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Axl-dependent signaling: A clinical update

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Abstract

Axl is a receptor tyrosine kinase that was originally cloned from cancer cells. Axl belongs to a TAM (Tyro3, Axl and Merck) family of receptor tyrosine kinases. Growth arrest specific gene 6 (Gas6) is a ligand for Axl. Activation of Axl protects cells from apoptosis, increases migration, aggregation and growth through multiple downstream pathways. Upregulation of Gas6/Axl pathway is more evident in pathology compared to normal physiology. Recent advances in Axl receptor biology are summarized here. The emphasis is given to translational aspects of Axl-dependent signaling under pathological conditions. In particular, inhibition of Axl reduces tumorigenesis and prevents metastasis as well. Axl-dependent signals are important for progression of cardiovascular diseases. In contrast, deficiency in Axl in innate immune cells contributes to pathogenesis of autoimmune disorders. Current challenges in Axl biology are related to functional interactions of Axl with other members of the TAM family or other tyrosine kinases, mechanisms of ligand-independent activation, inactivation of the receptor, and cell-to-cell interactions (with respect to immune cells) in chronic diseases.

Keywords

Axl; receptor tyrosine kinase; signal transduction; cancer; autoimmunity; vascular diseases

INTRODUCTION

Axl, a receptor tyrosine kinase, derives its name from a *Greek* word *anexelektō* that means uncontrolled. Axl was initially discovered in cancer cells over two decades ago [1]. Axl is a founder of a unique TAM (Tyro3, Axl and Merck) family of receptor tyrosine kinases (RTKs). Axl is broadly expressed with an onset of expression in late embryogenesis [2]. Over-expression and an increase in Axl activity are evident in a number of chronic pathological conditions. Two ligands that activate TAM receptors are growth arrest-specific protein 6 (Gas6) and Protein S [3]. Gas6 has the highest affinity for Axl among TAM receptors and is often called Gas6/Axl pathway. Axl receptor regulates various functions including survival, growth, aggregation, migration and anti-inflammation in multiple cells. Gas6/Axl pathway has been predominantly studied in cancer [4]. However, growing evidence supports a pathophysiological role for Gas6/Axl pathway in chronic immune disorders [5]. Finally, activation of Axl is implicated in progression of cardiovascular diseases [6]. The major aim of this review is to summarize recent advances in Axl receptor

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DISCLOSURES

None

biology. More attention is given to translational aspects of Axl-dependent signaling in three pathological conditions: cancer, chronic immune disorders and cardiovascular diseases.

STRUCTURE AND REGULATION OF AXL RECEPTOR

Axl is a transmembrane receptor of molecular weight between 100 and 140kDa that contains an extracellular (N-terminal) domain and an intracellular (C-terminal) tyrosine kinase domain (Fig. 1). The extracellular domain of Axl has two immunoglobulin (Ig) and two fibronectin (FN) type III motifs (Fig. 1). Presence of Ig-like and FN type III extracellular domains differentiate Axl (along with Tyro3 and Mertk) into a TAM family of RTKs. Axl is a highly conserved gene across species (20 exons) but has two alternative variants due to splicing site of exon 10 within the trans-membrane domain [2, 7, 8]. RNA *in situ* hybridization analyses showed onset of Axl expression in late embryogenesis at day 12.5 *post coitum* [2]. Three single nucleotide polymorphisms (SNPs) within introns 6 and 10 of the Axl gene were validated in humans recently [9]. Initial genomic studies on Axl showed that its promoter region is guanine-cytosine (GC) rich and contains recognition sites for a variety of transcription factors, including specificity protein 1 (Sp1), activating protein 2 (AP-2) and the cyclic adenosine monophosphate (cAMP) response element-binding protein [10]. Recent experiments in cancer cell lines showed multiple transcriptional mechanisms leading to Axl expression [11–13]. As predicted originally, expression of Axl is regulated by Sp1/Sp3 transcription factors and methylation of C-phosphate-G (CpG) sites within specific Sp1 motifs that modulates Axl gene expression [11]. In addition, myeloid zinc finger 1 (MZF1), a SCAN domain family transcription factor, can bind to the Axl promoter and trans-activate Axl expression that results in progression of colorectal and cervical tumor metastases [12]. Finally, the same group recently showed two specific microRNAs (miRs) that targeted 3'-UTR of the Axl gene in several cancer lines [13]. Specifically, miR-34a, miR-199a, and miR-199b can inhibit expression and functions of Axl in cancer. Taken together, Axl is a very unique receptor tyrosine kinase that can be induced via multiple molecular mechanisms.

