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c-Kit - The Novel Receptor: Physiological Roles, Downstream Signaling and Implications in Cancer

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Received Date: 19-09-2021, Accepted Date: 18-10-2021, Published Date: 25-10-2021

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Abstract

The belief that adult mammalian heart lacks regenerative potential was challenged by the identification of c-kit positive Cardiac Progenitor Cells (CPCs) in the heart. SCPIO; a phase I clinical trial; has shown that autologous c-kit positive CPCs improve cardiac function and quality of life when transplanted into ischemic heart disease patients. c-Kit is a type III receptor tyrosine kinase and a common stem cell antigen. Stem Cell Factor (SCF) is the only known ligand for c-kit. Activation of this membrane bound receptor is known to play a crucial role in many physiological processes like hematopoiesis, immune regulation, pigmentation, gametogenesis, intestinal motility, vasculogenesis, lung and brain development. Likewise, aberrant c-kit stimulation results in several pathological conditions, including tumors of the gut, blood, lung, breast, germ cells, melanocytes and mast cells. Several pro-growth, pro-survival signaling pathways like the MAPK, PI-3K and the phospholipase C and D pathways are involved in mediating the downstream effects, following the activation of this receptor. The goal of this article is to provide a comprehensive review of the structure, function, signaling mechanisms of c-kit activation and its role in normal and pathological conditions.

Vajravelu BN | Volume 2, Issue 3 (2021) | JCMR-2(3)-041 | Research Article

Citation: Vajravelu BN, et al. c-Kit - The Novel Receptor: Physiological Roles, Downstream Signaling and Implications in Cancer. Jour Clin Med Res. 2021;2(3) :1-36.

DOI: <http://dx.doi.org/10.46889/JCMR.2021.2304>

Keywords

Stem Cell Antigen; c-Kit Positive Cells; Cardiac Progenitor Cells; Stem Cell Factor, c-Kit Antigen

Introduction

Cellular Kit (c-kit) (also known as stem cell factor receptor or CD117) is a transmembrane glycoprotein with a molecular weight of 145 kDa [1-3]. Categorized as a type III receptor tyrosine kinase, c-kit was discovered in 1986 as the cellular homolog of the viral oncogene v-kit. It was identified to be the transforming gene of the Hardy-Zuckerman 4 feline sarcoma virus and hence named “kit”, an abbreviation for “kitten” [2]. Soon after the discovery of c-kit, studying the phenotype of c-kit mutants revealed that it is allelic with the dominant white spotting Locus (W) of mice [4]. A similar phenotype was found in mice with mutation in the Steel locus (SL) leading to the discovery of the only known ligand for c-kit called the steel factor or the Stem Cell Factor (SCF), the product of the Sl locus [5,6].

Structure of c-Kit

The c-kit protein has approximately 976 aminoacids and is comprised of five extracellular immunoglobulin-like (Ig-like) domains, a single transmembrane helix and a short cytoplasmic transmembrane domain (Fig. 1). The intracellular region of c-kit has two kinase domains separated by a short kinase insert and ends in the COOH terminus [2]. The c-kit gene is located in the chromosome 4q11 in humans and has 21 exons covering around 34 kb of DNA. Exon 1 encodes the signal peptide and translational initiation codon, exons 2-9 encode the five extra cellular Ig-like domains, exon 10 encodes the short transmembrane region and exons 11-21 encode the intracellular region comprising the two kinase domains separated by a short kinase insert and the terminal carboxy terminus [2,7]. c-Kit mRNA undergoes alternate splicing generating four isoforms in humans and two isoforms in mice. Two of the isoforms in humans differ by a short amino acid sequence GNNK. Although GNNK- is the predominant form, the GNNK+ and GNNK- are co-expressed in most tissues. Additionally, based on the presence of serine in the kinase insert, two more isoforms were identified [8-10]. Truncated kit (tr-kit), a shorter transcript, has only the second kinase domain and the carboxy terminus. This shorter transcript was found to play a critical role in gametogenesis by activating kinases of the Src Family and Phospholipase C (PLC γ), probably by acting as a scaffold [11,12]. Also, although the isoforms do not differ in their ligand binding capacity, it was found that they differ in their pattern of tyrosine phosphorylation, with the GNNK- isoform getting highly phosphorylated than its counterpart [13].

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Figure 1: Schematic representation of the structure of c-kit: The type III receptor tyrosine kinase has five extracellular Ig-like domains, followed by a short transmembrane domain. The intracellular region has two kinase domain separated by a short kinase insert and ends in a COOH terminus. The Stem Cell Factor (SCF) is the only ligand that can bind to and activate c-kit.

Steel Factor or the Stem Cell Factor

Stem Cell Factor (SCF) or the steel factor is the only known ligand for c-kit. It exists as both a membrane bound isoform with 220 amino acids and as a soluble isoform with 248 amino acids (Fig. 2) [14]. The encoding gene is present on chromosome 12 in humans and has 9 exons producing two isoforms [15,16]. The isoforms differ in the presence or absence of exon 6 that has the cleavage site and both are initially produced as membrane bound proteins. The soluble isoform with exon 6 containing the cleavage site gets cleaved by proteases like Matrix Metalloproteases (MMPs), chymase-1 as well as ADAM family of proteases to yield a 165 amino acid soluble SCF which gets released in the extracellular space [17-19]. Both the isoforms are biologically active, however, they exhibit functional differences. Notably, the binding of soluble SCF to the c-kit receptor induces a rapid but transient activation of the receptor, whereas the membrane bound isoform has a more prolonged effect [20].

Several cell types express SCF and c-kit including endothelial cells, fibroblasts and bone marrow stromal cells where they serve roles in proliferation, survival, migration and differentiation [6,14,21]. However, the regulation of SCF expression is poorly understood.

