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SOCS6 is a selective suppressor of receptor tyrosine kinase signaling

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Abstract

The suppressors of cytokine signaling (SOCS) are well known negative regulators of cytokine receptor signaling. SOCS6 is one of eight members of the SOCS family of proteins. Similar to other SOCS proteins, SOCS6 consists of an uncharacterized extended N-terminal region followed by an SH2 domain and a SOCS box. Unlike other SOCS proteins, SOCS6 is mainly involved in negative regulation of receptor tyrosine kinase signaling. SOCS6 is widely expressed in many tissues and is found to be down-regulated in many cancers including colorectal cancer, gastric cancer, lung cancer, ovarian cancer, stomach cancer, thyroid cancer, hepatocellular carcinoma and pancreatic cancer. SOCS6 is involved in negative regulation of receptor signaling by increasing degradation mediated by ubiquitination of receptors or substrate proteins and induces apoptosis by targeting mitochondrial proteins. Therefore, SOCS6 turns out as an important regulator of survival signaling and its activity is required for controlling receptor tyrosine kinase signaling.

Keywords: SOCS, FLT3, KIT, INSR, SCF, Insulin, FL.

Introduction

Signals mediated by growth factors are involved in diverse cellular processes including cell proliferation, differentiation, cell cycle progression and gene expression. Growth factors transduce signals through their corresponding receptors which upon growth factors binding dimerize, get activated and autophosphorylated. Aberrant and sustained activation of growth factor receptor signaling has been associated with several disorders including autoimmunity and cancer. The negative feedback regulation plays an important role by preventing over-activation of growth factor signaling thus maintaining a balance in proliferative signals. Loss of function of negative feedback regulators results in sustained proliferative signals leading to malignancies. The critical signaling attenuators in these processes include phosphatases that dephosphorylate receptors and shut down signaling cascades, ubiquitin ligases that destabilize and regulate receptor turnover, and adaptor proteins that compete with signaling molecules or recruit negative regulators to the receptors. One class of E3-ubiquitin ligase called suppressors of cytokine signaling (SOCS) has been extensively studied in regulation of cytokine receptor. Among the different SOCS family proteins SOCS6 displays unique characteristic in regulation of growth factor receptor signaling. This review discusses the latest findings on the function of SOCS6 and comments on the mechanisms of regulation.

SOCS family proteins

The SOCS family consists of eight structurally similar proteins named SOCS1-7 and CIS (cytokine inducible SH2-containing protein). The most common features of this family of proteins are presence of a functional Src-homology 2 (SH2) domain and a C-terminal SOCS box [1]. The SH2 domain is predominantly involved in the interaction with certain phospho-tyrosine residues of signaling proteins, and the SOCS box is involved in recruitment of the ubiquitin ligase machinery. Therefore, SOCS family proteins can bind to tyrosine-phosphorylated receptors or signaling proteins and then link to the ubiquitination machinery directing proteins to ubiquitin-mediated degradation [2-5]. Although the C-terminal part of the different SOCS family proteins display structural similarity, the N-terminal region is highly divergent. SOCS1-3 have comparatively shorter N-terminal region, while SOCS6 and SOCS7 have a longer still uncharacterized N-terminal region [1]. Studies suggest that the extended N-terminal region is involved in the interaction with several SH3 domain containing proteins [6, 7].

Although SOCS family proteins have been widely studied in regulation of cytokine signaling, recent studies suggest that these proteins also play a role in receptor tyrosine kinases signaling. For example, SOCS1 is involved in the regulation of several receptors including platelet-derived growth factor receptor (PDGFR), colony-stimulating factor 1 receptor (CSF1R), FMS-like tyrosine kinase 3 (FLT3), stem cell factor (SCF) receptor (KIT), insulin receptor (INSR), proto-oncogene MET and AXL [8-14]. Similar regulatory roles were described for SOCS2, SOCS3 and SOCS6 as well [1]. Since these receptors are widely known for contributing to regulation of malignant signaling, it is possible that SOCS proteins play a role in the regulation of malignancies. Recently the relationship between SOCS1 expression and progression of cancer has been established [15]. However, other SOCS proteins have not been extensively studied in relation to regulation of receptor tyrosine kinase signaling. Although SOCS1, SOCS2 and SOCS3 also contribute to regulation of cytokine signaling [16], we currently do not have any evidence that SOCS6 interferes with cytokine signaling [17]. Therefore, it is more likely that SOCS6 is a selective regulator of receptor tyrosine kinase signaling. SOCS6 is also involved in the regulation of cell proliferation, transformation and apoptosis. For example, overexpression of SOCS6 is sufficient to inhibit cell proliferation and colony formation of AGS and AZ-521 cells [18]. SOCS6 expression enhances apoptosis by targeting mitochondrial proteins [19]. However, unlike other SOCS proteins SOCS6 does not interact with JAK2 [20], the growth hormone (GH) receptor (GHR) [21] nor block prolactin receptor