ACTIVATION OF AXL RECEPTOR

Gas6 and Protein S are known ligands for TAM receptor family [3, 14]. However, Axl has the highest affinity for Gas6 compared to other members of TAM family, while Protein S predominantly binds Mertk and Tyro3 [15]. Both ligands are more than 40% similar in amino acid sequence and require a vitamin K-dependent γ -carboxylation of glutamate (Glu) to γ -carboxyglutamate (Gla) for biological functions. Gas6 has four epidermal growth factor (EGF)-like repeats and a C-terminal sex hormone binding globulin (SHBG)-like domain, which includes two globular laminin G-like (LG) domains, in addition to the Gla-domain [16, 17]. Ligand-dependent activation of Axl is incompletely understood. Currently, binding of Gas6 to Axl is viewed as a two-step process that involves initial formation of a high affinity 1:1 Gas6/Axl complex followed by dimerization of two 1:1 Gas6/Axl complexes (Fig. 2A). A ligand-receptor 2:2 assembly with two Ig-like domains of Axl cross-linked by the LG domain of Gas6 was only shown by crystal structure analyses of the Gas6/Axl complex [18]. It is likely that both Gas6 binding sites are necessary for Gas6/Axl signaling. In addition, a recombinant protein (Fc-Axl) that mimics the extracellular Ig binding domain of Axl neutralizes Gas6 and prevents downstream signaling [15].

It has been proposed that the Axl homodimer can form heterodimers with Tyro3 or Mertk based on co-expression profiles of TAM family [5]. No experimental data on heterodimerization across TAM receptors have been reported to date. A homophilic binding of extracellular domains of Axl expressed on neighboring cells leads to aggregation (Fig. 2B). This is a ligand-independent type of receptor activation that occurs with experimental

over-expression of Axl [19]. The kinase domain of Axl is not required for cell aggregation suggesting a distinctive mechanism as compared to the ligand-dependent activation. Finally, TAM family is capable of ligand-independent homophilic dimerization and autophosphorylation of Axl (Fig. 2C). For example, this type of auto-activation may occur after overexpression of Axl [20]. Our group found that reactive oxygen species (ROS) promoted phosphorylation of Axl in vascular smooth muscle cells (VSMCs), which was independent of Gas6 [21]. Therefore, ligand-independent activation of Axl is more typical during pathophysiological conditions with increases in oxidative stress and excess of receptor expression.

Release of a soluble form of Axl (sAxl), an extracellular domain of Axl, represents another important feature of Axl receptor biology (Fig. 2D). Formation of the sAxl/Gas6 complexes limits ligand-dependent signaling as previously described for cytokine and growth factor receptors. A specific proteinase that is responsible for proteolytic cleavage of sAxl has yet to be identified [22]. However, a metalloproteinase ADAM 17 could be a possible candidate since it mediates cleavage of soluble form of Mertk in macrophages [23]. In addition, a range between 100 and 140kDa in molecular weight of Axl might relate to posttranslational modifications (glycosylation, phosphorylation and ubiquitination) and will be discussed below.

