

The Stem Cell Factor Receptor/c-Kit as a Drug Target in Cancer

J. Lennartsson¹ and L. Rönnstrand^{2,*}

¹Ludwig Institute for Cancer Research, Uppsala University, Box 595, SE-751 24 Uppsala, Sweden; ²Experimental Clinical Chemistry, Dept. of Laboratory Medicine, Lund University, Malmö University Hospital, SE-205 02 Malmö, Sweden

Abstract: Tyrosine phosphorylation has a key role in intracellular signaling. Inappropriate proliferation and survival cues in tumor cells often occur as a consequence of unregulated tyrosine kinase activity. Much of the current development of anti-cancer therapies tries to target causative proteins in a specific manner to minimize side-effects. One attractive group of target proteins is the kinases. c-Kit is a receptor tyrosine kinase that normally controls the function of primitive hematopoietic cells, melanocytes and germ cells. It has become clear that uncontrolled activity of c-Kit contributes to formation of an array of human tumors. The unregulated activity of c-Kit may be due to overexpression, autocrine loops or mutational activation. This makes c-Kit an excellent target for cancer therapies in these tumors. In this review we will highlight the current knowledge on the signal transduction molecules and pathways activated by c-Kit under normal conditions and in cancer cells, and the role of aberrant c-Kit signaling in cancer progression. Recent advances in the development of specific inhibitors interfering with these signal transduction pathways will be discussed.

Keywords: Stem cell factor, c-Kit, receptor tyrosine kinase, signal transduction, transformation, cancer, leukemia.

INTRODUCTION

The surface of every cell is continuously exposed to a large variety of signals, e.g. in the form of growth factors. These signals are received by specialized receptor proteins. There are many different types of receptors and one large group is the receptor tyrosine kinases (RTK) superfamily. c-Kit belongs to the RTK superfamily, specifically to subclass III within this group. All members of subclass III RTKs, i.e. c-Kit, PDGFR α , PDGFR β , Flt3, and M-CSFR, have an intracellular tyrosine kinase domain which is split in two parts by an insert region, a single transmembrane domain which is believed to have a α -helical conformation and a ligand-binding extracellular domain composed of five immunoglobulin-like domains. c-Kit is specifically activated by its cognate ligand stem cell factor (SCF). SCF is expressed by fibroblasts and endothelial cells throughout the body whereas c-Kit expression is more restricted and predominantly found on primitive hematopoietic cells, mast cells, melanocytes, testis, brain, vascular endothelial cells, interstitial cells of Cajal, breast glandular epithelial cells and sweat glands [1-9].

c-Kit and Scf

Binding of SCF to the extracellular domain of c-Kit result in dimerization of two receptor monomers [10]. Bringing two c-Kit tyrosine kinases domains in close contact with each other leads to autophosphorylation of tyrosine residues mainly outside the kinase domain. However, tyrosine phosphorylation also occurs within the kinase domain on tyrosine residues 823 and 900 [11-13].

Autophosphorylation serves two purposes; first to increase the kinase activity and second to create high affinity interaction sites for proteins with Src homology 2 (SH2) or phosphotyrosine binding (PTB) domains [14]. Proteins interacting with the activated receptor may then in turn be phosphorylated and signal transduction is initiated. Besides tyrosine phosphorylation, c-Kit is also serine and threonine phosphorylated. The importance of this phosphorylation is not clear at present. However, in the case of PKC-dependent phosphorylation of S741 and S746 in the kinase insert this has been linked to inhibition of the tyrosine kinase activity of c-Kit, hence establishing a negative feed-back loop [15, 16].

There are four isoforms of c-Kit in humans due to alternative RNA splicing (figure 1) [17-19]. Two isoforms are characterized by the absence or presence of a serine residue in the kinase insert region. The function of this serine is not known. The difference between the other two splice forms is a stretch of four amino acids close to the plasma membrane on the extracellular side. The latter splice forms have been studied at the molecular level and display striking differences in their ability to induce signal transduction and in its tumorigenic potential [12, 20]. In general, the c-Kit isoform lacking the tetrapeptide sequence induces stronger but transient signals and has a higher transforming ability compared to the longer splice form. SCF exist as two splice forms, one containing a transmembrane domain and hence membrane associated and the other soluble [21]. The membrane associated form induces prolonged c-Kit phosphorylation compared to the soluble form [22].