Figure 2: Schematic representation of the structure of SCF: The ligand for c-kit, SCF has two isoforms; the membrane bound and the soluble isoform. Both are synthesized as a membrane bound form but the longer transcript gets cleaved by proteases due to the presence of the cleavage site, producing soluble SCF.

c-Kit Receptor Activation and Downregulation

Similar to other growth receptors, c-kit dimerizes upon ligand binding resulting in autophosphorylation and receptor activation [22]. The extra cellular Ig-like domain is critical for ligand binding and dimerization. SCF binds to c-kit at the first three Ig-like domains and induces a conformational change in the Ig-like domain 4 and 5 thereby bringing contiguous two c-kit monomers in close proximity. In its inactive state, the juxtamembrane domain of c-kit interacts with the kinase domain, thus inhibiting its enzyme activity [22-24]. However, upon dimerization, Tyr-568 and Tyr-570 in the juxtamembrane domain get phosphorylated, causing a conformational change which abolishes its inhibitory effect on the kinase domain. Subsequently, the kinase domain phosphorylates other tyrosine residues (autophosphorylation) on the opposite c-kit monomer, resulting in receptor activation and downstream signaling [24,25].

Upon continuous activation of c-kit, down regulation of c-kit occurs through three main processes

1. Receptor internalization and degradation
  2. Negative regulation by protein kinase C
  3. Tyrosine dephosphorylation by phosphatases
- Receptor internalization and degradation: Following the binding of ligand and c-kit receptor activation, the E3 ubiquitin ligase c-Cbl, binds directly or indirectly through other proteins like Grb2, APS, p85 subunit of phospho inositol 3 kinase (PI3-kinase), CrkL [26-30]. Binding of this ubiquitin ligase leads to the covalent attachment of a single ubiquitin moiety on the receptor which marks it for internalization through clathrin coated pits followed by proteosomal and lysosomal degradation [31]. Internalization and degradation of the receptor after ligand binding is followed by the repopulation of new c-kit molecules at the membrane [32]. For the ubiquitination process to occur, the c-Cbl has to be phosphorylated and activated by the Src family kinases [26,33-35]. Recent studies have shown that other E3 ubiquitin ligases like SOCS6 are also involved in the downregulation process [36,37].
  - Negative regulation by protein kinase C: The serine/threonine kinase protein kinase C (PKC) has been shown to play a role in c-kit inactivation [38-40]. SCF dependent c-kit activation results in a PI3-kinase dependent activation of phospholipase D leading to the release of phosphatidic acid. This in turn can be dephosphorylated forming Diacylglycerol (DAG), a potent activator of PKC. Activation of PKC leads to the phosphorylation of two critical serine residues present in the kinase insert of c-kit (S741 and S746). This causes inhibition of the receptor's kinase activity and hence, termination of signal transduction [41].

- Tyrosine dephosphorylation by phosphatases: Receptor tyrosine kinases can also be inactivated by dephosphorylation of their tyrosine residues by phosphatases. It has been shown that the phosphatase SHP1 can interact with c-kit through one of its SH2 domain and dephosphorylates via its carboxy terminal protein tyrosine phosphatase domain [42, 43].

Signal Transduction

c-Kit activation is followed by the recruitment and activation of several downstream signaling molecules including Erk 1/2, Grb2, p38 MAPK, SFK, p85 subunit of PI3K and PLC γ) [44-46]. Fig. 3 depicts an overview of each of the main pathways involved in c-kit activation. It is important to remember that SCF dependent activation of c-kit can simultaneously activate more than one of these pathways. The combination of activated pathways and its downstream effects may differ depending on the species and on cell type (Fig. 3).

Figure 3: Signal Transduction in SCF Activated c-Kit Receptor: The ligand for c-kit, SCF binds to the c-kit receptor causing receptor dimerization and subsequent phosphorylation on key tyrosine residues. These residues act as a binding site for signal transduction molecules.

MAP Kinase Pathway

The Mitogen Activated Protein Kinase (MAPK) pathway is one of the major pathways activated by c-kit stimulation in melanocytes, mast cells and human leukemia cells [47-49]. The MAPK pathway is an essential pathway that promotes proliferation and survival of mammalian cells [50,51]. Although c-kit activation can activate all four types of MAP kinases namely ERK 1/2, p38 MAPK, ERK5 and JNK, activation of only ERK1/2 MAP kinase has been well characterized [52,53]. Ligand activated c-kit binds to the adapter protein Grb-2, which binds to the Guanine Nucleotide Exchange Factor (GEF) SOS. SOS then interacts with the membrane bound protein Ras and catalyzes the exchange of Ras-bound GDP with GTP. Subsequently, GTP activated Ras binds and activates several effector proteins including B-Raf. B-Raf activates Mek 1/2 kinase which in turn activates the pro-survival/pro-proliferation protein ERK, as evidenced by studies in melanoma cells [20,54]. Eventually, phosphorylated ERK 1/2 activates several downstream regulatory proteins including the ribosomal protein S6 Glycogen Synthase Kinase 3 (GSK3) and Microphthalmia Transcription Factor (MITF), thereby regulating cell survival and development [55-57].

PI3-Kinase Pathway

The PI3-kinase pathway is another important pathway activated by c-kit. Studies on SCF mediated c-kit activation in mast cells have shown that PI3K pathway is an important pathway regulating genes that are critical for cell survival, proliferation and differentiation [20,58]. Auto-phosphorylation of c-kit activates PI3K through phosphorylation of tyrosine 719 (Y721 in human) in mice [59]. Although there are four major classes of PI3 kinases, with the class I further divided into class 1A and 1B, much of the published articles address mainly class 1A. Structurally, class 1A PI3K has a regulatory subunit p85 and an enzymatic subunit p110, which can phosphorylate phosphoinositides [60]. p85 has SH2 domains through which it binds the phosphorylated c-kit receptor either directly at Tyr-712 or indirectly through the adapter protein GAB2 [61-64]. The binding induces a conformational change thereby activating the enzymatic p110 subunit. PI3K then translocates to the cell membrane and activates membrane bound Phosphoinositol 4, 5 biphosphate (PIP2) to form Phosphoinositol 3, 4, 5 trisphosphate (PIP3). Then PIP3 activates proteins with Plekstrin Homology (PH) domains, including AKT. AKT is a serine/threonine kinase that regulates many downstream signaling proteins pertaining to growth, proliferation and survival [65,66]. It promotes cell survival by inactivating the pro-apoptotic protein Bad via phosphorylation [67]. AKT also promotes survival through promoting the proteosomal degradation of the fork head transcription factor (FoxO) proteins which would otherwise promote the expression of pro-apoptotic genes [68]. Furthermore, AKT activation has been shown to activate mammalian Target of Rapamycin (mTOR), which in turn

activates ribosomal S6 kinase leading to the induction of pro-survival and anti-apoptotic genes [69,70].