We have limited knowledge on mechanisms of inactivation of Axl. Receptor tyrosine phosphatase C1 domain-containing protein (C1-TEN) was shown to bind Axl and affect Axl-dependent downstream signaling pathways in HEK293 cells [24]. However, authors were unable to show direct dephosphorylation of Axl by C1-TEN or increases in C1-TEN enzymatic activity. Endocytosis and lysosomal degradation are likely mechanisms of Axl deactivation upon Gas6 binding to Axl that involves interaction of Axl with the ubiquitin ligase c-Cbl [25]. Importantly, ROS (hydrogen peroxide) induced Axl tyrosine phosphorylation but not its ubiquitination, suggesting that oxidative stress may inhibit Axl downregulation. A proteolytic cleavage of sAxl is another possible mechanism of receptor inactivation (Fig. 2D). Thus, it is clear that more efforts should be put forward to understand Axl receptor biology especially in pathophysiology.

AXL RECEPTOR SIGNAL TRANSDUCTION

Typically, binding of Gas6 to the extracellular domain of Axl leads to dimerization of the Gas6/Axl complexes (Fig. 2A). The latter results in autophosphorylation of tyrosine residues on the intracellular tyrosine kinase domain of Axl (Fig. 1). Autophosphorylation of RTK may increase phosphorylation activity by Axl substrates or formation of signaling complexes with phosphotyrosine-binding domains. There are three known autophosphorylation sites (Y779, Y821, Y866) on the intracellular domain of Axl (Fig. 1). These residues are involved in binding Axl with subunits of phosphatidylinositol 3-kinase (PI3K), phospholipase C (PLC) and growth factor receptor-bound protein 2 (Grb2) [26, 27]. In addition, Axl interacts with other signaling molecules such as C1-TEN, NCK adaptor protein 2 (Nck2), Ran binding protein in microtubule organising centre (RanBPM), and suppressor of cytokine signaling 1 (SOCS-1) [28]. Activation of PI3K and its downstream target, serine/threonine protein kinase Akt (Akt), is a central step in Axl-dependent signal transduction (Fig. 3). The Gas6/Axl/PI3K/Akt pathway protects cells from apoptosis via multiple mechanisms. In particular, Akt activates ribosomal protein S6 kinase (S6K) of the mechanistic target of rapamycin (mTOR) pathway and phosphorylates BCL2-associated agonist of cell death (Bad), a pro-apoptotic protein [29]. Akt inhibits pro-apoptotic caspase 3 and phosphorylates nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) that increases expression of the anti-apoptotic proteins B-cell lymphoma 2 (Bcl-2) and B-

cell lymphoma-extra large (Bcl-xL) [30]. In addition, Akt phosphorylates α IIb β 3 integrins, which is triggered by Gas6/Axl pathway [31].

In some cell types Axl activates ERK pathway and contributes to proliferation (Fig. 3). Stimulation of mitogen-activated protein (MAP) kinases (ERK, p38, JNK) by Axl is attributed, in part, to its ability to bind to the adapter protein Grb2 [26]. Stimulation of Axl also regulates Rho family GTPase and actin cytoskeletal reorganization in gonadotropin-releasing hormone neuronal cell migration [32]. Activation of p38 and phosphorylation of heat shock protein 25 (HSP25), a regulator of actin remodeling, is downstream of Axl (Fig. 3). Interestingly, Gas6/Axl pathway can inhibit other growth factor signals as it was shown for vascular endothelial growth factor receptor (VEGFR) 2 in endothelial cell morphogenesis [33]. Activation of the tyrosine phosphatase SHP-2 is responsible for Gas6/Axl-mediated VEGFR2 inhibition (Fig. 3). Gas6/Axl pathway is an important inhibitory mechanism for toll-like and cytokine receptor signaling in innate immune cells [34, 35]. Specifically, Gas6/Axl co-activates INFAR/STAT1 pathway which increases expression of suppressors of pro-inflammatory signals such as Twist1, SOCS1 and SOCS3 (Fig. 3). Taken together, Axl-dependent signaling is responsible for cell survival, aggregation, migration and growth. However, the specific pathophysiological response varies depending on stimuli, cell types and binding partners.