In addition to the above described splice forms, a truncated form of c-Kit, resulting from the usage of a developmentally controlled promoter element within intron 16, has been described in murine testis. It consists of 12 amino acids encoded by intron 16 plus the carboxyterminal tail of c-Kit, lacking kinase activity [23]. It has recently been

*Address correspondence to these authors at the Experimental Clinical Chemistry, Wallenberg Laboratory, Entrance 46, 5th floor, Malmö University Hospital, SE-205 02 Malmö. Tel. +46 40 33 72 22; Fax: +46 40 92 90 23; URL: <http://www.expklkemi.mas.lu.se>; E-mail: Lars.Ronnstrand@med.lu.se

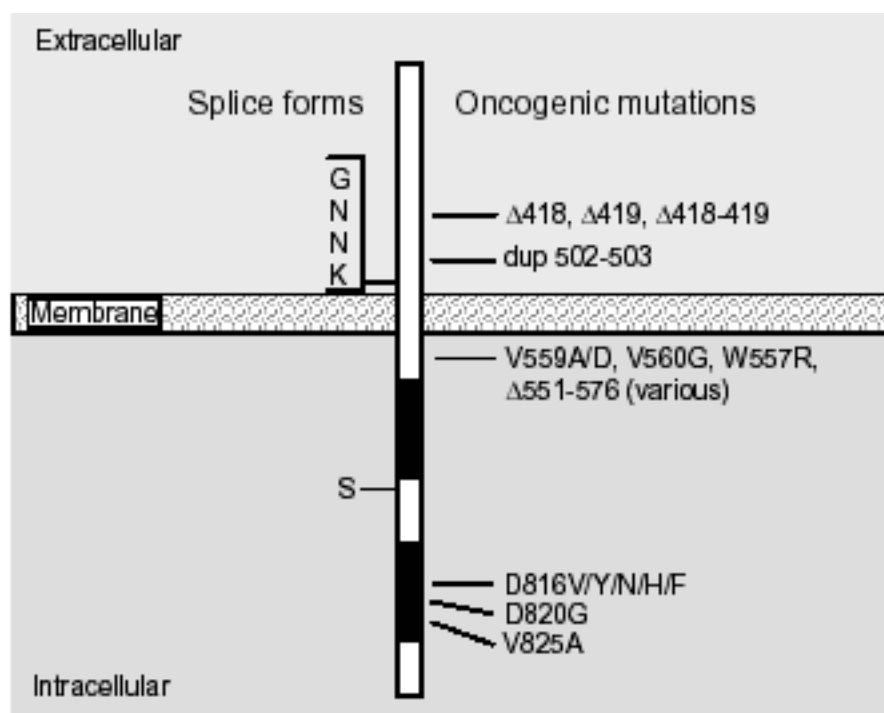


Fig. (1).

described to be ectopically expressed in human prostatic cancer, where it is associated with the more advanced stages of tumor [24]. However, other researchers have claimed that the testis specific truncated transcript of c-Kit only exists in mice, and not in humans [25]. Future research is needed to further elucidate the potential role of tr-Kit in human malignancies.

SIGNAL TRANSDUCTION

Stimulation of c-Kit activates a wide array of signaling pathways. Some of these are briefly discussed below. For a more in depth discussion of pathways downstream of c-Kit please refer to these recent reviews and reference therein [26, 27].

PI3-Kinase

Phosphatidylinositol 3'-kinase (PI3-kinase) phosphorylates as its name suggests the 3'-hydroxyl group in the inositol ring of phosphatidylinositol (PtdIns) lipids in the cell membrane. The preferred substrate *in vivo* appears to be PtdIns(4,5)P₂ (PIP₂) generating PtdIns(3,4,5)P₃ (PIP₃) [28]. This will increase the negative electric potential of the lipid. Patches in the cell membrane containing PIP₃ can interact with proteins containing Pleckstrin homology (PH) domains. This results in translocation of these PH domain containing proteins from the cytoplasm to the plasma membrane. One important PH domain containing protein in c-Kit signaling is the serine/threonine kinase Akt. Translocation of Akt to the plasma membrane results in its activation. Activation of Akt is critical for the ability of SCF to protect from apoptosis. This has been demonstrated in cell culture systems where expression of a mutant form of c-Kit not capable of binding PI3-kinase (c-Kit^{Y719F}) was unable to induce Akt-mediated phosphorylation of the pro-