SRC Family Kinase Pathway

The Src-Family Kinases (SFK) are a group of kinases that have been shown to mediate the effects of c-kit activation [52,71-73]. Some SFKs are ubiquitously expressed (Src, Yes, Fyn) whereas others are mostly expressed in hematopoietic cells (Lek, Hck, Fgr, Lyn, Blk). SFKs have an amino terminus, SH3 and SH2 domains that are involved in membrane targeting, a tyrosine kinase domain and a COOH-terminus. Inactive SH2 and the SH3 domains interact with the kinase domain and the phosphorylated COOH terminus, thus maintaining them in the inactive configuration. However, binding of the SH2 domain to a phosphorylated tyrosine residue or the SH3 domain to a proline rich region activates the enzyme and removes the inhibition of the kinase domain. The kinase activity of SFKs modulates the activity of multiple intracellular enzymes that play a role in cell proliferation, survival, differentiation and migration [74]. In addition, activation of SFKs regulates other survival/proliferation pathways including Erk 1/2, p38 MAPK, JNK and PI3K- AKT [52,71,72,73,75]. Indeed, SFKs have been shown to promote the proliferation of Mo7e cells, mast cells, melanocytes and also hematopoietic cells [73,76-78]. Interestingly, SFKs are also implicated in promoting the migration of primary hematopoietic cells like mast cells via phosphorylation of FAK and paxillin (a focal adhesion protein) [79-82].

Phospholipase C and D Pathways

There are 13 isoforms of Phospholipase C (PLC) [83-86] and two isoforms of phospholipase D (PLD) in humans [87,88]. Both these enzymes function to produce Diacylglycerol (DAG), a potent activator of the intracellular calcium releasing Protein Kinase C (PKC) that plays a significant role in cell proliferation, migration, survival [89]. Among the PLCs, the PLC γ has been extensively studied as a c-kit effector signaling pathway. PLC γ , which binds to Y730 residue on the activated c-kit receptor [46,90] hydrolyzes PIP₂ to form Inositol 1, 4, 5 trisphosphate (IP₃) and the membrane bound second messenger, DAG [91,92]. On the other hand, PLD produces DAG in an alternate route. It hydrolyzes the membrane lipid phosphatidylcholine or phosphatidylethanolamine to produce phosphatidic acid and choline. Phosphatidic acid hydrolase then hydrolyzes the phosphatidic acid to DAG. IP₃ functions by mobilizing Ca²⁺ from the endoplasmic reticulum whereas DAG activates PKC [93]. The resulting increase in intracellular Ca²⁺ has been found to mediate many cellular events like migration, proliferation and apoptosis [94].

JAK/STAT Pathway

JAK/STAT pathway is involved in cellular proliferation as evidenced in fetal liver cells [95], as well as in other cellular functions like differentiation, survival and proliferation as shown in mast cells [96-98]. JAKs are cytoplasmic tyrosine kinases that are bound to the Receptor Tyrosine Kinases (RTKs). Upon ligand mediated receptor activation, JAKs phosphorylate more tyrosine residues on the receptor creating a binding site for proteins with SH2 domains, like STATs. Upon binding, STATs then are phosphorylated at their tyrosine residues by JAKs and subsequently form homo or hetero dimers. Dimerization of STATs are followed by their translocation into the nucleus, where they bind to the DNA through their DNA binding domains, thereby activating the transcription of genes that are involved in cellular proliferation, survival and differentiation [99].

Physiological Role of c-Kit

c-Kit plays an important role in regulating cellular characteristics of various cell types. For instance, Matsui et al. analyzed c-kit Signaling in vascular endothelial cells and found a significant role of c-kit in promoting survival, migration and differentiation [100]. Several studies have shown that c-kit participates in regulating the survival, maintenance, self-renewal and engraftment of hematopoietic stem cells [101-103]. In addition, a recent study has demonstrated that c-kit is involved in pancreatic islet development by promoting differentiation and proliferation of insulin and glucagon-producing cells [104-111]. The results from the study indicates a role of c-kit in normal hematopoiesis, pigmentation, gametogenesis, immune response, intestinal motility, vasculogenesis, lung compliance and brain development. Interestingly, c-kit mutations are also associated with various types of tumors such as mast cell tumors, Gastrointestinal Stromal Tumors (GIST) and leukemia, making it a proto-oncogene [112-114].

Hematopoiesis

SCF-c-kit signaling has an essential role in maintaining normal hematopoiesis. Homozygous c-kit mutations in mice are embryonically lethal due to the development of severe anemia [105-107]. Indeed, hematopoietic cells in the bone marrow express c-kit as early as day 8 post-gestation in the embryonic yolk sac. The expression increases until day 15 post-gestation and then gradually decreases [108]. In support of c-kit role in hematopoietic cells, a study showed that transplanting the bone marrow of c-kit-mutant mice could not replenish cellularity in irradiated hosts [109]. The bone marrow of these mutants were found to contain significantly less Colony Forming Units Spleen (CFU-S), Colony Forming Unit Erythroid (CFU-E) and

Vajravelu BN | Volume 2, Issue 3 (2021) | JCMR-2(3)-041 | Research Article

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DOI: <http://dx.doi.org/10.46889/JCMR.2021.2304>

Colony Forming Unit-Granulocyte-Macrophage (CFU-GM) when compared to their control littermates [110-113]. In a recent study by Kimura, et al., knocking out c-kit in adult mice using the Cre-lox system resulted in marked hematopoietic failure [114]. Furthermore, exogenous addition of SCF was able to promote the proliferation and survival of Hematopoietic Stem Cells (HSCs) with no effect on self-renewal [115]. Another study by Leary, et al., showed that SCF (in combination with IL-3) shortened the G0 phase in HSCs' cell cycle and thus promoted their division [116]. A third study revealed that treating an enriched population of long-term repopulating hematopoietic stem cells increase their survival for at least 7 days compared to control cells [115]. Interestingly, SCF exhibits a synergistic effect with other cytokines like Erythropoietin (Epo), G-CSF, GM-CSF in promoting the proliferation and the colony size of BFU-E, CFU-GM and CFU-GEMM [117-119]. The findings of the aforementioned studies confirm that c-kit is essential for the maintenance and regeneration of Hematopoietic Stem and Progenitor Cells (HSPC). Not only does c-kit play a role in proliferation, but it is also involved in the interaction of HSPCs with the niche cells like the endothelial cells and the bone marrow stromal cells [114].