Examples implicating Axl-dependent signaling in three pathological conditions are discussed below (Fig. 4).

AXL-DEPENDENT SIGNALING IN CANCER

It is logical to start discussion of the role of Axl in oncology, since the receptor was cloned from human cancer cells [1]. A large body of data suggests the importance of the TAM family in cancer. Over-expression and activation of Axl protein and not Axl gene mutations are responsible for tumor growth in mesothelioma [36]. Tumor cell survival and growth, increased migration and angiogenesis are likely mechanisms by which Axl signaling regulates tumorigenesis [37]. Up-regulation of Axl was documented in a vast majority of tumors and was summarized recently [4]. For example, Axl is one of the most common RTKs detected in human breast cancer [38]. In fact, Axl expression correlates with metastasis and poor prognosis in breast cancer [39]. It was shown that estrogen-induced Axl expression increased cell's survival via PI3K/Akt pathway in breast cancer [40]. A recent study suggested that intermediate filament protein vimentin-dependent cell migration requires Axl in breast tumor formation [41]. In addition to a significant role in tumor growth, anti-apoptosis, migration and metastasis Axl was also implicated in angiogenesis [42]. Finally, Gas6/Axl signaling may also affect tumor-stromal cells interactions via changes in immune response during tumorigenesis. Previous experiments suggest that communications between multiple cell types, including vascular and immune cells, are required for Gas6/Axl-dependent immune responses [43]. Inhibition of Axl significantly reduced expression of pro-inflammatory cytokines, which are important mediators of metastasis [44]. Thus, Gas6/Axl pathway increases cell survival, promotes proliferation, aggregation and migration and is necessary for angiogenesis and immune cell activation in cancer.

AXL-DEPENDENT SIGNALING IN CHRONIC IMMUNE DISORDERS

Gas6/Axl pathway plays a crucial role in immune biology [5]. Specifically, TAM receptors protect innate immune cells (macrophages, dendritic and NK cells) from apoptosis and are involved in phagocytosis of apoptotic bodies. Insights from triple TAM knockout mice revealed the importance of the TAM family in the immune system [45]. Autoimmunity phenotypes in TAM knockout mice suggest the protective role for Axl receptor in chronic

immune disorders such as rheumatoid arthritis, systemic lupus erythematosus, etc. Negative regulation of pro-inflammatory signals by TAM and phagocytosis in innate immune cells is the proposed mechanism in autoimmune disorders. In particular, activation of interferon- α/β receptor (INFAIR) triggers the Gas6/Axl pathway that inhibits toll-like and cytokine receptor signaling via suppressors of pro-inflammatory signals such as Twist1, SOCS-1 and SOCS-3 in innate immune cells [34, 35]. It was suggested that the autoimmune phenotypes are linked to TAM functions in innate immune cells, e.g. macrophages, dendritic and NK cells. Previous data showed that Axl is undetectable in lymphocytes and granulocytes [7]. Recent experiments in Axl/Mertk double knockout mice showed a greater ability of antigen presenting cells to drive Th1 response via increases in pro-inflammatory cytokine levels [46]. However, recent data in chronic lymphocytic leukemia suggests that a phosphorylated form of Axl is derived from B cells [47, 48]. In addition, Axl inhibition decreased expression of pro-inflammatory cytokines in breast cancer [44]. These findings show complexity of Axl-dependent signaling in homeostasis of the immune system vs. pathological stimulation. Alternatively, up-regulation of other members of TAM family (e.g., Mertk) and/or ligands (Protein S vs. Gas6) determine immune cell populations' functions as was shown for Mertk-dependent phagocytosis [49]. A complex mechanism of interactions among multiple cell types was recently shown in 26T model of colon cancer [50]. In particular, authors found a paracrine mechanism of tumor growth, which was driven by infiltrating leukocytes producing Gas6. Taken together, Axl-dependent signaling is involved in immunosuppressive pathways in innate immune cells. However, more studies are required for careful investigation of the effects of Axl on cell populations in chronic immune diseases (Fig. 4).