apoptotic protein Bad and as a consequence incapable of protecting U2-OS from apoptosis [29]. Recently, it was demonstrated that SCF also contributes to survival by inactivating the transcription factor FOXO3a, which occurs through Akt-mediated phosphorylation [30, 31]. Consequently, this reduces the expression of the proapoptotic protein Bim, which in addition is functionally downregulated by Mek-dependent phosphorylation. Furthermore, using knock-in methodology to produce mice expressing c-Kit^{Y719F} it was shown that male mice were sterile due to a block in spermatogenesis and extensive apoptosis [32]. Also the female mice displayed reduced fertility [33]. It should be noted that besides the importance in protection from apoptosis, PI3-kinase is also important for SCF-induced proliferation, regulation of the actin cytoskeleton and cell migration [34, 35]. SCF has a significantly reduced mitogenic activity in mast cells from mice deficient in p85 α subunit of PI3-kinase compared to normal cells [36]. In fact, PI3-kinase is essential for growth and tumorigenicity of hematopoietic cell lines expressing a constitutive active oncogenic form of c-Kit [37, 38]. Thus, PI3-kinase is central for both proliferation and protection from apoptosis, underscoring the importance of these events for tumor formation and progression.

The above discussion focuses on class I PI3-kinase (or classical PI3-kinase). However, c-Kit constitutively associates with PI3KC2 β which belongs to class II. PI3KC2 β is activated following SCF stimulation and contributes to Akt activation downstream of c-Kit [39]. Interestingly, it was recently shown that C2 domains can interact with phosphotyrosine residues [40] providing a possibility of interaction between PI3K-C2, that lacks SH2 domains, and the activated c-Kit. However, detailed knowledge of the involvement of class II PI3-kinases in c-Kit signaling is currently lacking.

Src Family Kinases

Src family kinases (SFK) are cytoplasmic tyrosine kinases involved in plethora of biological functions including proliferation, chemotaxis and survival [41]. It is well established that c-Kit activates SFKs but the function in terms of signal transduction is less clear [42, 43]. On the cell biological level it has been demonstrated that SFK are involved c-Kit internalization [44]. Furthermore, using Lyn^{-/-} bone marrow mast cells it was shown that Lyn contributes to c-Kit phosphorylation as well as phosphorylation of STAT3 and JNK [45]. Interestingly, Lyn negatively regulate PI3-kinase/Akt signaling by an unclear mechanism. SFK have been demonstrated to participate in SCF-induced chemotaxis and proliferation in the megakaryoblastic cell line MO7e and primary hematopoietic progenitor cells [44, 46]. Using a chimeric c-Kit receptor with all tyrosine residues mutated to phenylalanine and then adding back specific tyrosines Hong *et al.* (2004) could show that adding back the SFK binding site restored survival, chemotaxis and partially proliferation [47]. This data suggest a critical role for SFK in most effects downstream of c-Kit, however, it should be noted that the SFK binding site also interacts with other signaling molecules including APS, SHP1 and SHP2 [48, 49]. This makes the analysis of data from a c-Kit mutant lacking the SFK binding site difficult to interpret. As in the case of PI3-kinase, knock-in experiments have been performed with mutant c-Kit unable to interact with SFK (c-Kit^{Y567F} and c-Kit^{Y567/569F}) to clarify the *in vivo* role. Phenotypic analysis of these mice indicated a role for SFK in c-Kit signaling in lymphocytes. Specifically, mice expressing the c-Kit^{Y567F} mutant displayed a reduction in pro-B and pro-T cells with increasing age [50, 51]. Moreover, if the double c-Kit^{Y567/569F} mutation was introduced, these mice revealed problems with mast cell development, splenomegaly and pigmentation in addition to the lymphocyte defect. One important general conclusion that can be drawn from the knock-in experiment of mutant c-Kit unable to interact with certain signaling proteins (i.e. PI3-kinase and SFK) is that the dependence on a specific signaling pathway is cell type specific. Hence loss of a specific pathway can in most tissues be compensated for by redundant signals. The nature of these redundant signals and why they cannot compensate in all cell types remains to be defined.

Ras-Erk Pathway

Activation of the MAP-kinases Erk1 and 2 has an important role for cell proliferation, in addition to other cellular responses such as survival and differentiation. c-Kit activates these proteins by first recruiting the guanine nucleotide exchange factor Sos to the plasma membrane where its substrate, the small GTPase Ras is located. Sos promotes the exchange of the guanine nucleotide phosphate bound to Ras from GDP to GTP, which leads to Ras activation. Active Ras translocate the serine/threonine kinase Raf-1 to the plasma membrane where it is activated by an unclear mechanism. Activated Raf-1, in turn, phosphorylates and activates another serine/threonine kinase Mek which is an activator of Erk. Activated Erks have substrates both in the cytoplasm and in the nucleus resulting in both altered protein activity and changes in gene expression [52].