It is noteworthy that c-kit in the hematopoietic compartment is essential not only under steady state conditions, but also under conditions of stress, such as ischemia. For instance, Fazel, et al., have reported an increase in the myocardial expression of the c-kit ligand (SCF) following ischemic cardiac injury which led to the recruitment of hematopoietic progenitor cells to the site of injury and improved cardiac recovery. On the other hand, deletion of c-kit caused cardiac failure following the ischemic insult [120].

Immune Regulation:

Mast Cells

The role of c-kit in regulating the cellular characteristics of mast cells has been previously studied. c-kit has been reported as a key regulator of proliferation, survival, migration, differentiation and even secretory functions of mast cells. Mice with mutation in the W locus was found to have profound mast cell deficiency indicating a critical role of c-kit in mast cell development [121]. Furthermore, published studies have shown that depleting SCF from the growth medium of cultured mast cells induced apoptosis within 48 hours, as identified by DNA fragmentation. Interestingly, a subsequent replenishment with SCF rescued the dying cells, indicating an anti-apoptotic role of SCF [122].

In addition, the mitogenic role of SCF has been established in Bone Marrow Derived Mast Cells (BMMCs). SCF-treated BMMCs had significantly faster rate of proliferation than the control cells and doubled in number within only 48 hours of incubation. The group reported that SCF treatment is required for BMMC's cell cycle entry and survival [123].

In addition to its protective and proliferative roles, c-kit-activation by SCF in combination with IL-3 activation has been shown to facilitate mast cell differentiation [124]. SCF can also promote the migration and release of inflammatory mediators by cutaneous and lung mast cells. It was reported that SCF enhances IgE- dependent histamine and leukotriene C4 release from human lung mast cells [125], histamine and prostaglandin release from human cutaneous mast cells [126]. Migration of mast cells along a gradient of SCF was found to be essential in regulating mast cell biology [127].

Dendritic Cells

Unlike in mast cells, the role of c-kit in Dendritic Cells (DC) is still unclear. It is however known that the expression of c-kit is maintained in mature DCs and mast cells unlike other mature cell types which lose c-kit upon differentiation [128, 129]. Studies have reported that treating DCs with Cholera Toxin (CT) or House Dust Mite (HDM) has resulted in increased expression of IL-6, which promoted the differentiation of DCs linked to c-kit expression [130]. c-Kit activation in DCs was associated with the activation of jagged-2, an interacting partner of Notch-2. Confirming the significance of c-kit and DCs, c-kit-mutant mice significantly reduced expression of IL-6 and jagged-2 resulting in defective differentiation of DCs [131].

Lymphopoiesis: Although the development and functions of B-lymphocytes are not affected by c-kit mutation, the development, proliferation and functions of T-lymphocytes are dependent on c-kit [132,133]. Indeed, very early precursors of the T-lymphocytes which are CD4-CD8-CD3- (Triple Negatives; TN) highly express c-kit [134]. Neonatal mice with mutations in c-kit were not able to contribute to thymopoiesis [135]. In addition, when these cells had their c-kit receptor blocked with an antibody, they were not able to properly reconstitute the T- cell compartment upon transplantation of thymic explants [134]. Also, SCF-c-kit signaling was found to potentiate the Mixed Lymphocyte Reaction (MLR) which is a measure of lymphocyte response to allogenic determinants presented by the antigen presenting cells of the host organism [136]. In combination with IL-7, SCF also increases the proliferation of early thymocytes [137]. Studies in c-kit mutant W/W^v mice have documented a defective intestinal immune system in these animals reiterating the importance of c-kit in the maturation of the intestinal immune system [138].

Pigmentation

The role of c-kit and SCF in the development and migration of melanocytes is well established. Luo, et al., have shown that SCF-dependent activation of c-kit in cultured melanocytes induces the transcription of the tyrosinase gene which is involved in melanogenesis [139]. Studies on

the expression patterns of c-kit among different species have suggested that although to varying degrees, melanocytes are dependent on kit activation for the migration of their precursors (neural crest cells) and for their proliferation and survival [140]. Indeed, the neural crest cells in the head express c-kit as early as embryonic day 9.5. These precursors then migrate towards the eyes, inner ears and into the second brachial arch [141,142]. When kit is mutated, the migration of melanocyte precursors is blocked and they undergo apoptosis. Another study on mouse Hair Follicle (HF) melanocytes indicated that the proliferation of melanocytes is dependent on c-kit expression [143-145]. Interestingly, immunohistochemical analysis of HF melanocytes for Ki-67 and c-kit revealed that only the c-kit expressing subset were actively dividing. Also, SCF overexpression in mice significantly increased the number of actively dividing melanocytes and the use of a c-kit blocking antibody significantly reduced the number of these proliferating cells [143].

Gametogenesis

c-Kit and its ligand SCF play a crucial role in regulating reproduction in organisms. Phenotypic analysis of the White spotted and steel mice has revealed sterility of these mice due to a defect in the early development of Primordial Germ Cells (PGCs) which are required for normal spermatogenesis and oogenesis [146]. c-Kit mRNA expression can be detected in PGCs as early as 7 days post-coitum [147]. The SCF-c-kit signaling is involved in the proliferation and migration of PGCs from the hindgut to the genital ridge [148-150]. In the testes, c-kit expression can be detected in the postnatal interstitial cells of Leydig, primary spermatocytes and spermatogonia. Also, the truncated c-kit isoform (tr c-Kit) is found to be expressed in round spermatids [151]. Although this truncated c-kit contains no kinase activity as it has only the second kinase domain and the COOH terminal, it could cause the metaphase-anaphase transition when injected in mouse eggs [11]. Results of the study published by Packer, et al., have shown that the SCF-Kit signaling regulates the survival of germ cells *in-vivo*. Administration of an anti-c-kit antibody was found to promote apoptosis of primary spermatocytes and spermatogonia as identified by TUNEL staining [152]. On the other hand, in the post-natal ovary, c-kit is highly expressed in all oocytes at different stages of development. Using in-situ hybridization, the primordial oocytes were found to have a strong c-kit expression followed by interstitial theca cells [151]. The expression of both isoforms of SCF by the sertoli cells is critical for the survival and proliferation of spermatogonia. It is known that the c-kit protein expressed on spermatogonia interacts with the SCF expressed on sertoli cells leading to c-kit phosphorylation and activation [153]. Point mutation analysis of the c-kit receptor has identified that this activation leads to an increased proliferation of the differentiating type A spermatogonia through the activation of PI3K-AKT pathway [154].

