male mice (Blume-Jensen et al, 2000 ; Kissel et al, 2000). In the work by Kissel et al. also female mice had a reduced fertility (Kissel et al, 2000). These studies suggest that c-Kit in germ cell biology is involved in the processes of oogenesis, folliculogenesis and spermatogenesis. Furthermore, the function of c-Kit in germ cells is strictly dependent on its ability to activate PI3-kinase. Interestingly, these mice displayed no other phenotype, indicating that other cell types have redundant systems to compensate for the loss of PI3-kinase signaling downstream of c-Kit. In addition there exists a truncated form of c-Kit (tr-Kit) that is produced by an alternative intronic promotor which is active during spermatogenesis (Rossi et al, 2003). Even though tr-Kit lacks kinase activity it can activate the mouse egg if microinjected, in a SFK- and PLC- dependent manner (Sette et al, 1997, 1998, 2002). It is likely that tr-Kit becomes phosphorylated by intracellular kinases, *e.g.* Src family kinases, and functions as an adaptor protein. However, the molecular function is presently not clear. The fact that tr-Kit was recently discovered in human prostate cancer (Paronetto et al, 2004) warrants further investigation of this protein also in relation to cancer.

C. Pigmentation

The observation that mice with reduced c-Kit function have defective pigmentation is believed to be linked to the ability of SCF to induce proliferation and guide migration of melanocytes from the neuronal crest to the dermis (Scott et al, 1994; Mackenzie et al, 1997; Wehrle-Haller, 2003). A recent study from Bernstein and co-workers demonstrated that the two tyrosine residues 568 and 570 in the juxtamembrane region of c-Kit, which among other proteins couple the receptor to SFKs, are needed for normal pigmentation (Kimura et al, 2004). Moreover, loss-of-function mutations in c-Kit have been detected in the majority of humans with Piebaldism syndrome which is characterized by areas of hypopigmentation on the stomach, extremities and hair due to a lack of melanocytes (Spritz, 1994).

D. Gastrointestinal tract

In the *W* and *Sl* mice there is a depletion of Interstitial cells of Cajal (ICC). ICC, also known as pacemaker cells, regulate the gut movement by their ability to communicate with both nerve and smooth muscle cells. Loss of ICC is connected with several human diseases involving defects in gut movement such as slow transit constipation (Lyford et al, 2002). ICC express c-Kit and in the *W* and *Sl* mice there is a constipation phenotype suggesting an important role for c-Kit in ICC development and/or function (Huizinga et al, 1995; Ward et al, 1995; Ward et al, 1994). In addition, treatment of mice with an inhibitory c-Kit antibody leads to loss of ICC and consequently impaired gut movement. (Maeda et al, 1992)

E. Nervous system

Studies using *W* and *Sl* mutant animals have established a role for c-Kit signaling in the spatial learning function of the hippocampal region of the brain (Motro et al, 1996; Katafuchi et al, 2000). Moreover, c-Kit expression has been found in neuroproliferative zones in the adult rat brain as well as in neuronal cultures (Jin et al, 2002). Furthermore, administration of SCF leads to proliferation of primitive neurons *in vivo*. It was recently demonstrated that c-Kit signaling is important for the migration of neuronal stem- and progenitor cells to injured areas of the brain (Sun et al, 2004). The precise role of c-Kit signal transduction for the development and function of the nervous system is not clear and needs further investigation.