(PRLR) [22], leukemia inhibitory factor (LIF) [23] or GH signaling [21]. These studies suggest that SOCS6 in many cases is a negative regulator of survival signaling while it is not a negative regulator of cytokine signaling.

SOCS6 gene and protein

Although *SOCS6* is the official gene symbol for SOCS6 protein, the gene is also known as CIS4, SSI4, CIS-4, SOCS4, STAI4, SOCS-4, SOCS-6, STAT4 and HSPC060. The gene is located on human chromosome 18q22.2 spanning 40 kb and encodes a 535 amino acid polypeptide [24]. Deletion of this region has been shown to be associated with congenital anomalies [25]. However, direct involvement of this gene has not yet been demonstrated. SOCS6 is highly conserved in many species including mouse, chicken, frog and zebrafish (Fig. 1) suggesting that SOCS6 might be involved in critical biological processes. Furthermore, the functional domains present in SOCS6, such as the SH2 domain and the SOCS box, are highly conserved between the species which further suggests that SOCS6 function is highly conserved. The N-terminal poorly characterized domain is less conserved compared to the other two domains and might play a slightly different function. This domain contains several highly conserved proline-rich motifs such as PXPWP, PLRP, PLSP and PLLPP, suggesting that SOCS6 may associate with SH3 domain containing proteins and thus creates multi-protein complexes.

SOCS6 expression in different tissues and relationship to human disease

SOCS6 displays a tissue-specific expression pattern (Table 1). Normal tissue sample from uterus, thyroid gland, and pancreas express higher level of SOCS6 mRNA, while tissues from the ovary, lung, stomach, skin, bladder, small intestine, prostate and vulva express relatively low level of SOCS6 [24, 26] indicating that SOCS6 plays specific roles in selective tissues. Besides the expression in normal tissues, SOCS6 expression has also been described in malignant cells. Expression was identified in granulocyte-macrophage colony-stimulating-factor (GM-CSF)-dependent myeloid cell lines such as Mo7e, UT-7 and TF-1 [20]. These cells also express stem cell factor (SCF) receptor, KIT, and SCF stimulation increases SOCS6 mRNA expression [5]. Therefore, it is suggested that SOCS6 is involved in the regulation of hematopoietic malignancies. Stimulation of another hematopoietic growth factor receptor, FLT3, with its ligand (FL) also induces SOCS6 expression [3].

Insulin stimulation increased SOCS6 expression in mouse embryonic fibroblasts [17] and also stabilized the protein [27]. Similar observation was described where platelet-derived growth factor (PDGF) increased the stability of SOCS6 [27] suggesting that the activation of receptor tyrosine kinase signaling leads to transcriptional activation of the SOCS6 gene as well as stabilizes the protein through a still unknown mechanism. Treatment with the tumor promoter phorbol ester Phorbol 12-myristate 13-acetate (PMA) also stabilizes SOCS6 in transfected HEK293T cells [27]. PMA is a well-known activator of the protein kinase C (PKC) family of proteins, which have been reported to be up-regulated in many cancers and play critical roles in cancer progression [28-32]. The small molecule drug risperidone, an antipsychotic drug mainly used to treat schizophrenia, induces SOCS6 expression in the human SH-SY5Y neuroblastoma cell line [33]. However, the exact role of SOCS6 in PMA-induced or risperidone-inhibited signal transduction remain mostly unknown. It is known that SOCS6 is required for partial inhibition of insulin and leptin signaling by risperidone [33].