AXL-DEPENDENT SIGNALING IN CARDIOVASCULAR DISEASES

Gas6/Axl pathway is equally important for cardiovascular system, especially under pathological conditions (Fig. 4). Late onset of up-regulation of Gas6 and Axl was shown in rat carotid arteries after balloon injury [51]. Our findings in Axl knockout (Axl $^{-/-}$) mice further support a role of Axl in vascular remodeling [21, 52]. Smooth muscle cell response to injury is complex and regulated through multiple autocrine growth mechanisms [6]. Induction of growth factors (PDGF, ET-1, IL-6, Gas6, etc.) and growth factor receptors (PDGFR, Axl) mediates long-term pathophysiological adaptations (e.g., restenosis) in arteries. In fact, G protein-coupled receptor agonists (angiotensin II (AngII) and thrombin) increased Axl expression in VSMCs *in vitro* [51]. Mechanistic studies showed that Gas6/Axl activates PI3K and Akt that protects VSMCs from apoptosis [53]. Similar anti-apoptotic mechanisms have been described for Axl in endothelial cells [30, 54]. It is important to note that Axl signaling may direct not only VSMCs survival but also migration depending upon cellular microenvironment. Specifically, the lower molecular weight isoform of Axl (114kDa) activates the PI3K/Akt/mTOR pathway and survival in low glucose, while the higher molecular weight isoform of Axl (140kDa) leads to increased ERK1/2-mediated migration in high glucose conditions [55]. Gas6/Axl pathways not only increase survival but also protect VSMCs from calcium deposition *in vitro* [56]. However, we observed no evidence of calcification in arteries with increased apoptosis in Axl $^{-/-}$ mice (Gerloff and Korshunov, unpublished data). Activation of Axl is regulated by oxidative stress in VSMCs [21], which could be a common pathophysiological mechanism in all discussed pathologies. We recently found a novel posttranslational redox modification that leads to Axl-dependent migration of VSMCs [57]. Glutathiolation (a reaction of glutathione with cysteine residues) of non-muscle myosin heavy chain-IIB interacts with Axl in response to ROS and increases migration during vascular remodeling. In fact, vascular oxidative stress and vasoreactivity were improved in Axl $^{-/-}$ compared to Axl wild-type littermates in a mouse model of hypertension [58]. It is also possible that Axl controls phagocytosis that, in combination with pro-survival effects, determines vascular remodeling [52]. Finally, Gas6 gene

polymorphisms are associated with stroke and acute coronary syndrome in humans [59–61]. Gas6/Axl pathway is shown to regulate thrombosis, which is crucial for cardiovascular events. In particular, Gas6/TAM transgenic mice were protected from experimental thrombosis and Fc-Axl treatment protected wild type mice against fatal thromboembolism [31]. Plasma concentrations of Gas6 and sAxl proteins correlated with large abdominal aortic aneurysms in humans [62]. Not surprisingly (as noted in cancer) a weak causal relationship was reported between Gas6 or Axl gene mutations and human atherosclerosis recently [9]. Further exploration of the Gas6/Axl pathway with respect to cell origin (vascular, blood and immune) will be important for clinical studies on cardiovascular patients. In summary, Gas6/Axl pathway is critical for progression of cardiovascular pathology via regulation of survival, proliferation and migration of vascular cells, and various functions of circulating blood cells.