Intestinal Motility

It is well known that loss of function mutations of the c-kit receptor in humans can cause piebaldism, a pigmentation disorder. In the same individuals, c-kit mutation also causes constipation due to the defective development of Interstitial Cells of Cajal (ICCs); the pacemaker cells of the intestine; responsible for its motility [155,156]. Interestingly, although these cells develop from the mesoderm, they establish an anatomic and functional connection with the enteric neurons during the later stages of their development [157]. The ICCs relay the electric impulses between the nerve cells of the intestine and the smooth muscles [156,158] and thus are required for the generation and propagation of autonomic gut motility. Defective development of the ICCs affects the enteric nervous system causing various dysfunctions. In the small intestine, c-kit⁺ paneth cells are the supporting cells that form the niche for the Lgr5⁺ intestinal stem cells. These paneth cells support the proliferation, survival and differentiation of the intestinal stem cells into secretory goblets or enteroendocrine cells. Similarly, the colon was found to have c-kit⁺ paneth-like goblet cells that support the Lgr5⁺ stem cells. These are the cells found at the base of the intestinal crypt that undergo cell division and differentiation to replace the epithelial lining of the colon [159]. c-Kit is also a key molecule in regulating mucosal immune responses. Upon stimulation with IL-1 β , a subset of c-kit⁺ Innate Lymphoid Cells (ILCs) that are present in the intestine produces IL-22, an important cytokine secreted by the small intestinal cells to protect themselves against bacterial infection. This IL-22 was found to have a protective role against Inflammatory Bowel Disease (IBD) besides promoting the proliferation and survival of intestinal epithelial cells [160].

Vasculogenesis

c-Kit has an important role in vasculogenesis, under both physiological and pathological conditions. It was found to be important for the mobilization of hematopoietic and endothelial stem cells that are required for angiogenesis. A study by Heissig, et al., in a mouse model has illustrated that mobilization of stem cells upon injection of some chemotactic agents like SDF-1, G-CSF occurs through an induction of MMP-9 that increases the levels of soluble SCF in the blood [17]. In addition, SCF has a protective effect on Vascular Smooth Muscle Cells (VSMC) from oxidative stress induced apoptosis and it is also found to be essential for VSMC proliferation. For instance, published results of Wang, et al., have shown that treating VSMCs with SCF can significantly lower the amount of cleaved caspase-3 in hydrogen peroxide (H₂O₂) treated VSMCs [161]. This pro-survival effect of SCF on VSMCs was found to be mediated through activation of the PI3K-AKT pathway leading to upregulation of the anti-apoptotic protein Bcl-2 that acts by inhibiting the release of cytochrome-c from the mitochondria to the cytosol [161]. Besides playing a direct role in mobilizing cells that are required for angiogenesis, the kit ligand also has an indirect role through the activation of mast cells. For

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example, it was found that SCF can induce mast cells to release VEGF, a potent angiogenic factor [162]. Matsui, et al., have clearly established that treatment of HUVEC cells with SCF promoted its survival, migration and capillary tube formation through the downstream activation of both PI3K-AKT and ERK 1/2 pathways [100]. Also under normoxic conditions, the interaction of SCF with its receptor caused induction of hypoxia-inducible factor 1 α (HIF-1 α), which enhances angiogenesis [163].

Lung Compliance

One of the critical functions of c-Kit was found to be the maintenance of lung compliance. Investigations in c-kit-deficient mice showed that the deficiency causes prominent enlargement of alveoli, a significant reduction in alveolar volume density and alveolar surface density, which are parameters of proper lung function. There was a significant alteration in the pressure-volume changes due to c-kit mutation with the c-kit mutant mice having a larger lung residual volume and static lung compliance [164]. Some of the abnormalities in the alveolar histology can be ascribed to a defective alveolar epithelium in which c-kit has been implicated to play a role in regulating its growth [164]. Also as discussed earlier, c-kit is essential for proper development, survival and maturation of HSCs, which contributes to the regeneration of the lung parenchyma following injury induced by radiation or by administering lipopolysaccharides, elastase and/or radiation [165-167]. In a murine model of elastase-induced emphysema, investigators found that instillation of hepatocyte growth factor repaired the injury and was accompanied by an increase in the levels of bone marrow derived c-kit⁺ cells, highlighting the beneficial reparative role of c-kit [168]. In another study by Ding, et al., the authors found that SCF-c-kit interaction can promote migration of c-kit⁺ bone marrow cells to the bleomycin injured lung and that migratory potential is reduced by inhibiting c-kit using imatinib (c-kit inhibitor) [169]. The same study found a subset of lung fibroblasts expressing c-kit which were highly responsive to SCF. Also co-culturing BM-derived c-kit⁺ cells with myofibroblasts promoted their differentiation through a paracrine effect [169]. The findings of the above studies indicate that c-kit is required for the maintenance of normal alveolar architecture and that its expression on alveolar epithelial cells and lung fibroblasts is indispensable for proper maintenance of lung homeostasis.