Sos forms a stable complex with the adaptor protein Grb2 which interacts directly with tyrosine residues 703 and 936 in c-Kit [53]. However, the Grb2/Sos complex can also interact indirectly through ShcA or SHP2 [54, 55]. The contribution to Erk activation by direct binding of Grb2 to c-Kit or indirect through ShcA is not known, and may vary between different cell types. Some studies have suggested an important role for SFK in Erk activation [47, 56-58]. However, other studies have failed to do so, suggesting cell type specificity [59]. This cell type specificity may involve differences in cell differentiation status, variations in the expression of c-Kit splice forms and by which route Erk is activated downstream of c-Kit, e.g. direct or indirect binding of Grb2/Sos. In fact, SCF-induced Erk activation in hematopoietic progenitor/stem cells is dependent on PI3-kinase [60]. In contrast, in more differentiated hematopoietic cells PI3-kinase is not needed. This demonstrates that the differentiation status of the cell can have significant impact on which proteins are used for signal transduction. In regards to splice forms, Voytyuk *et al.* (2003) found that Erk1/2 are robustly phosphorylated after activation of the c-Kit splice form lacking the GNNK amino acid sequence in the juxtamembrane extracellular region compared to the splice form containing this sequence [12].

It is well established that SCF exerts many of its effects on hematopoietic cells in conjunction with other cytokines and growth factors. In fact, these interactions are in many cases synergistic. Several studies have implicated synergistic activation of Erk1/2 after concurrent stimulation with SCF and cytokines such as GM-CSF or Epo [61-63]. Interestingly, for both these cytokines PI3-kinase is essential for the synergistic Erk1/2 phosphorylation.

c-Kit mediated activation of Erk1/2 has direct relevance for cancer treatment since it has been suggested that SCF-mediated Erk activation up-regulates survivin expression in HL60 cells, resulting in resistance towards radiation induced apoptosis [64].

Other Pathways

The above described signaling pathways are by no means the only ones activated by c-Kit. Other important pathways and signaling proteins include JAK/STAT pathway, phospholipase C γ , adaptor proteins (e.g. Grb2, Grb7, ShcA, Gads, APS, Gab and Crk), cytoplasmic tyrosine kinases (e.g. Tec, CHK, Fer and Fes), protein tyrosine phosphatases (e.g. SHP1, SHP2 and PTP-RO) and transcription factors (e.g. Mitf, Slug and STAT) [11, 48, 49, 53, 65-75]. The molecular function of these proteins in the context of c-Kit signaling is characterized to varying degrees. The large number of signaling proteins affected by c-Kit probably lies behind the many and diverse functions of c-Kit in different tissues. In addition, c-Kit often functions in co-operation with other growth factors and cytokines further complicating the signaling networks.

TARGETED CANCER THERAPIES

As a result of research performed over the last decades our molecular understanding of cancer has increased and it is clear that a multitude of tumors overexpress and depend on

kinases for disease progression. This makes proteins in the kinase superfamily a prime target for molecular cancer therapeutics. Strategies employed to target these proteins include antibodies e.g. trastuzumab (Herceptin®) targeting the extracellular domain of HER2 and low molecular weight kinase inhibitors targeting the enzymatic activity e.g. gefitinib (Iressa®) inhibiting the EGFR activity. Antibodies can only affect proteins with extracellular domains but in return are very specific. In contrast, low molecular inhibitors can target both transmembrane and intracellular proteins but the specificity may be limited. Most kinase inhibitors bind to the enzymatic domain and compete with ATP. Experience has shown that although all kinases bind ATP, the binding pockets are unique enough to allow development of inhibitors with reasonable specificity.

A general phenomenon in cancer management is the occurrence of resistance towards the drug(s) used. Resistance may be due to mutations in the target protein reducing the binding between drug and kinase or the cell may overexpress transport protein(s) reducing the intracellular concentration of the drug. In addition, other oncoproteins may replace or compensate for the inhibition of the drug target. In the case of imatinib mesylate (Gleevec® (USA), Glivec® (Europe) or STI571) in the treatment of CML clinical resistance has been noted involving both increased Bcr-Abl expression and mutations in the kinase domain rendering imatinib mesylate unable to bind effectively [76].