IV. c-Kit as a therapeutic target in cancer treatment

The tyrosine kinase receptor c-Kit is associated with several malignant human diseases. As mentioned above, c-Kit is expressed in the hematopoietic system, in certain cells in the gastrointestinal system (*i.e.* ICC), nervous system, germ cells, and melanocytes. Indeed, c-Kit driven tumors in these tissues have been observed. Mechanisms leading to uncontrolled activation of c-Kit involve both autocrine loops as well as mutational activation. Autocrine loops, *i.e.* the same cells produce both c-Kit and its ligand SCF, have been found in small cell lung cancer (SCLC), gynecological tumors and neuroblastomas (Inoue et al, 1994; Krystal et al, 1996; Vitali et al, 2003). Mutational activation of c-Kit has been found in patients with mastocytosis, gastrointestinal stromal tumors (GIST) and acute myeloid leukemia (AML). These mutations are predominantly located within the kinase domain or in the juxtamembrane region of c-Kit. The juxtamembrane mutations are believed to release an inhibitory alpha-helix and thereby result in uncontrolled receptor activation (Ma et al, 1999). In a study using molecular modelling Torrent et al. showed that kinase domain mutations in c-Kit and FLT3, a close relative to c-Kit, result in constitutive activation by destabilizing the inactive conformation, and not by directly stabilizing the active structure (Torrent et al, 2004). Unregulated c-Kit activity probably contributes to malignancies through increased proliferation and suppression of apoptosis. In all tumors in which c-Kit is the driving force for cellular transformation its kinase activity is essential. This is irrespective of the mode of kinase activation, whether it is activated by mutation(s) or by autocrine stimulation. Thus, inhibitors that specifically inhibits c-Kit enzymatic activity should be promising therapeutic agents. Several of these have been developed and perhaps the clinically approved STI-571 is the best known. This inhibitor was developed to target the kinase activity of the fusion oncoprotein Bcr-Abl, which is a result of the Philadelphia chromosome translocation and is commonly associated with chronic myelogenous leukemia. Subsequently STI-571 has been found to efficiently inhibit also c-Kit and the PDGFRs (Buchdunger et al, 2000). Recently, STI-571 was co-crystallized with the c-Kit kinase domain (Mol et al, 2004). This study showed that STI-571 binds to c-Kit in a way that is similar to its interaction with the Abl tyrosine kinase, *i.e.* near the active site of the inactive kinase and thereby stabilizing this conformation (Nagar et al, 2002). The crystal structure

of STI-571 in complex with c-Kit also revealed that optimization of the STI-571 structure, removing unfavorable sterical interactions, could lead to a c-Kit inhibitor with higher affinity and specificity (Mol et al, 2004). Notably, STI-571 interacts with the inactive conformation of the kinase and hence will have limited efficacy on c-Kit with mutations in the kinase domain resulting in constitutive activity. Indeed, it has been shown that STI-571 does not effectively inhibit the c-Kit^{D816V} mutant (Frost et al, 2002; Ma et al, 2002). Therefore, the development of an inhibitor that targets the activating mutants of c-Kit would be beneficial for the treatment of many c-Kit driven tumors. Optimally this inhibitor will only inhibit the mutant form of c-Kit but not the wild-type protein, resulting in minimal side-effects. Effective treatment with kinase inhibitors requires exact knowledge regarding the presence or absence of specific mutations in c-Kit. Thus, development of methods to diagnose the c-Kit status in tumor tissues at the molecular level, *e.g.* using DNA sequencing as well as probing tumor tissues with antibodies raised against active and/or mutated forms of c-Kit, will be critical for successful treatment of these patients.

As with most, if not all, enzymatic inhibitors used in cancer treatment the tumors eventually become tolerant or even resistant to the drug. This can occur through different mechanisms including increased expression of the oncoprotein, point mutations and/or expression of drug-resistance-proteins that can for example remove the drug from the cell or interact with the drug in a way that blocks its therapeutic activity. In the case of STI-571 resistance has been observed due to increased oncoprotein expression as well as point mutations within the kinase domain (Gorre et al, 2001; Weisberg and Griffin, 2001; Shah and Sawyers, 2003). Strategies to overcome problems with resistance include combinational therapy using several drugs that simultaneously target the receptor and/or downstream signal transduction pathways. Thus, a detailed understanding of the cell biological and biochemical properties of receptors and their downstream effectors is critical for the development of novel targeted therapeutic interventions. Below follows a brief account of some of the cancers in which c-Kit has been implicated. The list is by no means complete and is constantly growing as more tumors are found to express c-Kit.

A. AML

AML is exceedingly variable with relation to cell morphology and genetic changes. Expression of c-Kit has been found in about 85% of human AML cells (Heinrich et al, 2002). Since c-Kit primarily is expressed on stem- and progenitor cells in the hematopoietic system this suggests that AML cells are in a primitive differentiation stage. In many AML samples c-Kit is constitutively phosphorylated. This is achieved through different mechanisms. First, SCF stimulates proliferation of AML cells *in vitro* and a co-expression of SCF and c-Kit has been detected in AML blasts (Ikeda et al, 1991, 1993; Kanakura et al, 1993; Pietsch, 1993). Thus, autocrine stimulation is probably involved in driving tumorigenesis. Second, activating mutations have been detected in the c-Kit kinase domain (D816V or D816Y) (Ferrao et al, 1997). Interestingly, the mutant c-Kit is often found in combination with mutations in the core-binding transcription complex (Beghini et al, 2000). However, a functional connection between c-Kit and the core-binding mutations has not been established. It has been speculated that the mutation(s) in the core binding factor result in defects in differentiation and c-Kit activation provides anti-apoptotic and proliferative signals complying with the 2-hit hypothesis of AML etiology (one hit affecting differentaition and one hit affecting proliferation) (Reilly, 2002). Notably, also the c-Kit related tyrosine kinase FLT3 has been found activated in AML (O'Farrell et al, 2003). Using the low molecular weight inhibitors SU5416 and SU6668, that target c-Kit, FLT3 and VEGFR-2, leads to reduction of c-Kit phosphorylation and increased apoptosis in human AML blasts (Smolich et al, 2001; O'Farrell et al, 2004). These data support the idea that c-Kit (and/or FLT3 and VEGFR-2) can drive AML blast proliferation and survival. A recent phase I trial of SU11248, an inhibitor of FLT3, c-Kit, VEGFR-2 and PDGFRs, in treatment for AML showed partial short term remission and reduced c-Kit, Stat3, Akt and VEGFR-2 phosphorylation (Fiedler et al, 2004). In two studies analyzing the effect of STI-571 on *bcr-abl*-negative AML patients, one showed a hematological response whereas the other did not (Cortes et al, 2003; Kindler et al, 2004). However, in the study that failed to observe a response the investigators used a lower dose (400mg/d) compared to the study where a response was seen (600mg/d). Thus more work is needed before we can fully appreciate the efficacy of STI-571 in the treatment of *bcr-abl*-negative AML patients. For detailed information on c-Kit and FLT3 in leukemogenesis refer to (Reilly, 2002).

