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Review article

Axl inhibitors as novel cancer therapeutic agents

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ABSTRACT

Overexpression and activation of Axl receptor tyrosine kinase have been widely accepted to promote cell proliferation, chemotherapy resistance, invasion, and metastasis in several human cancers, such as lung, breast, and pancreatic cancers. Axl, a member of the TAM (Tyro3, Axl, Mer) family, and its inhibitors can specifically break the kinase signaling nodes, allowing advanced patients to regain drug sensitivity with improved therapeutic efficacy. Therefore, the research on Axl is promising and it is worthy of further investigations. In this review, we present an update on the Axl inhibitors and provide new insights into their latent application.

1. Introduction

Cancer, which is responsible for a million deaths each year, is universally feared, and approximately 50% of newly diagnosed cases can be cured [1]. The existing cancer treatments, including surgery, chemotherapy, radiotherapy or a combination of them, are quickly losing efficacy. The low cure rate, serious side effects, and continuous rising morbidity and mortality rates necessitate the need for novel therapy strategies. The TAM family (Tyro3, Axl, Mer) has been reported to regulate different biological processes, including intracellular signaling, cytoskeletal functions, and gene expression. The TAM family shares a unique KWIAIES conserved sequence, and the transcription products of TAM genes show homology in the tyrosine kinase domain. TAMs are widely expressed in the heart, liver, hippocampus, and cerebellum of the brain, as well as in platelets, monocytes, and endothelial cells. Moreover, increasing lines of evidence have highlighted the carcinogenicity of TAMs to promote the proliferation, apoptosis, survival, and metastasis of neoplasms [2]. Axl (ARK, TYRO7, and UFO) receptor tyrosine kinase, an important member of the TAM family, has been shown to be aberrantly overexpressed in a variety of malignancies [3] (Table 1). Overexpression or activation of AXL is strongly linked to cell proliferation, survival, migration, and invasion by activating the oncogenic signaling pathways, including PI3K/Akt and/or MAPK/Erk pathways [4–6]. In addition, AXL can serve as a protector of blood vessels, promoter of cell differentiation in the erythrocyte lineage, sweeper of apoptotic cells, and regulator of pro-inflammatory cytokines [7]. AXL is also associated with hematopoiesis, platelet aggregation, and angiogenesis [8]. Mer has been implicated in a variety of malignancies [9–11], the oncogenic potential of MerTK has been proved [12]. The Mer is also related to the down-regulation of the immune system and phagocytosis after apoptosis [13]. Tyro3 is known to be restrictedly expressed in tissues and is associated with myelination in the brain [14] and metastasis in cancers [15]. Recently, small molecule tyrosine kinase inhibitors targeting Axl have been demonstrated to be significantly effective. This review article updates the understanding of Axl inhibitors and presents a new approach for their potential application.

2. Axl receptor tyrosine kinase

2.1. Axl and cancer

AXL is derived from the Greek word “anexelekto,” which means “uncontrolled” [16], and is a receptor tyrosine kinase (RTK) that belongs to the TAM family. AXL was first identified and reported to be a transforming gene in chronic myeloid leukemia (CML) in 1988 [17], and it was then isolated from a case of chronic myeloproliferative disorder (CMPD) in 1991 [18]. Accumulating lines of evidence indicate that the overexpression of AXL is associated with a poorer prognosis and higher risk of metastasis [19–21]. The AXL gene resides on chromosome 19q13.2 and is encoded by 20 exons. The protein structure of Axl includes an extracellular domain that contains a combination of two immunoglobulin-like domains and two fibronectin type III repeats, conserved intracellular kinase domain, and transmembrane domain,

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Table 1
Overexpression or ectopic expression of AXL in different *in vitro* trials.