Efforts towards c-Kit targeting have exclusively utilized the low molecular inhibitor approach where imatinib mesylate which also inhibits Abl and PDGFRs, is a

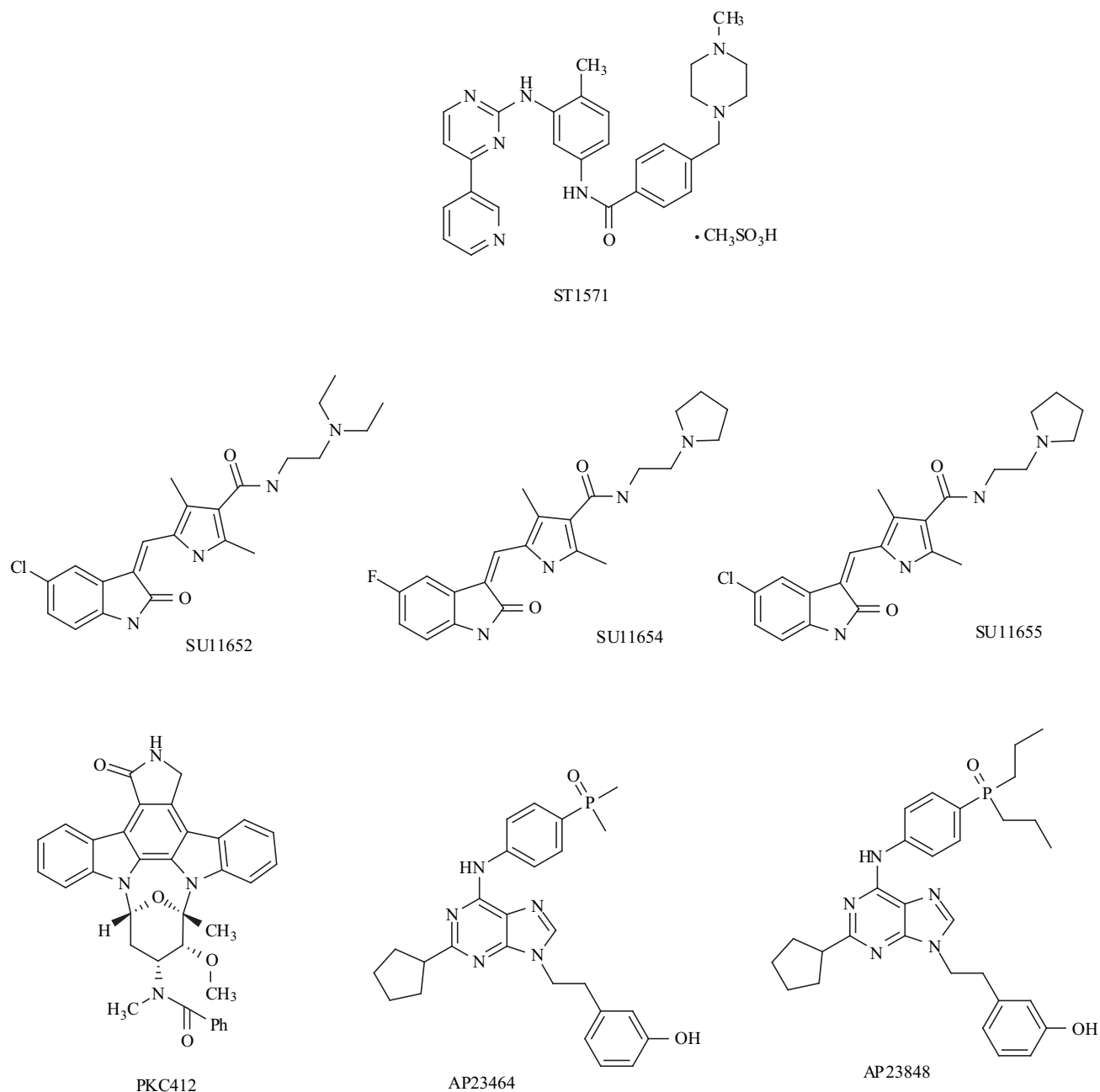


Fig. (2).

clinically used example. Below we discuss c-Kit involvement in tumor development and introduce clinical or experimental inhibitors in this context. The available structures of c-Kit inhibitors mentioned in the text are depicted in figure 2.

C-KIT IN TUMORS

The role of c-Kit in cancer is somewhat ambiguous. On one hand, a number of tumor types are associated with activation of c-Kit, either through overexpression, co-expression of its ligand, or mutations. On another hand, there are also tumor forms, such as breast cancer, thyroid carcinomas and melanomas in which progression into a malignant phenotype occurs concomitantly with a loss of c-Kit expression. In fact, forced expression of c-Kit in highly metastatic melanomas has been shown to lead to c-Kit induced apoptosis [77]. In contrast, All-Ericsson *et al.* (2004) found uveal melanomas to proliferate in a c-Kit dependent manner, and treatment with the kinase inhibitor imatinib mesylate lead to induction of apoptosis [78]. In a recent study, Willmore-Payne *et al.* [79] found an activating mutation of c-Kit, L576P, in a small subset of malignant melanomas. However, those represented only 2 % of the highly metastatic malignant melanomas. Melanomas illustrate the complex and sometimes poorly understood involvement of c-Kit in tumorigenesis.