The human SOCS6 gene is located at a locus (18q22.2) which is frequently associated with malignant diseases. Allelic loss of this locus often occurs in several cancers. Loss of gene copy number of SOCS6 has been identified in colorectal cancer but did not display any correlation with disease free survival or overall survival [34]. SOCS6 expression has been found to be down-regulated in gastric cancer. Loss of SOCS6 expression is caused by the epigenetic modifications such as hyper-methylation of the promoter

region [18]. SOCS6 copy number loss has also been reported in primary lung squamous cell carcinoma and has been shown to be associated with poor survival [35] suggesting that SOCS6 expression can be used as a prognostic marker in this disease. Ovarian, stomach and lung cancer tissues express comparatively high levels of SOCS6; however, its role in these cancers has not yet been defined [26]. Although normal thyroid expresses higher level of SOCS6, reduced SOCS6 expression was reported in patients with thyroid cancer [26]. SOCS6 is downregulated in hepatocellular carcinoma and a correlation between SOCS6 expression and progression of hepatocellular carcinoma has been established [36]. Reduced SOCS6 expression was associated with elevated tumor recurrence risk and poor disease free survival. Although loss of the SOCS6 gene has been shown to be the result of epigenetic modification, a recent report suggests that micro RNA miR-424-5p plays a role in the suppression of SOCS6 expression in pancreatic cancer [37]. Another report suggests that micro RNA, miR-431 downregulates SOCS6 expression in medulloblastoma and glioblastoma cells [38]. Higher expression of miR-17-5p was identified in gastric cancer resulting in down-regulation of SOCS6 expression [39]. Taken together, the current data support the idea that SOCS6 acts as a tumor suppressor in multiple cancers.

SOCS6 is a regulator of insulin receptor signaling

The insulin receptor (INSR) is probably a target of all SOCS proteins although current data suggest that SOCS1, SOCS2, SOCS3, SOCS6 and SOCS7 associate with and regulate INSR signaling [1]. By overexpressing both SOCS6 and INSR, it has been demonstrated that SOCS6 associates with INSR in response to insulin in rat hepatoma cells [40]. Furthermore, SOCS6 could pull down endogenous INSR in HepG2 cells treated with insulin [40]. These studies demonstrate that activation of the INSR is indispensable for its interaction with SOCS6. Tyrosine residues of activated receptor are most likely the binding sites for the SH2 domain of SOCS6.

Activation of INSR leads to activation of several signaling cascades resulting in phosphorylation-dependent activation of AKT and ERK proteins [41]. Overexpression of SOCS6 in rat hepatoma cells results in partial inhibition of the insulin-induced AKT and ERK1/2 phosphorylation [40] suggesting that SOCS6 counteracts insulin signaling, however the mechanism of suppression of insulin signaling is still unclear. Although SOCS6 acts as a ubiquitin ligase, there is no direct evidence available in favor of SOCS6-mediated INSR degradation [40]. Another possibility of controlling INSR signaling could be inhibition or destabilization of INSR substrates. Other SOCS family members, such as SOCS1 and SOCS3, interact with insulin receptor substrate 1 (IRS1), and through ubiquitination and degradation of IRS1 they limit the availability of substrate [9]. Another study suggests that SOCS6 interacts with IRS4 in response to insulin [42] and SOCS7, a close homolog of SOCS6, also associates with both INSR and IRS1 [43]. It has also been shown that overexpression of SOCS6 leads to inhibition of tyrosine phosphorylation of IRS1 indicating that SOCS6 not only regulates substrate turnover, but it also competes with the substrate for association to the INSR [40]. Therefore, it is suggested that SOCS6 associates with both INSR and its substrates, and thereby decreases the stability of the substrate as well as partially blocks substrate phosphorylation (Figure 2). These events in turn partially block activation of INSR downstream signaling.

SOCS6 binds with PI3K component

Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), an important mediator of survival signals downstream of receptor tyrosine kinases, has two subunits. The regulatory subunit is an 85 kDa protein and is named as p85. The other subunit is a catalytic subunit which is a mass of 110 kDa hence named p110. SOCS6 binds to the regulatory subunit of PI3K and this association is induced by insulin or insulin-like growth factor 1 (IGF1) stimulation [17, 42]. These findings are also supported by *in vivo* studies on