DIAGNOSTIC AND THERAPEUTIC POTENTIAL OF INHIBITION OF AXL RECEPTOR

Gas6/Axl pathway is a plausible target for diagnostic test development given its role in an array of chronic disorders. Detection of Gas6, the Axl ligand, is challenging due to low concentration of Gas6 in human plasma [63]. However, measurements of sAxl could be an alternative diagnostic strategy for evaluation of Gas6/Axl pathway (Fig. 2D). In fact, Gas6 circulates in a complex with sAxl in human plasma [64]. It was proposed that sAxl acts as a decoy for Gas6 systemically and the Gas6:sAxl balance shifted towards Gas6 in chronic inflammatory and vascular diseases [62, 65]. While these studies are encouraging, systemic versus local activity of the Gas6/Axl pathway remains an open question especially in a cell- and disease-specific manner.

Despite limitation of the detection of abnormal Axl signaling in humans we have a number of established preclinical approaches to inhibit the Axl receptor (Fig. 2). First, a decrease in Gas6/Axl binding can be achieved in two ways. Decreased activity of Gas6 can occur by the well known anticoagulant warfarin that inhibits vitamin K dependent γ -carboxylation of Gas6 [66]. Although, the narrow therapeutic index and low specificity of warfarin clearly limits such an approach. Alternatively, administration of the recombinant protein Fc-Axl (extracellular domain of Axl) blocks interaction of Gas6 with Axl [15]. One report shows that Fc-Axl significantly protected mice against pulmonary thrombosis [31]. Second, targeting the Axl receptor with inhibitory antibodies can prevent downstream signaling. Two reports showed that anti-Axl antibodies affected not only tumor cells but also modulated tumor-associated vasculature and immune cell functions [67, 68]. The anti-Axl antibodies are highly specific but have limitations due to poor pharmacokinetic in humans. Third, utilization of Axl-specific small molecule inhibitors (SMI) is the best therapeutic strategy currently. Again, majority of reports on Axl inhibition by SMI are shown in cancer [4]. Among several inhibitors, R428 (Rigel Pharmaceuticals) was effective in multiple animal models of tumorigenesis and also has favorable pharmacokinetic profiles and specificity to Axl [44]. We recently found that R428 strongly inhibited Axl signaling in VSMCs [69]. In addition, we observed that R428 was more effective than Fc-Axl in ligand-independent activation of Axl in response to oxidative stress in VSMCs. One possibility could be a posttranslational redox modification that leads to Axl signal transduction in VSMCs [57]. Chronic administration of R428 also promoted adipocyte hypertrophy, enhanced macrophage infiltration and apoptosis in a murine obesity model [70]. These data suggest that R428 is an effective and selective compound that inhibits Axl signaling in multiple cell types. All possible Axl inhibition approaches need to be carefully evaluated in humans. For example, specificity of the Axl inhibitors to other members of TAM receptor family may lead to harmful effects on immunity as was shown in TAM knockout mice [45]. Thus, recent

progress in translational aspects of Axl signaling should be further evaluated in a wider spectrum of chronic diseases.

CONCLUSIONS

The Gas6/Axl pathway is highly regulated in chronic pathological conditions. Axl is a very unique receptor tyrosine kinase that can be induced via several molecular mechanisms. Axl-dependent signaling is responsible for cell survival, aggregation, migration and growth through multiple downstream pathways. Axl signaling has mostly been implicated in cancer. Naturally, translational advances of Gas6/Axl (diagnostics and drug development) are seen in cancer. However, a rapid increase in publications supports the significance of Axl in other chronic pathological conditions (immune and cardiovascular). It appears that over-expression of the Axl receptor in cardiovascular pathology dramatically affects signaling as compared to normal physiology. Axl-dependent signals are important for physiological homeostasis of immune system (Fig. 4). Despite these discrepancies, targeting of Axl is beneficial in advanced stages of cancer and more effective to overcome chemoresistance [71]. Current challenges in Axl biology are related to functional interaction of the receptor with other members of the TAM family or other tyrosine kinases, mechanisms of ligand-independent activation, inactivation of the Axl receptor, and cell-to-cell interactions (with respect to immune cells) in chronic diseases.