In certain tumors, e.g. GIST, activating mutations in c-Kit is believed to be the causative molecular event for tumor occurrence. Targeting c-Kit in these tumors with imatinib mesylate result in a dramatic increase in survival (70-80% survival after 2 years) compared to earlier treatment regimes [80]. In contrast if c-Kit activation is not the causative event, treatment targeting c-Kit is expected to have less dramatic results.

Autocrine Loops of c-Kit and SCF

In several receptor systems the concomitant overexpression of receptor and ligand, leading to autocrine loops, has been shown to contribute to transformation (for review, see [81, 82]). Tumor types that have been shown to simultaneously express c-Kit and its ligand include breast carcinomas, colorectal carcinomas, small cell lung carcinoma (SCLC), gynecological tumors and neuroblastomas (reviewed in [83]). Recently, an osteosarcoma cell line established from a primary osteosarcoma lesion in the distal femur was shown to express both SCF and c-Kit, indicating the presence of an autocrine loop [84]. Malignant mesothelioma is often resistant to chemotherapy. In a recent study this resistance was connected with Slug-mediated upregulation of c-Kit and SCF thus establishing a protective autocrine loop [85]. In summary, autocrine loops may support cancer development and progression by promoting proliferation and/or by protecting the tumor cell from death.

Ectopic Expression of c-Kit

While only a minority of hematopoietic cells express c-Kit under normal physiological circumstances, certain leukemia cells, such as blasts from patients suffering from acute myeloid leukemia express c-Kit [86, 87] and in these

cases c-Kit is suggested to contribute to the malignant phenotype. A recent study analyzing c-Kit expression in renal tumors demonstrated c-Kit overexpression in renal oncocytoma and chromophobe renal carcinoma with an average 7.4-fold increase compared to normal renal tissue [88]. In contrast, c-Kit overexpression was not found in other types of renal cancers. In general, c-Kit overexpression will result in hypersensitivity towards ligand and if the overexpression level is high enough ligand-independent activation.

Gain-of-Function Mutations

Gain-of-function mutations of receptor tyrosine kinases is a widely occurring event in the progress towards cancer (for review, see [89]). Although gain-of-function mutations of tyrosine kinases as a cause of cancer has been known since the beginning of the 1980's [90], the first gain-of-function mutation of c-Kit identified in 1993 by Furitsu *et al.* in the human mast cell line HMC1 were V560G and D816V [91], located in the juxtamembrane region and in the tyrosine kinase domain, respectively. Interestingly, both mutations cause constitutive activation of c-Kit. This is also representative for the overall picture of mutations in c-Kit. Thus, gain-of-function mutations of c-Kit can roughly be divided into two classes: those residing in the juxtamembrane region and those in the kinase domain close to the activation loop (Figure 1). The juxtamembrane region has been shown to negatively regulate the kinase activity of c-Kit [92]. X-ray crystallographic data indicate that the autoinhibited conformation of c-Kit is stabilized by the juxtamembrane domain, which inserts into the active site of the kinase and disrupts formation of the activated structure [93]. Thus, mutations in this region disrupt the interaction of the juxtamembrane region with the kinase domain, thereby releasing the inhibition posed by the juxtamembrane domain. In addition, point-mutations in the juxtamembrane domain can induce constitutive c-Kit dimerization [94]. Both these events are believed to lead to activation of the kinase domain. The other hot spot for activating mutations of c-Kit, codon 816, resides in the second part of the kinase domain (corresponding to codon 814 in mouse). This aspartic acid residue has been found to be mutated to either a tyrosine, histidine, asparagine or valine residue with similar consequences; ligand-independent activation [95]. Molecular modelling of a kinase domain mutant suggests that this mutation destabilizes the inactive conformation and in this way indirectly promote the active configuration [96]. There are conflicting data whether the kinase domain mutants form dimers in the absence of SCF [94, 97, 98]. Moreover studies on both a juxtamembrane domain mutant (KΔ27, in frame deletion of amino acids 547-555) and a kinase domain mutant (D814Y) have indicated a shift in substrate specificity compared to wild-type c-Kit [99, 100]. This could have dramatic consequences for which signaling pathways are activated by the oncogenic forms of c-Kit, and hence provide therapeutic targets specific for mutated forms of c-Kit. However, more work is needed to explore these possibilities.