transgenic mice where association only occurs when p85 stays as monomer [17]. This suggests that SOCS6 acts as a physiologically relevant partner of p85. Although SOCS6 plays a negative regulatory role in INSR signaling (discussed above) it does not affect insulin-induced INSR auto-phosphorylation, IRS tyrosine phosphorylation or ERK1/2 phosphorylation in CHO-IR cells. Moreover, it enhances insulin-induced AKT phosphorylation suggesting that SOCS6 can play different roles in CHO cells. The effects observed in CHO-IR cells was independent of the lipid kinase activity of PI3K or turn-over of the p85/p110 dimer [17], indicating that SOCS6 transduces activating signals from INSR through p85 which is independent of PI3K. Although SOCS6 acts as an E3-ubiquitin ligase by recruiting the ubiquitin machinery to the interacting protein which subsequently leads to degradation in the proteasomes, association of SOCS6 with p85 did not induce p85 degradation following insulin stimulation but SOCS6 itself was degraded in the proteasomes in response to insulin stimulation [17]. Monomeric p85 can act as a negative regulator of INSR signaling [44, 45]. Negative regulation of p85 is probably mediated through association with IRS which limits access to the p85/p110 complex to the IRS and therefore reducing insulin response (Figure 3). Association of SOCS6 with p85 reduces free p85 and thus increases chance of interaction between IRS and p85/p110 complex resulting in accelerated AKT activation. It should be noted that although the primary function of p85 is to form a complex with p110, it can also form complexes with a number of signal transduction molecules, including CBL, GRB10 and CRK and thus influence signaling in a PI3K independent manner [46-48].

SOCS6 is a negative regulator of KIT signaling

KIT is a cell surface receptor widely used as a cell surface marker to characterize stem cells. KIT is overexpressed or mutated in many cancers including small cell lung carcinoma (SCLC), malignant melanoma, colorectal cancer, mastocytosis, acute myeloid leukemia, testicular germ cell tumors, gastrointestinal stromal tumors (GISTs). Oncogenic KIT has the ability of the activating survival signaling constitutively independent of ligand expression [49-52]. SOCS6 interacts with KIT in response to the KIT-ligand stem cell factor (SCF) stimulation suggesting that the interaction is tyrosine phosphorylation dependent [5]. Association is mediated by pY567 and pY569 (murine sequence) of KIT, and a peptide containing pY567 (pY567-V-Y-I-D-P-T) displayed higher affinity than pY569, indicating that pY567 (corresponding to pY568 in the human sequence) is the major SOCS6 binding site in KIT. A more recent study confirms that this residue selectively associates with the SOCS6 SH2 domain [4]. This interaction is involved in negative regulation of distinctive signaling cascade including phosphorylation of ERK1/2 and p38 but not of AKT and STAT and in negative regulation of cell proliferation but not chemotaxis [5]. Therefore, the regulation mediated through SOCS6 is not only due to the ubiquitination-directed degradation of KIT [4] but probably SOCS6 compete with signal transduction molecules for binding to KIT and also regulates other downstream regulators of KIT signaling which remain to be identified (Figure 4).

SOCS6 negatively regulates FLT3 signaling

FLT3 is a type III receptor tyrosine kinase sharing a similar structure as KIT [53]. FLT3 is a frequently mutated gene in acute myeloid leukemia and FLT3 mutations display prognostic significance in this disease [54]. Both normal and oncogenic FLT3 have been shown to associate with SOCS6 [3]. This association is mediated through the SOCS6 SH2 domain and phosphotyrosine residues on FLT3. Phosphotyrosine residues 591 and 919 on FLT3 were described as the major binding sites for SOCS6. Binding site specificity of the SOCS6 SH2 domain has been determined. A valine in pY+1 position has been shown to be highly selected, and hydrophobic residues are preferable in position +2 and +3 [42]. Known SOCS6 binding sites in KIT include pY(567)VYIDP and pY(569)IDPTQ, and in FLT3 include pY(589)FYVDF, pY(591)VDFRE and pY(919)IIMQS [3, 5]. Thus, based on these data it is more likely

that SOCS6 has a consensus sequence like pY-(V/I/F)-(pY/D)-followed by a hydrophobic residue. Another SOCS protein, SOCS2, also exhibit overlapping binding sites on FLT3 suggesting that SOCS proteins regulate FLT3 signaling in a similar fashion [2]. Binding of SOCS6 to the FLT3 receptor results in increased ubiquitination and degradation of the receptor and selective inhibition of the ERK1/2 signaling [3]. Like with the KIT receptor, it is still also a puzzle how SOCS6 selectively inhibits ERK1/2 signaling while it increases receptor degradation (Figure 5).