Brain Development

Both c-kit and SCF are expressed widely in the nervous system. In most part of the nervous system, the c-kit receptor and its ligand are found to be expressed in neurons that are connected synaptically. In the embryo, the cells forming the floor of the neural tube were found to have prominent SCF expression, whereas the cells on the dorsal surface of the neural tube were

strongly c-kit+ [170,171]. In the adult mouse brain, SCF expression is mostly confined to the Purkinje fibers in the cerebellum and the c-kit expression is found in the cells that are involved in synaptic connection with the Purkinje fibers namely, Golgi, stellate and basket cells. Apart from the cerebellum, a high level of SCF expression is also found in the thalamus and cerebral cortex, whereas high c-kit expression is found in the hippocampal region of the cerebral cortex [172]. In an *in-vivo* ischemic mouse brain model, SCF administration induced neurogenesis by promoting the proliferation of the neurons which was abolished by treating the cells with anti-c-kit antibody [173]. Neural stem/progenitor cells are important for the regeneration of the neural tissue after injury. In a ‘freeze’ brain injury model, SCF could cause migration of these neural stem/progenitor cells to the injured area both *in vitro* and *in-vivo* [174]. In the Dorsal Root Ganglia (DRG), c-kit expression can be detected on embryonic day 12.5 and persists during the post-natal period. c-Kit functions to support the survival of DRGs and SCF treatment can induce the growth of c-kit+ neuritis from the DRGs [175]. There are also reports suggesting that the c-kit receptor has a role in central and peripheral pain regulation [176].

Role in Tumorigenesis

The oncogenic role of c-kit is well established in a variety of tumors. Activating mutations of c-kit play a role in Gastrointestinal Stromal Tumors (GIST), germ cell tumors, malignant transformation of mast cells, breast tumors, malignant melanomas, small cell lung cancer and acute myeloid leukemia.

Gastrointestinal Stromal Tumors (GIST)

The interstitial cells of Cajal are the pacemaker of the intestine and have been implicated to be important for the motility of the intestine [156]. GISTs are tumors of the intestine that arise from these Cajal cells with most of them occurring in the stomach and the rest in the small intestine and rarely in the esophagus, colon, appendix and rectum [177-182]. Gain-of-function mutations of c-kit have been shown in approximately 95% of GISTs with the majority of these mutations being a deletion or a point mutation and occasionally an insertion [183]. These mutations result in constitutive activation of c-kit in the absence of its ligand, SCF. The intra-cellular juxta-membrane domain is the region where most of the mutations are reported to occur and is encoded by exon 11. The remaining mutations are reported to occur in the extra cellular region (exon 9) and in the intracellular kinase domain (exon 13) [184]. Mutations in the c-kit gene are classified as either enzymatic or regulatory in nature. The enzymatic mutations occur in the “enzymatic pocket” of the receptor affecting its enzymatic function whereas mutations in the intracellular juxta-membrane region which regulates the enzymatic pocket are considered regulatory [185]. GISTs with regulatory mutations of the c-kit receptor responds well to

Vajravelu BN | Volume 2, Issue 3 (2021) | JCMR-2(3)-041 | Research Article

Citation: Vajravelu BN, et al. c-Kit - The Novel Receptor: Physiological Roles, Downstream Signaling and Implications in Cancer. Jour Clin Med Res. 2021;2(3) :1-36.

DOI: <http://dx.doi.org/10.46889/JCMR.2021.2304>

imatinib treatment than tumors with enzymatic c-kit mutations and hence have a better clinical prognosis [185]. The clinical symptoms of GISTs vary widely depending on the size of the tumor and the site of occurrence. The patients can be asymptomatic if the tumor is smaller than 2 cm or may present with abdominal discomfort, altered bowel habits or dysphagia. Larger tumors can cause abdominal obstruction, perforation, acute or chronic intestinal hemorrhage [179,180,186]. Untreated tumors can also metastasize to the liver or abdomen and sometimes to the bones or lungs [186,187].

Germ Cell Tumors

SCF-c-kit Signaling is critical for normal spermatogenesis and oogenesis [148-150]. Mutations of c-kit have been found in Germ Cell Tumors (GCT) and are considered important in determining the pathogenesis and the response of the disease to treatment with imatinib [188-193]. GCTs are tumors of the primordial germ cells that form the male and female gametes. GCTs are broadly divided into seminomas/dysgerminomas and nonseminomas/nondysgerminomas. Activating mutations of c-kit were identified in seminomas and dysgerminomas and were found to be not related to nonseminomas/nondysgerminomas [194,195]. Seminomas can be further classified into unilateral and bilateral seminomas based on its occurrence. c-Kit mutations associated with these tumors were found to be Y823D, D816V, D816H and N822K. Almost all these mutations were detected in the intracellular kinase domain encoded by the exon 17 of the c-kit gene [196]. These mutations cause constitutive phosphorylation on the tyrosine residues of the receptor, thereby leading to prolonged activation of the downstream pro-survival and mitogenic signaling pathways, resulting in cellular transformation [192]. However, there are contradicting reports on the association of c-kit mutation in unilateral versus bilateral seminomas. Some studies have reported that mutations in c-kit are associated with bilateral seminomas [190,197] whereas others have reported no association [198]. The contradiction can be ascribed to differences in sample size or the mutation analysis technique used in each study. Like the seminomas, the dysgerminomas also have a strong association with c-kit expression. c-Kit overexpression was found in around 85% of dysgerminomas studied and 30% of those samples had a mutation in their c-kit gene [199]. More interestingly, c-kit overexpression was found to have a high correlation with advanced pathological stage of the disease [200].

Mast Cell Tumors

Mast cells are derived from CD34⁺ hematopoietic progenitor cells [201,202]. Immature mast cells reside in the bone marrow and peripheral blood [203]. They migrate to the tissues and differentiate to form mature mast cells. As mentioned in the discussion on the physiological

role of c-kit, mast cell differentiation, survival, proliferation and migration is dependent on SCF-c-kit signaling [123-127]. Mast cells have intra cytoplasmic granules filled with pro-inflammatory and vasoactive mediators that are released upon IgE mediated immune response to an external stimulus [204]. The clinical features presented in mast cell neoplastic transformation (mastocytosis; MC) are essentially due to the release of these mediators in the blood or due to the scavenging of mast cells in various tissues. The World Health Organization (WHO) has classified MC under three major groups

1. Cutaneous MC which is mostly prevalent among children and regresses as they reach adulthood
2. Systemic MC (SM), which is further classified into indolent SM, SM with an associated clonal hematologic non-mast cell-like disease, aggressive SM and mast cell leukemia
3. Extra cutaneous mastocytoma [205]. The D816V c-kit mutant was found to be associated with around 90% of the SM cases with the other mutations of exon 17 contributing to the remaining 10% [206-208].