Tumors	Cell lines
Breast cancer [48,49]	Highly invasive cell lines (BT549, Hs578T, MDA-MB-435s, MDA-MB-157, MDA-MB-436, and MDA-MB-231)
Chronic lymphocytic leukemia [50]	CLL B cells
Colorectal cancer [51,52]	Invasive colorectal cancer cell lines (HCT116, HKH-2, LoVo, and Colo 205)
Cutaneous squamous cell carcinoma [53]	Spontaneously immortalized SCC cells (SCC-IC1)
Endometrial cancers [54]	Endometrial cancers patients (G1, G2, and G3 EC cell lines)
Esophageal cancer [55,56]	Esophageal adenocarcinoma cell lines (SK-GT-4, OE19, OE33, and FLO-1)
Gastric cancer [57]	Human gastric cancer cell lines (GCIY, AGS, TMK1 KATO3, MKN1, MKN7, MKN28, MKN45, and MKN74)
Glioblastoma [58–60]	Glioblastoma cell lines (U251, A172, and SF188)
Hepatocellular carcinoma [61,62]	HCC cell lines (H2P, MHCC97L, MHCC97H, Hep3B, and PLC/PRF/5)
Lung cancer [63–65]	NSCLC cell lines (-H2126, -H2077, -H2009, -H1573, NCI-H23, -H820, -H125, U1752, LCLC-103H, U1810, LCLC-97TM1, and EPLC-32M1)
Malignant pleural mesothelioma [66]	Tissues
Melanomas [67–69]	Melanomas (501mel, G361, WM9, and MeWo cell lines)
Myeloid leukemia [70–72]	AML cell lines (HL60, OCI-AML5, MV4-11, KG-1, THP-1, and Kasumi-1) and BMDSC cell lines
Ovarian cancer [73,74]	Metastatic ovarian cell lines (MESOV, HEYA8, OVCAR-8, SKOV3, and ES-2)
Pancreatic adenocarcinoma [75,76]	Pancreatic adenocarcinoma cell lines (MIA PaCa-2, Hs766T, BxPC-3, ASPC-1, Capan-1, Capan-2, CFPAC-1, Panc-1, Panc-3, Panc-28, and Panc-48)and a human pancreatic ductal epithelial cell line (HPDE)
Prostate cancer [77–79]	Prostate cancer cell lines (LNCaP, CW19, CW22, PC-3, and DU145) and a clonal cell line (CL1)
Renal cell carcinoma [80,81]	The ccRCC cell lines (786-O, Caki-2, SKRC-7, -17, -21, and SKRC-52),papillary RCC cell line, and chromophobe RCC cell line
Thyroid cancer [82,83]	Thyroid papillary cancer cell lines (BCPAP, NIM, and TPC1) and anaplastic thyroid cancer cell lines (C643, SW1736, OCUT-1, ACT-1, 850–5C, CAL62, U-HTH7, and U-HTH83)

and the structure is the same as that of Tyro3 (RSE, SKY, TIF, DTK, and BRT) and Mer (MERTK, TYRO12, and RP38) [6]. The protein ligand growth arrest-specific protein 6 (Gas6, an extracellular ligand) binds to phosphatidylserine (PtdSer) located on cell membranes and forms an extracellular lipid-protein complex [22–24]; following the unique and maximal activation of TAMs, tumor cells can survive in an anti-inflammatory, immunosuppressive microenvironment [25]. The affinity of Axl for the ligand Gas6 is the highest among the TAMs. Gas6 is the common ligand of TAMs, but Axl can be also activated by Tubby-like protein 1 (TULP-1) [26]. Following the binding to Gas6, the dimerization of the TAM receptors leads to the activation of the invasion, proliferation, angiogenesis, and drug resistance of cancer cells [6]. Drug resistance is observed in multiple cancers, and it leads to the failure of therapies. Axl is an alternative effector or activator of distinct signaling networks [27]. Axl mediates the failure of various targeted drugs that inhibit Erk [28], BRAF [28], PI3Kα [29], Alk [30], EGFR [31–34], or VEGFR [35]. Targeted inhibitors of the Axl pathway restore the sensitivity to multiple therapies. Axl is a node that is involved in the bypassing of EGFR signaling, leading to resistance to EGFR inhibitors [36]. In HER-2-positive breast cancer, Axl contributes to lapatinib resistance [37]. Epithelial-to-mesenchymal transition (EMT) induces stem cell-like properties; thus, resistance to anti-proliferative agents is observed [38]. EMT also promotes resistance to chemotherapeutic agents and targeted therapies [39]. Multiple studies have shown EMT may be an indirect but major mechanism in drug resistance [40,41]. Axl is overexpressed in mesenchymal EMT-like cells [42,43]. In the innate immune system, immunosuppression blocks antitumor activity. Axl promotes phagocytosis, supports the maturation of NK cells, and inhibits inflammation [44,45], allowing resistance to antitumor treatments to further develop. One of the functions of Axl is maintaining vascular integrity and promoting angiogenic properties. As a result, Axl can mediate resistance to antiangiogenic therapies [46,47]. In conclusion, the upregulation of Axl will lead to drug resistance. Thus, Axl inhibitors can enhance chemosensitivity and reduce the metastatic potential of cancer cells [25].