SOCS6 transgenic and knockout mice are mostly normal

Both SOCS6 transgenic and knockout mice appear to be healthy and overall normal without any abnormality apart from the fact that knockout mice experience a slight decrease in body weight (8 to 10%) [17, 42]. SOCS6 is ubiquitously expressed in the hematopoietic compartments, however, SOCS6 knockout mice did not display any changes in the peritoneal cavity, spleen and bone marrow. Although, SOCS6 associates to INSR, IRS1, IRS2 and IRS4 and thereby regulates insulin signaling, mice lacking the SOCS6 gene exhibited normal glucose metabolism and were not hyper-responsive to insulin [42], suggesting that SOCS6 function can be compensated by other family members. SOCS7 expression overlaps in many cases with SOCS6 expression, and since SOCS7 is its closest related family member, it is possible that mice lacking SOCS6 function can be compensated by SOCS7. Although SOCS6 knockout mice did not show any effect on glucose metabolism, SOCS6 transgenic mice displayed improved glucose metabolism [17]. SOCS6 transgenic mice exhibited a significant reduction in plasma glucose level, as compared to wild-type mice.

Subcellular localization

The subcellular localization of SOCS6 has been studied using fluorescent fusion proteins and subcellular fractionation. Yellow fluorescent protein (YFP) and cyan fluorescent protein (CFP) fusion proteins of SOCS6 localize to the nucleus and cytosol in HEK 293T cells [55]. Furthermore, fractionation studies showed that endogenous SOCS6 was localized to both cytosol and nucleus in MDA-MB231 breast cancer cells [56]. These findings suggest that SOCS6 plays a role not only in regulation of cytosolic or membrane-bound proteins, but also it can also reside in the nucleus and modulate the function of nuclear proteins. It has been shown that SOCS6 can reduce STAT3 protein level in both the cytosol and nucleus. Nuclear localization of SOCS6 is mediated through the uncharacterized N-terminal region of SOCS6 [56].

SOCS6 in regulation of apoptosis

SOCS6 inhibits cell growth and colony formation through eliciting intrinsic apoptotic pathways in gastric cancer cells [18]. Overexpression of SOCS6 reduces cell survival in oncogenic FLT3-dependent hematopoietic cells [3]. Since SOCS6 has the ability of recruiting E3 ubiquitin ligase to receptors resulting in degradation and suppression of mitogenic signaling [3, 5], another mechanism of regulation of cell growth has been proposed. Recently it has been demonstrated that loss of SOCS6 expression confers cells resistant to programmed cell death [19]. Ectopic expression of SOCS6 protein promotes apoptosis through increased conformational change of BAX and mitochondrial fragmentation. This process is mediated through SOCS6 mediated complex formation of DRP1 and the mitochondrial phosphatase PGAM5, thereby attenuating phosphorylation, as well as mitochondrial translocation of DRP1 [19]. The C-terminal SOCS-box is required for the mitochondrial targeting by SOCS6.

Conclusions

Like with other SOCS proteins, the presence of a SOCS box allows SOCS6 to act as an E3 ubiquitin ligase. SOCS6 binds to the elongin B/C complex through its SOCS box [42], and then recruits other components of the ubiquitination machinery [1]. SOCS6 also associates with haem-oxidized IRP2 ubiquitin ligase-1 (HOIL1) [57]. This association leads to stabilization of SOCS6 but induces degradation of SOCS6 interacting proteins. Therefore, SOCS6 can control cell signaling by inducing ubiquitin-directed degradation of signaling proteins. Since SOCS6 does not inhibit signaling mediated by growth hormone, leukemia inhibitory factor and prolactin and furthermore does not associate with JAK2 [21], it is likely that, unlike other SOCS family members, SOCS6 is not involved in the regulation of cytokine signaling. Furthermore, SOCS6 did not show any suppressive function in lymphocytes stimulated by interleukin-2, interleukin-4, interleukin-12, interferon-gamma and granulocyte/macrophage colony-stimulating factor or did not inhibit phosphorylation of STAT1, STAT2, STAT4, STAT5 and STAT6 [17]. Thus, SOCS6 is most likely not involved in control of cytokine signaling. However, it is now well established that SOCS6 regulates downstream signaling of several receptor tyrosine kinases including INSR, KIT and FLT3 (Figure 6). Although SOCS6 selectively inhibited KIT and FLT3 downstream signaling by inhibiting p38 and/or Erk1/2, it appears that INSR downstream signaling regulated by SOCS6 is more complicated. SOCS6 can either activate or block AKT phosphorylation thereby regulating two different pathways. Nevertheless SOCS6 controls FLT3 and KIT signaling by inducing ubiquitination followed by degradation of the receptor, but the question of how it selectively regulates distinct downstream signaling pathways is still unclear. Probably SOCS6 not only targets the receptor itself but also targets downstream signaling proteins. However, this needs to be further investigated.