Mutations of the juxtamembrane region including deletions, point mutations, tandem duplications or combinations thereof, are commonly seen in patients

suffering from gastrointestinal stromal tumors (GIST). Interestingly, mutations in the juxtamembrane region have also been described in the closely related receptor tyrosine kinase Flt3, where it is in about 30 % of all cases of acute myeloid leukemia is associated with constitutive activation (for review, see [101]). Additional tumor types found to involve mutations of the juxtamembrane region of c-Kit include sinonasal lymphomas [102] and rare cases of systemic mastocytosis [103]. Mutations in the kinase domain are commonly found in human malignancies such as mastocytosis [104, 105], mast cell leukemia, acute myeloid leukemia [106, 107], core factor binding leukemia [108], testicular germ cell tumors [109], ovarian dysgerminomas [110] and intracranial germinomas [111]. There is also a report of mutation of D816 in papillary renal carcinomas [112], although these findings have been questioned [113].

Interestingly, the juxtamembrane and activation loop mutations, respectively, differ in their sensitivity to inhibitors of wild-type c-Kit, including imatinib mesylate and SU9529. In the case of imatinib mesylate this has been addressed at the molecular level by co-crystallization of imatinib mesylate with the c-Kit kinase domain [93]. This study showed that imatinib mesylate binds and stabilizes the inactive configuration of the kinase domain. Activating mutations in the catalytic domain of c-Kit will force the enzyme into an active conformation that cannot bind imatinib mesylate with high affinity. Thus, imatinib mesylate will not inhibit the oncogenic kinase domain mutant forms of c-Kit. Indeed, this has been demonstrated experimentally [114, 115]. If the receptor is activated by another mechanism (e.g. autocrine loop, mutations in the juxtamembrane domain) the kinase domain, albeit often in an active conformation, will be in equilibrium with the inactive state which can bind imatinib mesylate. Binding of imatinib mesylate prevents the kinase domain to go back to the active conformation and inhibition is achieved. Therefore to predict whether a c-Kit driven tumor will respond to imatinib mesylate it is essential to have molecular knowledge of the reason(s) for c-Kit over-activity. However, four small molecule inhibitors have been shown to be effective against the D816 mutant. These drugs, SU6577, SU11652, SU11654 and SU11655, all indolinone derivatives, are active against the juxtamembrane mutants as well as the activation loop mutants [116, 117]. Furthermore, Growney *et al.* [118] demonstrated the efficacy of the inhibitor PKC412 against the D816Y and D816 V, despite their resistance to imatinib. As for most anti-cancer drugs, prolonged treatment with imatinib mesylate results in the emergence of tumor drug resistance. This can result from mutations at or close to the interaction site between the drug and the receptor or if the mutation changes the receptor conformation in a way that reduces binding to the drug. Therefore it is important to develop drugs, like the abovementioned, that interact with c-Kit in an alternative manner to use as second-line treatment for tumors that have obtained resistance towards the first-line drugs, for instance imatinib mesylate. Recently, Corbin *et al.* [119] described two novel ATP-based kinase inhibitors, AP2346 and AP23848, that selectively inhibited the activation loop mutants of c-Kit, while the wild-type and juxtamembrane mutant of c-Kit required significantly higher concentrations for inhibition. It is likely that this novel class of inhibitor

will cause less side effects in patients since they are selective for the mutated form of c-Kit. Table 1 summarizes the various oncogenic mutations found in certain cancers.

Table 1. c-Kit Mutations in Human Tumors

Tumor type	c-Kit mutation
Mastocytosis	D816V, D816Y, D820G, V560G
GIST	V559A, V559D, W557R, dup 502-503, various Δ between amino acids 551 and 576
AML	Δ 418, Δ 419, Δ 418-419, D816V, D816Y
Sinonasal NK/T cell lymphoma	V825A, D816N
Germ cell tumor	D816H

Δ = deletion, dup = duplication

Additional Mutations of c-Kit Found in Tumors

Core binding factor (CBF)- acute myeloid leukemia (AML) is a subtype of AML that often displays mutations in exon 8 of c-Kit, which corresponds to immunoglobulin-like domain 5. These mutations cause hyperactivation of the receptor in response to SCF-stimulation [120]. Thus, it seems to constitute an additional type of mutation that potentiates the normal, ligand-mediated activation of c-Kit.