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ABBREVIATIONS

TAM	<i>Ty</i> ro3, <i>Axl</i> and <i>Mertk</i>
RTKs	receptor tyrosine kinases
Gas6	growth arrest-specific protein 6
Ig	immunoglobulin
FN	fibronectin
RNA	ribonucleic acid
SNPs	single nucleotide polymorphisms
GC	guanine-cytosine
Sp1	specificity protein 1
AP-2	activating protein 2
cAMP	cyclic adenosine monophosphate
CpG	C-phosphate-G
MZF1	myeloid zinc finger 1
miRs	microRNAs
Glu	glutamate

Gla	γ -carboxyglutamate
EGF	epidermal growth factor
SHBG	sex hormone binding globulin
LG	laminin G-like domains
NK	natural killer cell
ROS	reactive oxygen species
VSMCs	vascular smooth muscle cells
sAxl	a soluble form of Axl
ADAM 17	metalloproteinase domain-containing protein 17
HEK293	human embryonic kidney 293 cell line
C1-TEN	C1 domain-containing protein
PI3K	phosphatidylinositol 3-kinase
PLC	phospholipase C
Grb2	growth factor receptor-bound protein 2
Nck2	NCK adaptor protein 2
RanBPM	Ran binding protein in microtubule organising centre
SOCS-1	suppressor of cytokine signaling 1
S6K	ribosomal protein S6 kinase
mTOR	mechanistic target of rapamycin (serine/threonine kinase)
Akt	serine/threonine protein kinase
Bad	BCL2-associated agonist of cell death
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B cells
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra large
MAP	mitogen-activated protein kinase
ERK	extracellular-signal-regulated kinase
p38	mitogen-activated protein kinase
JNK	c-Jun N-terminal kinase
HSP25	heat shock protein 25
VEGFR2	vascular endothelial growth factor receptor 2
SHP-2	protein-tyrosine phosphatase 2
INFAR	interferon- α/β receptor
STAT1	signal transducer and activator of transcription
Twist1	transcription factor twist homolog 1
PDGF	platelet-derived growth factor
ET-1	endothelin-1

IL-6	interleukin 6
PDGFR	platelet-derived growth factor receptor
AngII	Angiotensin II.

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Figure 1. Axl receptor structure

The extracellular domain of Axl has two immunoglobulin (Ig)-like (black bracket) and two fibronectin (FN) type III-like (blue) domains. An intracellular kinase domain (yellow) contains autophosphorylation sites (Y779, Y821, Y866). A product of growth arrest-specific protein 6 (Gas6; green) can activate Axl. A dimerization of two 1:1 Gas6/Axl complexes is required for signal transduction.

Figure 2. Mechanisms of Axl receptor activation/inactivation

A, Ligand-induced activation of Axl by Gas6 (green color). Initial formation of a high affinity 1:1 Gas6/Axl complex followed by dimerization of two 1:1 Gas6/Axl complexes. **B,** A homophilic binding of extracellular domains of Axl expressed on neighboring cells leads to aggregation, especially in excess of Axl. **C,** A ligand-independent homophilic dimerization of Axl and autophosphorylation in response to ROS (red color). **D,** A proteolytic cleavage of sAxl by unknown protease.

Figure 3. Axl receptor signal transduction

Axl controls cell survival, migration, proliferation and inflammation (red color). Major downstream targets of Axl are shown in circles and ellipses. Thick arrows show direction of signals. Thin lines show inhibitory effects of Axl. Activation of SHP-2 is responsible for Gas6/Axl-mediated VEGFR2 inhibition.

Figure 4. Axl signaling associates with pathological conditions

Axl is involved in immune cell functions that inhibit chronic immune activation (blue color). Axl affects tumor cell function and promotes cancer (green color). Activation of Axl is critical for advanced cardiovascular diseases (red color). Thin arrows show potential effects of Axl signaling on cell-to-cell interaction during three types of pathological conditions.