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Figure legends:

Figure 1: Alignment of SOCS6 proteins in different species. The SOCS Box sequence is the same in all species analyzed. The SH2-domain sequence is also highly conserved while the un-characterized region displays poor conservation.

Figure 2: Regulation of insulin signaling by SOCS6. Insulin binding to the INSR leads to activation of AKT and ERK1/2 signaling cascades. SOCS6 associates with the INSR or substrate proteins (IRS) and negatively regulates AKT and ERK1/2 activation. Association of SOCS6 with IRS results in reduced turnover of the proteins as well as reduced phosphorylation.

Figure 3: Regulation of PI3K signaling by SOCS6. Insulin binding to the INSR leads to activation of AKT through IRS and PI3K. Monomeric p85 binds with IRS substrate and blocks PI3K interaction with IRS resulting in negative regulation of AKT activation. Association of SOCS6 with monomeric p85 reduces number of free p85 available to block IRS-PI3K interaction and thus positively regulates AKT activation.

Figure 4: Regulation of KIT signaling by SOCS6. Association of SCF to KIT results in activation of the KIT receptor and phosphorylation on multiple tyrosine residues. SOCS6 associates with KIT through tyrosine residues and negatively regulates SCF-induced ERK1/2 and p38 signaling but not AKT signaling. SOCS6 association increases KIT ubiquitination and accelerates degradation.

Figure 5: Regulation of FLT3 signaling by SOCS6. FLT3 ligand (FL) induction leads to dimerization and auto-phosphorylation on several tyrosine residues on FLT3. SOCS6 associates with FLT3 through its SH2 domain and directs receptor for ubiquitin-mediated degradation and negative regulation of ERK1/2 activation.

Figure 6: Regulation of RTK signaling by SOCS6. Ligand binding to the receptor leads to activation of different signaling cascades leading to the expression of SOCS6 mRNA and subsequently protein. SOCS6 then associates with the receptor or substrate proteins. This association results in ubiquitination of SOCS6 binding proteins followed by their degradation. Thus, SOCS6 reduces the levels of receptors and their substrates which in turn leads to negative regulation of receptor signaling.

Table 1: SOCS6 expression in different tissues

Tissue/cell line	mRNA	Protein	Species	RTK involved	Note	Ref.
Bladder	+		Human			[26]
Brain	+		Human			[24]
Colon	+		Human			[24]
Epithelial cells	+		Human			[24]
Fibroblast	+	+	Mouse	INSR	Insulin-induction increases expression	[17, 27]
Fibroblast	+		Human			[24]
Heart	++		Human			[24]
Hematopoietic cell lines	+		Human			[24]
Hematopoietic cell line Ba/F3-FLT3	+		Mouse	FLT3	FL-stimulation increases expression	[3]
Kidney	++		Human			[24]
Liver	+		Human			[24]
Lung	+		Human			[24, 26]
Lymph node	+		Human			[24]
Myeloid cell lines Mo7e, UT-7, TF-1	+		Human	KIT	SCF-stimulation increases expression	[5, 20]
Ovary	+		Human			[26]
Pancreas	++		Human			[26]
Placenta	+++		Human			[24]
Prostate	+		Human			[24, 26]
Skeletal Muscle	+++		Human			[24]
Skin	+		Human			[26]
Small intestine	+		Human			[26]
Spleen	+		Human			[24]
Stomach	++/+		Human			[24, 26]
Thyroid	++		Human			[24, 26]
Trachea	+		Human			[24]
Uterus	++		Human			[26]
Vulva	+		Human			[26]