B. GIST

GIST are the most common cancer in the digestive tract with mesenchymal origin. However, this class of cancers only accounts for about 1% of all tumors in the gastrointestinal tract. There is evidence to suggest that GIST originate from ICC, which are known to express c-Kit (Wang et al, 2000; Duensing et al, 2004). Gain-of-function mutations found in GISTs are commonly located in the c-Kit intracellular juxtamembrane domain, but mutations have also been found in the extracellular domain (duplication of A501 and Y502) and in the kinase domain (K642E, N822K and N822H) (Lux et al, 2000: Rubin et al, 2001; Kinoshita et al, 2003). GISTs that contain mutated c-Kit have high cellularity and mitotic activity (Kim et al, 2004). In addition, the presence of c-Kit mutations indicates poor prognosis. However, all GISTs do not have mutations in c-Kit and it was recently shown that 35% of these c-Kit mutation negative patients had an activated mutation in the related PDGFR (Heinrich et al, 2003). Moreover, activating mutations in c-Kit or PDGFR appear to be mutually exclusive since no tumor has been found expressing both these oncoproteins.

Primary GIST can be treated with surgical removal of the tumor. In the case of metastatic GIST this treatment is combined with conventional chemotherapy. Lately the

tyrosine kinase inhibitor STI-571 that targets c-Kit, PDGFRs and Bcr-Abl fusion protein has been demonstrated to be therapeutically successful against GIST (Joensuu et al, 2001; Tuveson et al, 2001). Actually, STI-571 is the first effective treatment for GIST and more than 80% of patients respond to this drug. This highlights the importance of c-Kit signaling in this type of cancer. Recently, point mutations in the c-Kit kinase domain (V654A or T670I) making the GIST tumor resistant to STI-571 have been found in patients treated for a prolonged period of time with this drug (Chen et al, 2004; Tamborini et al, 2004). For detailed information on GIST biology and tumorigenesis please refer to Duensing et al, 2004 and Corless et al, 2004.

C. Mastocytosis

Mastocytosis is a disease characterized by accumulation of mast cells in various tissues. The disease shows large variations in clinical symptoms, with prognosis varying form non-lethal to lethal. c-Kit and its ligand SCF are critical for normal development and function of mast cells. In fact, mast cells are the only differentiated hematopoietic cell type that relies on c-Kit for its function. Activating mutations in the c-Kit kinase domain (D816V) have been identified in mast cells from patients with mastocytosis. This mutation renders c-Kit constitutively active. Notably, familial and pediatric mastocytosis may occur without mutations in the kinase domain of c-Kit indicating that the molecular reasons behind mastocytosis is variable and not exclusively dependent on a single oncoprotein. However, it is also possible that these patients have not yet identified mutations in other regions of c-Kit.

Currently, there is no effective treatment of mastocytosis and consequently pharmacological interventions aim to reduce discomfort. Unfortunately, the catalytic domain mutant of c-Kit, most often found in mastocytosis patients, is not well inhibited by STI-571 rendering this drug inefficient in treatment of mastocytosis (Zermati et al, 2003). Thus, there is a need to develop therapeutic agents that target the kinase activity of the c-Kit^{D816V} mutant. Recently Corbin et al. published a study demonstrating that the kinase inhibitor MLN518 could inhibit the activity of c-Kit^{D816V} (Corbin et al, 2004). Moreover, MLN518 showed low toxicity in a phase I trial for AML (DeAngelo et al, 2003). Thus, this is a promising compound for treatment of mastocytosis expressing activating mutation in c-Kit kinase domain. Furthermore, it has also been demonstrated that inhibition of NF- B using IMD-0354 reduced proliferation of mast cells expressing c-Kit V560G and/or D816V but not normal mast cells (Tanaka et al, 2004). This suggests that interfering with NF- B function might provide a novel therapeutic approach for mastocytosis. For detailed discussion of mastocytosis refer to Castells, 2004.