2.2. Structure of Axl

Structurally, Axl possesses two Ig domains, two FNIII moieties in the extracellular domain, a transmembrane portion, and a conserved cytoplasmic kinase domain with intrinsic tyrosine kinase activity [6]. The established natural ligands for the TAMs are Gas6 (all three TAMs) and protein S (Tyro3 and Mer). These two vitamin K-dependent proteins have similar domain structures [84–86]. As the most common ligand of Axl, Gas6 has an N-terminal g-carboxy-glutamic domain, followed by four EGF-like sequences, and a double globular C-terminal domain with a sex hormone-binding globulin (SHBG)-like structure [22,23,87,88] (Fig. 1). After binding to Gas6, Axl receptor dimerization and autophosphorylation are triggered, then tyrosine residues in its kinase domain are phosphorylated, and finally signaling adaptors bind to its multiple substrate docking sites [89].

2.3. Regulation of AXL

2.3.1. Receptor activation

The mechanisms that activate Axl are unique; a bridging protein ligand and an extracellular lipid moiety are needed to achieve maximal stimulation [13]. The types of Axl activation are described below.

(a) Gas6 ligand-dependent activation of Axl. The binding of Axl and Gas6 forms a dimer complex, consisting of two Axl molecules and two Gas6 molecules. Gas6-mediated Axl dimerization occurs with a high-affinity 2:2 stoichiometry, and the proximity of the kinase domains of Axl enables the formation of a dimeric RTK–ligand signaling complex [24]. Trans-autophosphorylation of tyrosine residues occurs on the Y779, Y821, and Y866 of Axl on its cytoplasmic tails [89]. Signaling molecules such as PLCg (phospholipase C-g), PI3K, and growth factor receptor-bound protein 2 (Grb2) can dock on the cytoplasmic tails [89,90] (Fig. 2a).
(b) Ligand independent activation of Axl [91]. This tends to be triggered by the overexpression of AXL or oxidative stress and mostly occurs in vascular smooth muscle cells [92] (Fig. 2b).
(c) Heterophilic activation of Axl with a non-TAM family protein [93]. VEGFR1 is observed to undergo GAS6-independent heterophilic dimerization with TAM kinases, indicating that this Axl activation is an alternative resistance mechanism and that VEGFR1-targeted therapies should be developed [94] (Fig. 2c).
(d) Heterophilic activation of Axl with Mer or Tyro3. It has been hypothesized that Axl can undergo heterophilic activation with either Tyro3 or Mer. Although the Axl/Mer interactions have not been proved [95], Axl and Tyro3 have been found to heterodimerize or coexist in B-cells of patients with chronic lymphocytic leukemia [96] (Fig. 2d).
(e) Transcellular ligand independent activation of Axl. It has been