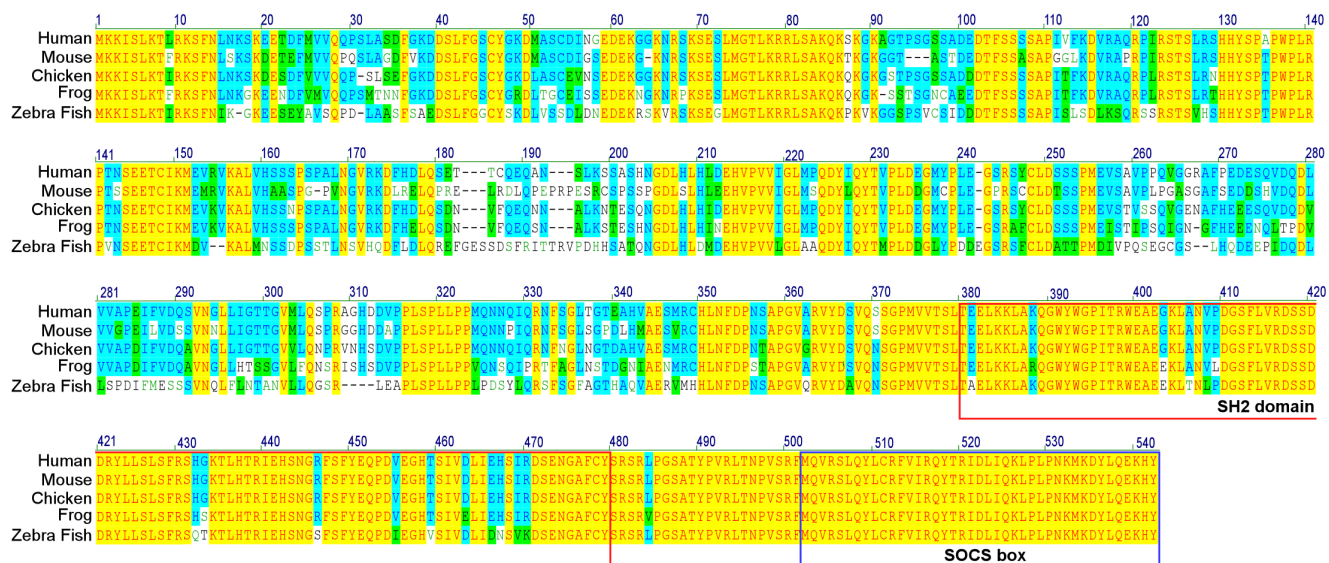


Figure 1

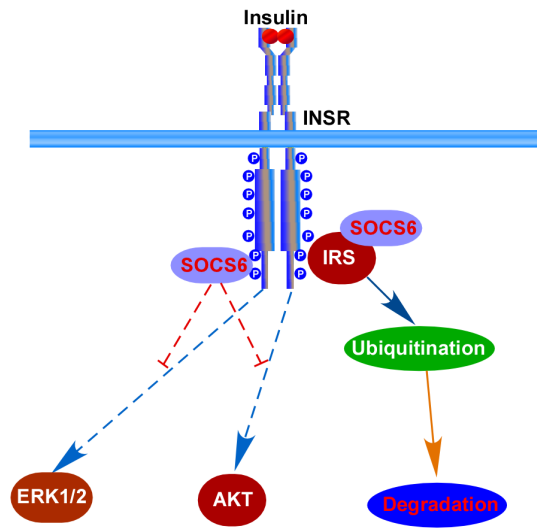


Figure 2

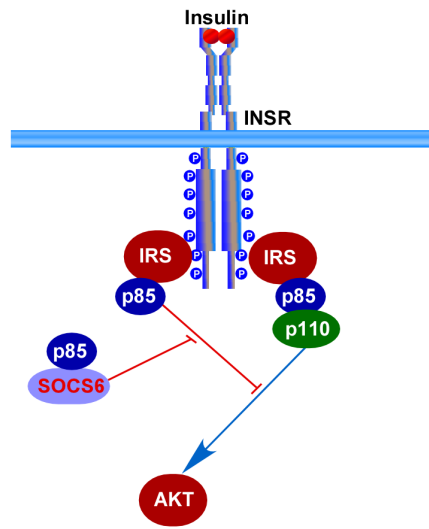


Figure 3