D. Melanoma

The genetic changes that occur in melanoma are tremendously variable, but a common characteristic is an increased presence of tyrosine phosphorylated proteins compared to normal melanocytes (McArdle et al, 2003). One obvious phenotype of *W* and *Sl* mice is their lack of fur pigmentation, which is due to a loss of melanocytes (Lev et al, 1994). Thus melanocytes depend on c-Kit for their function and therefore melanomas have been investigated in regard to c-Kit involvement (Wehrle-Haller, 2003). In general, it appears that c-Kit is down-regulated during tumor progression towards metastatic cutaneous melanoma (Easty and Bennett, 2000). In addition, re-expression of c-Kit into certain melanoma cell lines leads to reduced tumor growth and SCF-induced apoptosis (Huang et al, 1996). Hence it is possible that loss of c-Kit allows the transformed melanocytes to escape from apoptosis. In contrast, c-Kit expression has been detected on choroidal and uveal melanoma cell lines (Mouriaux et al, 2003; Lefevre et al, 2004). Furthermore, a significant c-Kit phosphorylation has been found in uveal melanoma cell lines, but no activating mutations (All-Ericsson et al, 2004; Lefevre et al, 2004). However, co-expression of SCF was observed thus establishing an autocrine/paracrine loop. Therefore the efficacy of STI-571 was tested on these cell lines and the results are encouraging with reduced proliferation (All-Ericsson et al, 2004; Lefevre et al, 2004). Welker et al. demonstrated reduced expression of membrane-associated SCF but not soluble SCF in melanoma cell lines, particularly those that metastasize *in vivo*, compared to normal melanocytes (Welker et al, 2000). Thus, it is possible that the membrane-restricted form of SCF makes it difficult for melanocytes to move. Loss of this anchor allows the transformed melanocyte to metastasize to distant regions of the body.

In conclusion, the involvement of c-Kit in melanoma is complex and varies between different types of melanoma, *e.g.* cutaneous versus choroidal and uveal melanomas. Hence, further investigation is needed to clarify the importance of c-Kit as a therapeutic target in choroidal and uveal melanomas.

E. SCLC

SCLC represents about 20% of all lung cancers, and if untreated it is a very aggressive form. The molecular biology behind SCLC is not clear, but it has been reported that about 70% of small-cell lung cancer cell lines overexpress c-Kit (Krystal et al, 1996; Sekido et al, 1991). Indeed c-Kit kinase inhibitors, such as STI-571, SU11248, SU5416 and SU6597, have been shown to reduce growth of SCLC cell lines and human SCLC xenografts in mice (Krystal et al, 2000; Wang et al, 2000: Krystal et al, 2001; Abrams et al, 2003). The *in vivo* activity of STI-571 on SCLC needs to be addressed in patients with tumors that are positive for c-Kit overexpression. Notably, a recent study failed to detect a correlation between the expression or mutation status of c-Kit and SCLC survival (Boldrini et al, 2004). Moreover, Haas and co-workers showed in a retrospective study of 203 patients with SCLC that lack of c-Kit expression was associated with shorter survival compared to patients with tumors that expressed c-Kit (Rohr et al, 2004). In contrast, Micke et al. found that high c-Kit expression is correlated with poor prognosis (Micke et al, 2003). Thus the involvement and importance of c-Kit

in SCLC is not clear and further investigations are justified.

Since SCLC is generally sensitive to both chemo- and radiotherapy these are the standard therapies for this disease (Stupp et al, 2004). However, the overall prognosis of SCLC is poor due to its tendency to metastasize early in the disease (Jafri et al, 2003). Autocrine and paracrine loops are believed to be important for the ability of SCLC to form metastases. Thus, development of strategies that interfere with the autocrine loops could result in significantly better long-term prognosis for patients with SCLC. A recent study found that simultaneous targeting both c-Kit/SCF and IGF-1R/IGF-1 signaling using the inhibitors AG1024 and AG1296 synergistically reduced growth and increased apoptosis in SCLC cells (Camirand and Pollak, 2004).