It is important to remember that this mutation is not exclusively found in SM and hence cannot be used as a diagnostic criterion for SM. Studies in canine CM models have illuminated that c-kit mutation in CM is associated with increased proliferation and poor prognosis. Although activating mutations of c-kit is highly correlated with CM and SM, the expression of c-kit has no role in determining the pathogenesis of MC [209,210].

Breast Tumors

The role of c-kit in the pathogenesis of breast tumors is not well established and hence it is mostly of diagnostic and prognostic value. There are conflicting results on the expression of c-kit in tumors of the breast [211-215]. Most of the studies found an inverse relationship between c-kit expression and tumor transformation of breast tissue. There is a strong expression of c-kit in the normal mammary epithelium and in benign breast tumors. However, this expression gradually diminishes and is lost completely upon malignant transformation in females [211,212,216]. It is interesting to note that there is a difference in the expression of c-kit between male and female malignant breast conditions. Although the membrane and cytoplasmic expression of the receptor is absent in malignant breast tumors in females, a strong expression of c-kit has been identified in male malignant breast tissue samples. This indicates that c-kit has a pivotal role in the differentiation of mammary epithelium and is lost when the cells become more undifferentiated upon malignant transformation [216]. The difference in c-kit expression between male and female breast conditions indicates a significant difference in the molecular mechanisms of breast cancer development between genders. However, the pathways involved in the malignant transformation of human breast tissue are not clear. Unlike

other tumors in which c-kit plays a role, none of the benign or malignant tumors of the breast are associated with mutations of c-kit [214,217].

Malignant Melanomas

SCF-c-kit axis also plays a critical role in the development, migration and proliferation of melanocytes [139-142,218]. Constitutively active c-kit promotes migration of melanocytes but also cause pigmentation and proliferation defects [219]. The reduction in pigmentation is due to increased degradation of the Microphthalmia- Associated Transcription Factor (MITF) caused by increased c-kit Signaling [54]. MITF is a transcription factor that is important for regulating the survival and proliferation of melanocytes [57]. Increased polyubiquitination and proteosomal degradation of MITF under constitutive c-kit activation reduces the survival of melanocytes and hence affects proper pigmentation [54]. Studies conducted early in the 1990s largely dismissed the role of SCF-c-kit axis in melanoma initiation and progression. These investigations highlighted the loss of c-kit expression when the melanoma become invasive and that expressing c-kit in invasive melanoma cell lines induces apoptosis and reduction in tumor growth [220-223]. However, later studies have shown a strong evidence of the involvement of c-kit in the transformation of melanocytes and in melanoma progression. These studies have highlighted that melanomas are highly heterogenous group of tumors. Although a majority of melanomas carry mutations in BRAF or NRAS leading to constitutive activation of the MAPK pathway and tumor transformation [224,225], there is a significant subset of melanomas that do not harbor BRAF or NRAS mutation but however, has a mutation in the c-kit gene [226,227]. A study conducted by Bastian showed that while BRAS and NRAS mutations are more common in melanomas occurring in areas with less sun exposure like the cutaneous melanomas, activating mutations of c-kit and gene amplification is more common in melanomas with chronic sun damage, acral and mucosal melanomas [226]. Subsequent studies have identified that 30% of c-kit mutations in melanoma are also accompanied by c-kit gene amplification and there are also reports of isolated gene amplification in melanomas without c-kit mutation, further confirming the heterogeneity in the pathway of tumor transformation in melanocytes [228-231].

Small Cell Lung Cancer

Small Cell Lung Cancer (SCLC) is an aggressive type of lung cancer contributing to 15-20% of all lung tumors. SSLCs have clinically poor prognosis and are not responsive to chemotherapy [232]. This necessitates finding better treatment strategies to benefit the patients suffering from the disease. For a better understanding of the disease and to find new treatment options, different molecular markers that were implicated in the pathogenesis of the disease

Vajravelu BN | Volume 2, Issue 3 (2021) | JCMR-2(3)-041 | Research Article

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were studied. One of such markers is the tyrosine kinase receptor, c-kit. The expression of c-kit on the SSLC cells analyzed using immunohistochemistry varies from 37% [233] to as high as 87% [234]. An interesting finding is that the tumor cells also co-express SCF [235,236] suggesting the existence of an autocrine loop playing a role in the tumor growth and survival. However, clinical studies conducted on patients with SCLC failed to support the above hypothesis. While some studies completely reject c-kit as a possible therapeutic target and a useful prognostic factor based on their results [237,238], other studies provide evidence to use c-kit as a marker to predict the clinical outcome [233,234,239]. The difference could be due to the sample size and the patient population selected for the study. Hence, the role of c-kit in SCLC is still debatable and warrants more clinical studies with the selection of appropriate patient populations.

Acute Myeloid Leukemia

Studies on the pathogenesis of Acute Myeloid Leukemia (AML) have identified two types of mutations that are responsible for the disease genesis. Type I includes the mutations of the receptor tyrosine kinases resulting in constitutive activation of the receptor and aberrant signal transduction leading to uncontrolled proliferation and survival of the transformed cells. Type II includes loss of function mutations of the transcription factors that are critical for the differentiation of myeloblasts [240]. Around 60-80% of myeloblasts express c-kit and mutations of c-kit are observed in around 17% of AML patients [241-244]. In core binding factor AML; a subtype of AML; around 52% of the patients have c-kit mutation [242], mostly in the extra cellular ligand binding domain encoded by exon 8 or in the kinase insert region encoded by exon 17. The only other RTK mutation that is observed at a higher frequency than c-kit is the mutation in FLT3 gene [245]. Although the role of c-kit in the pathogenesis of AML and as a prognostic factor is still unclear, several studies have suggested that mutations in the c-kit gene have an adverse outcome in AML patients [246-248]. Patients with c-kit mutations are reported to have higher relapse rate and poor prognosis, both in adults and in children [249-251].

c-KIT Inhibition in Cancer Treatment and Therapy Resistance

Among the currently available Tyrosine Kinase Inhibitors (TKIs), there are at least 16 drugs that are known to inhibit c-kit, albeit with variable potencies and specificities. This includes, axitinib, dovitinib, dasatinib, imatinib, motesanib diphosphate, pazopanib, sunitinib malate, masitinib, vatalanib, cabozantinib, tivozanib, amuvatinib, Ki8751, telatinib, dovitinib, tyrphoston [252]. These inhibitors competitively bind to c-kit and prevent the activation of downstream signaling cascade. Among the potent inhibitors of c-kit are dasatinib, sunitinib,

masitinib and pazopanib[253, 254]. Most of these drugs also inhibit survival (BCR/ABL, FLT3), and/or angiogenesis (VEGF and PDGF) pathways. These drugs were initially designed to block pathways involved in malignant cell survival, proliferation and/or vasculogenesis, with the ultimate goal of combating cancer [255].