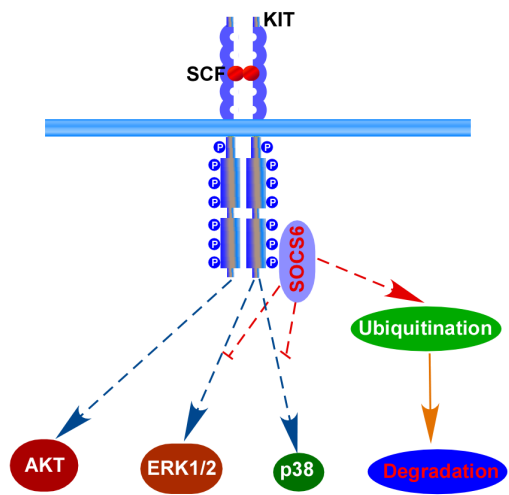


Figure 4

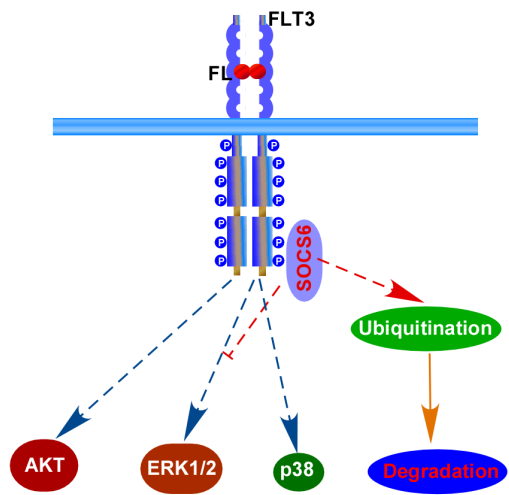


Figure 5

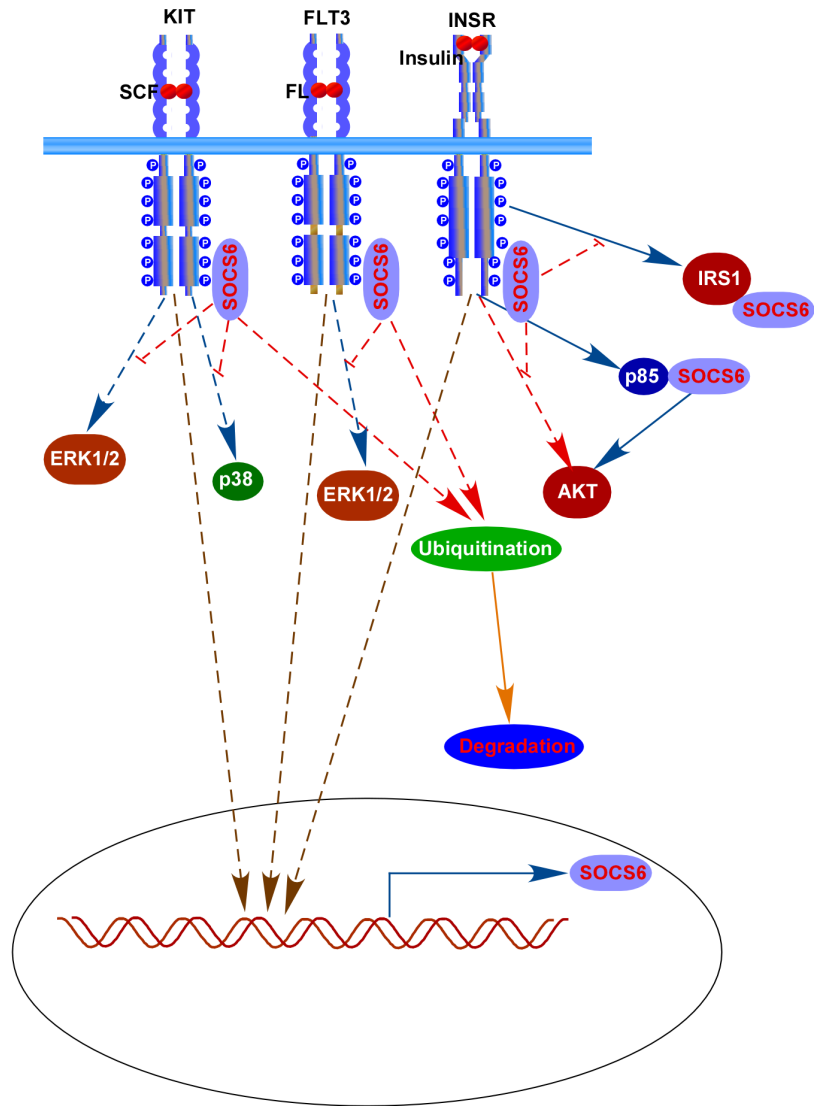


Figure 6