F. Other tumors

Expression of c-Kit has been detected in several other tumors not described above including neuroblastoma, cervical, testicular, thyroid, breast, colon, bladder and renal carcinoma (Arber et al, 1998; Bellone et al, 2001; Caceres-Cortes et al, 2001; Hines et al, 1999; Lin et al, 2004; Pan et al, 2004; Tian et al, 1999; Vitali et al, 2003).

In the case of breast cancer, c-Kit expression is lost during transformation from normal breast tissue to invasive breast cancer (Ulivi et al, 2004; Yared et al, 2004). Thus monitoring c-Kit expression can be of prognostic value.

A recent report identified expression of the tr-Kit in a prostate cancer cell line and in 28% of primary prostate cancers (Paronetto et al, 2004). Tr-Kit has been thought only to be expressed in sperm. Therefore tr-Kit might provide an almost tumor specific target.

Two recent investigations regarding the involvement of c-Kit in neuroblastoma have highlighted the complexity of this disease. In one study it was shown that c-Kit expression indicates a good prognosis (Krams et al, 2004). c-Kit expression was associated with differentiation and this is a possible explanation for c-Kit being a favorable prognostic marker. On the other hand, Beppu et al, demonstrated using neuroblastoma cell lines that STI-571 treatment leads to reduced proliferation and viability (Beppu et al, 2004). Furthermore, STI-571 treatment was associated with reduced c-Kit and PDGFR phosphorylation as well as reduced VEGF expression. In order to target c-Kit clinically these discrepancies between *in vitro* and *in vivo* studies must be resolved.

V. Concluding remarks

Since the discovery of SCF as the ligand of c-Kit almost fourteen years ago, numerous studies have contributed to our knowledge about the mechanism of action of c-Kit. A multitude of signaling pathways are activated by SCF leading to diverse biological responses, such as chemotaxis, proliferation, differentiation and survival. Using a number of different cell systems, investigators have many times found similar mechanisms of action of c-Kit, but sometimes also differences. The exact reason for these discrepancies is not fully understood. Some studies were conducted on transfected fibroblasts that express a different repertoire of signal transduction molecules than hematopoietic cells, possibly giving rise to activation of different signal transduction pathways. Also the differentiation state of hematopoietic cell lines is likely to influence the response elicited by SCF stimulation. Several splice forms of c-Kit have been demonstrated to exist, with sometimes different signaling capabilities, both quantitatively and qualitatively. Very little is known about how the expression of these different splice forms is regulated during development and differentiation. It is not unlikely that differences in signaling shown in the literature might be due to differences in expression of various splice forms of c-Kit in different cell types. Furthermore, the qualitative differences in signaling of the membrane bound versus soluble form of SCF have been demonstrated in a number of studies. The use of transgenic animals with targeted deletions of individual signal transduction molecules and the use of so-called knock-in methodology to introduce specific mutants of c-Kit in animals, have proven invaluable tools for our understanding of c-Kit signaling. In order to be able to study c-Kit signaling in hematopoietic development, more sensitive methods for the study of signaling in individual cells will be of utmost importance. Understanding of the mechanisms of synergy between SCF and various cytokines is also a challenging field for future research. Increased knowledge of molecular mechanisms of c-Kit signaling in diseases, such as cancer, is paramount to the potential development of targeted therapies. It is likely that c-Kit signal transduction, resulting in suppression of apoptosis and enhanced proliferation, at least contributes to the progression of many tumors. Hence, c-Kit is a clinically relevant target for drug development. Importantly, in cases where c-Kit activation is due to overexpression or an autocrine loop, the tumors are likely to respond to drugs that target wild-type c-Kit, *e.g.* STI-571. In contrast, tumors that contain c-Kit with mutations in the catalytic domain will not respond well to STI-571. Therefore we would like to emphasize the importance to develop new therapeutic agents which target the catalytic domain mutant of c-Kit that can be applied in the clinic. Moreover, it is important to identify differences in signal transduction pathways downstream of normal and oncogenic forms of c-Kit since these would be excellent targets for therapeutic intervention, with minimal effect on the function wild-type c-Kit, in order to complement drugs acting directly on the receptor. It is likely that simultaneous targeting of different signaling molecules will lead to synergistic anti-cancer effects and circumvent or at least delaying the emergence of drug resistance.

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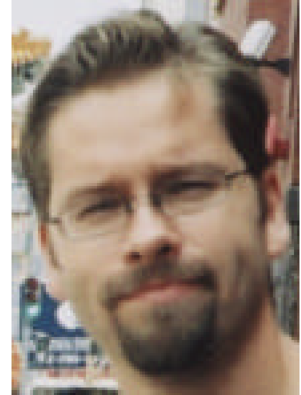
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