A considerable number of patients with sinonasal NK/T cell lymphomas have been described to have mutations in codon 825 of c-Kit which also resides in the second part of the kinase domain of c-Kit [102]. A small proportion of patients with myeloproliferative disease carry a D52N mutation located in immunoglobulin-like domain 1 in the extracellular domain of c-Kit [121, 122]. Finally, mutations in exon 9 of c-Kit are found in 5-10 % of GISTs [123, 124], which otherwise usually are associated with mutations in exon 11. Hirota *et al.* (2001) described a duplication of the codons 501 and 502, coding for alanine and tyrosine, respectively, in patients with GIST [125]. It was demonstrated that the duplication leads to constitutive activation of c-Kit. The mechanism for this activation is not known. However, several studies have indicated that the rotational orientation of two receptor tyrosine kinases in a functional dimer could be critical. By insertion of an artificial dimerization motif in different positions in the PDGF β -receptor transmembrane region, Bell *et al.* (2000) could demonstrate a periodic shifting in the kinase activity of the receptor as the dimerization motif was moved along an alpha-helical region [126]. Thus, in some orientations the kinase activity of the dimer was almost obliterated, while in others the kinase activity was very strong. Since Ala-501 and Tyr-502 reside in a region with predicted alpha helical structure, an insertion of two amino acids would presumably lead to an altered orientation of the two receptor subunits in a dimer, and maybe put the in a more optimal position for activation than in the wild-type receptor. Two alternative splice forms of c-Kit that differ in the absence or presence of four amino acids, GNNK, in the same region as Ala-501 and Tyr-502, display both quantitatively and qualitatively different activation and signaling downstream of c-kit [12,

20]. However, the functional significance of the abovementioned mutations remains to be shown.

Mutations in the transmembrane region of c-Kit have been found in a few cases. A mutation of codon 530 was detected in a patient suffering from core-factor binding leukemia [127]. Also in a variant of mastocytosis a mutation at codon 522 has been described [128]. However, the functional impact of these mutations on the kinase activity of c-Kit has not been investigated. However, in other receptor systems mutations within the transmembrane domain has been associated with constitutive activation of kinase activity. The most well known example is HER2 which was originally found as a transforming oncogene in neuroblastomas in rats induced by chemical mutagenesis [129], which was found to carry a mutation of a glutamic acid for a valine residue in the transmembrane region, leading to constitutive activation of its tyrosine kinase activity and transformation.

CONCLUDING REMARKS

In most tumors where c-Kit is a driving oncogene its tyrosine kinase domain is activated in an improper manner. A consequence of unregulated c-Kit activity is subsequently over-activation of downstream signaling pathways. c-Kit probably contributes to cancer formation and progression by inappropriately promoting survival and proliferation. In tumors that depend on c-Kit over-activity for progression this receptor is a prime target for anti-cancer therapies. A strategy that has been used for c-Kit driven tumors are low molecular weight inhibitors of the c-Kit kinase activity, thus inhibiting its signaling capability. One such inhibitor already in clinical use is imatinib mesylate. Besides c-Kit, imatinib mesylate also efficiently inhibits the PDGFRs, and Abl tyrosine kinases. In general, low molecular weight inhibitors are not completely specific for a certain kinase, but rather inhibit subsets of kinases. Future work is likely to produce new inhibitors with even narrower specificity, which is important to avoid inhibition of kinases not involved in tumor progression. In fact, the structure of the c-Kit kinase domain in complex with imatinib mesylate showed that affinity and specificity may be increased by removing sterical unfavorable interactions between c-Kit and imatinib mesylate [93]. One important aspect regarding inhibitors is what the optimal specificity is. It is possible that a very specific drug will easily provoke resistance since only minor changes of the target structure will reduce affinity for the drug. Moreover, perhaps a drug with a several targets will have more significant impact on tumor growth. Thus the specificity of the drug must be balanced in way that gives maximal effect on the tumor but can still be tolerated by non-transformed cells. Furthermore, to increase efficacy and to reduce the problem with drug resistance we believe that combination therapies using a panel of inhibitors (or classical chemotherapy) targeting various aspects of cell signaling (i.e. both receptor and downstream pathways) will prove most effective in cancer treatment. Indeed, several studies have indicated the power of combination therapies e.g. AEE788 which inhibits EGFR and VEGFR in combination with CPT-11 (i.e. chemotherapy) is effective against colon cancer [130], and the EGFR kinase inhibitor gefitinib combined with cisplatin (i.e. chemotherapy) is

effective in apoptosis induction in a human head and neck cancer cell line [131]. However, to be able to develop new effective targeted combination treatments, knowledge about the molecular mechanisms resulting in resistance to the primary drug is vital. This information may be used to identify relevant additional targets. Moreover, an important question is whether one should treat cancer with one drug and only add/change drug when resistance is observed, or if treatment with both drugs simultaneously from the beginning is a better strategy. Future will have to determine this, but it is possible that results may vary between different drug combinations and tumor types.

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