The question of using selective c-kit inhibitors to combat cancer has been debatable. However, while it is widely accepted that using selective c-kit inhibitors may result in blocking a pro-oncogenic pathway and should result in fewer side effects, there is increasing body of literature showing that proliferation and invasiveness of tumors are dependent on multiple pathways. This indicates that even if c-kit is inhibited by a small molecule, tumors may prove resistant to antineoplastic therapy via activating other proliferative pathways to keep growing. Thus, a multi-targeted therapy that inhibits multiple kinases are more likely to provide better clinical efficacy against tumors [256].

Another reason for cancer cell's ability to resist the effect of c-kit inhibition by small molecules is the inability of these drugs to bind to the active conformational form of c-kit. For instance, although imatinib- a well-established tyrosine kinase inhibitor- has the ability to inhibit c-kit, some c-kit mutants were proved refractory to imatinib treatment because it was not able to bind active c-kit [255]. Thus, a new generation of c-kit inhibitors, such as sunitinib and regorafenib, have been designed to ensure their ability to bind both the non-activated and activated forms of c-kit [254,257,258]. Mutations in c-kit have also been implicated in inducing resistance of tumors to TKIs. For instance, GIST patients who are responsive to second generation sunitinib cease to respond to therapy if tumor cells acquire a D816H/V mutation in c-kit [259].

c-kit and Cancer Cell Stemness

Tumor recurrence either at the primary site or at the secondary site (following metastases) is still an unresolved issue in medical practice, leading, at times, to therapy failure. This resistance to therapy may be attributed to the activation of cancer stem cells in both sites. These cancer stem cells that remain after tumor resection are postulated to drive tumor recurrence in the primary cancer site. On the other hand, circulating cardiac stem cells are thought to drive recurrence in secondary sites. And because these cells are ultimately stem cells, they may also express c-kit on their cell membranes, just like regular non-oncogenic stem cells. Thus, it has been proposed that SCF/CD117 pro-survival and pro-proliferative signaling cascades on cancer stem cells may possibly play a role in promoting cancer progression despite chemotherapy, conferring resistance to treatment with antineoplastic agents [260,261].

In accordance, several reports have shown that c-kit is expressed in recurrent tumors [262-264] and that c-kit over-activation is seen in GIST, AML, mastocytosis and melanoma [265,266]. Also, it was shown that c-kit was expressed in 88% of surveyed cases where GIST had

metastasized to bone [267]. In addition, higher c-kit expression in ovarian carcinomas appeared to result in poorer prognosis, peritoneal metastasis and shorter life expectancy [268]. Indeed, c-kit has been proposed to confer resistance to ovarian cancer cells against chemotherapy [269]. Also, c-kit expression in circulating cancer cells predicts advanced prostate cancer [262]. Increased expression of c-kit accompanied prostate cancer cells' metastases to secondary sites; potentially through a downstream cross-talk that eventually resulted in BRCA2 downregulation in these cells [270]. In breast carcinomas and small cell lung cancers, c-kit was also a major regulator of cancerous cell survival, growth and proliferation [271].

Since c-kit activation in a cancer cell initiates multiple downstream signaling pathways that are believed to induce stemness (e.g. RAS/ERK, PI3-kinase, SRC, JAK/STAT and WNT), c-kit is thus implicated in maintaining cancer resilience. Indeed, it has been shown that activation of tyrosine kinase SRC in acute AML cells resulted in crosstalk with Akt-mTOR and promoted stemness of cells [272,273]. Similarly, Ras/ERK pathways have been implicated in conferring stemness to colon carcinoma and synovial sarcoma cell lines [274]. Taken together, these data suggests that c-kit is involved in cancer cell stemness and may be used as biomarker for cancer stem cells.

c-Kit Clinical Trials

The pleiotropic roles of c-kit as a protooncogene and a cell-surface marker has invited significant amount of research about its utility in cancer treatment and as a stem-cell marker through multiple clinical trials. Thus far, more than hundred clinical trials have been completed and more underway offering hope for better cancer treatments in the near future. Most of them have tested c-kit inhibitors like imatinib mesylate, nilotinib, dasatinib in cancers with constitutive c-kit activation such as melanomas, Gastrointestinal Stromal Tumors (GIST), acute myeloid leukemia, myelodysplastic syndromes and mastocytosis [275-278]. Few clinical trials have utilized c-kit positive stem cells for myocardial regeneration [279,280]. Although the outcomes of these clinical trials are promising, most of them still lack a more conclusive phase III testing.

Concluding Remarks

Since the discovery of c-kit in 1986, numerous studies have established its pivotal role in both physiological settings and in disease processes. RTK inhibitors such as imatinib has significantly improved the outcome of cancers due to c-kit mutation. However, there are still many areas that need further research. For example, any difference in the downstream effects of the splice variants of c-kit is largely unstudied and the problem of tumors developing

resistance of c-kit inhibitors remain. A deeper understand about the interplay between specific c-kit mutation and the signal dysregulation is currently lacking. Also, whether c-kit binds or can be activated by a ligand other than SCF is unknown. Future studies focusing on elucidating these challenges will make this receptor a target for the treatment of various tumorous conditions.

Acknowledgement

We thank Dr. Kyung Hong for his guidance and Dr. Afsoon Moktar for the helpful discussion.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Vajravelu BN | Volume 2, Issue 3 (2021) | JCMR-2(3)-041 | Research Article

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