Targeting Axl and Mer Kinases in Cancer

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Abstract

Receptor tyrosine kinases (RTK) are cell-surface transmembrane receptors that contain regulated kinase activity within their cytoplasmic domain and play an important role in signal transduction in both normal and malignant cells. The mammalian TAM RTK family includes 3 closely related members: Tyro-3, Axl, and Mer. Overexpression or ectopic expression of the TAM receptors has been detected in a wide array of human cancers. Growth arrest-specific gene 6 has been identified as the major ligand for these TAM RTKs, and its binding to the receptors has been shown to promote proliferation and survival of cancer cells in vitro. Abnormal expression and activation of Axl or Mer can provide a survival advantage for certain cancer cells. Inhibition of Axl and Mer may enhance the sensitivity of cancer cells to cytotoxic agents and would potentially be a therapeutic strategy to target cancer cells. This review elucidates the role of Axl and Mer in normal cellular function and their role in oncogenesis. In addition, we review the potential to inhibit these RTKs for the development of therapeutic targets in treatment of cancer. Mol Cancer Ther; 10(10); 1763–73. ©2011 AACR.

Introduction

Receptor tyrosine kinases (RTK) are a large family of transmembrane proteins exhibiting great diversity in their extracellular regions, although sharing in common a highly conserved intracellular tyrosine kinase domain. They function as sensors for extracellular ligands, the binding of which triggers receptor dimerization and activation of the receptor’s kinase activity. This activation leads to the recruitment, phosphorylation, and activation of multiple downstream signaling proteins, which ultimately change the physiology of the cell. RTKs regulate cellular processes, including survival, growth, differentiation, adhesion, proliferation, and motility. Fifty-eight known RTKs in the human genome are classified into 20 families by amino acid sequence identity within the kinase domain and structural similarities within their extracellular regions. One subfamily is referred to as the TAM family, identified in 1991, comprising Tyro-3 (also called Sky), Axl, and Mer. The TAM receptors are characterized by a combination of 2 immunoglobulin-like domains and dual fibronectin type III repeats in the extracellular region and a cytoplasmic kinase domain (Fig. 1A). The primary ligand for TAM receptors is growth arrest-specific 6 (Gas 6), a fairly large (75 kDa) vitamin K–dependent protein known to activate downstream signaling (1).

Axl also called Ark and Ufo, was originally detected in 1988 from 2 patients with chronic myelogenous leukemia (CML) as an unidentified transforming gene. Axl was later cloned from patients with CML and chronic myeloproliferative disorders (2). The name Axl is derived from the Greek word anexelektos, which means “uncontrolled.” The human axl gene is located on chromosome 19q13.2 and encodes 20 exons (Fig. 1A; ref. 2). Axl is ubiquitously expressed and has been detected in a wide variety of organs and cells, including the hippocampus and cerebellum, monocytes, macrophages, platelets, endothelial cells (EC), heart, skeletal muscle, liver, kidney, and testis (3–5). Subsequent to its original identification in CML, Axl overexpression has been reported in several human cancers including colon (6), esophageal (7), thyroid (8), breast (9), lung (10), liver (11), and astrocytoma-glioblastoma (12).

The second member of the TAM family was isolated from the chicken retrovirus RLP30 and was named v-ryk and later cloned from embryonic chicken brain and renamed to c-eyk (13). It was later named c-Mer after being cloned from the human B-lymphoblastoid expression library, as it was found in monocytes, epithelial, and reproductive tissues (4). It is also called MerTK and mapped to chromosome 2q14.1 and contains 19 exons (Fig. 1A; ref. 14). Mer is expressed in hematopoietic lineages such as monocytes, macrophages, dendritic cells, natural killer (NK) cells, megakaryocytes, and platelets (3, 4). Since its original detection in B- and T-cell leukemias (15), it has also been detected in mantle cell lymphomas (16), gastric cancer (17), pituitary adenomas (18), melanoma (19), prostate cancer (20), and breast cancer (9, 21, 22).
Figure 1. A, DNA structure of axl and mer aligned with their functional protein domains (top 2 diagrams). Human axl gene encodes 20 exons, whereas mer encodes 19 exons. Domain structure of Gas 6, ligand for Axl and Mer (bottom). B, TAM receptors and their ligands along with signaling pathways for Axl and Mer. Ig, immunoglobulin; FN III, fibronectin type III; GLA, gamma-carboxyglutamic acid.
The biological ligands for Axl and Mer are 2 highly similar vitamin K–dependent proteins, Gas 6 and protein S (Fig. 1A). Both proteins have an N-terminal region containing a modified γ-carboxyglutamic acid residue (Gla), which has the ability to interact with negatively charged membrane phospholipids to mediate the binding of both Gas 6 and protein S to apoptotic cells. The Gla domain mediates Ca²⁺-dependent binding to negatively charged membrane phospholipids exposed on the surface of apoptotic cells. The Gla domain is followed by a loop region, 4 epidermal growth factor (EGF)-like repeats, and 2 C-terminal globular laminin G (LG)-like domains, which house a globular sex hormone binding globulin (SHBG)-like region. This SHBG domain binds directly to and activates Axl, and a supporting role has been implicated for the Gla region in the function of Gas 6 (23, 24). Gas 6 has 3- to 10-fold lower affinity for Mer than Axl (23). Studies using cultured cell lines have shown an Axl-mediated effect of exogenous Gas 6 on cell survival, proliferation, migration, and adhesion (25–28). Currently, no studies show any connection between protein S and proliferation, migration, and adhesion (25–28). Currently, no studies show any connection between protein S and proliferation, migration, and adhesion (25–28).

Axl and Mer Signaling Pathways

Fms-Mer receptor chimera and EGF-Axl receptor chimera studies were the first to elucidate the TAM receptor signaling pathways. Signaling pathways downstream from the Mer and Axl kinases include growth factor–mediated proteins such as phosphoinositide 3-kinase (PI3K), RAS sarcoma (RAS), and extracellular signal regulated kinase (ERK; Fig. 1B).

Studies using chimeric Mer receptors expressed in NIH3T3 fibroblasts linked downstream signaling pathways, such as PI3K, phospholipase C-γ (PLCγ), and ERK, to Mer activation. Gas 6–dependent activation of Mer stimulates phosphorylation of ERK1/2, leading to cellular transformation and increased proliferation and DNA synthesis. The ultimate downstream targets of the pathway differ according to cell type and tissue microenvironment. In leukemia cells, ligand-dependent activation of EGF receptor (EGFR)–Mer chimeric receptor stimulates phosphorylation of Akt, ERK 1/2, and p38 mitogen-activated protein kinases (MAPK), which results in decreased apoptosis but no change in proliferation (30). Expression of CD8-Mer chimera in pro-B cells results in transcriptional activation of NF-kB via PI3K/Akt. Additional activation of p38/MAPK and mitosis-specific serine/threonine protein kinase 1 (MEK1) occurs via CD8-Mer, leading to protection from apoptosis. Some atypical signaling pathways involving in cell survival have been studied as a link between Mer and the actin cytoskeleton via growth factor receptor-bound protein 2 (Grb2), Shc, and Vav1. Downregulation of the proapoptotic tumor suppressor WW domain-containing oxidoreductase (Wwox) may be a mechanism by which activated Cdc42-associated kinase 1 (Ack1) and Mer relay survival signals in cancer cells (31).

An early study that screened an expression library revealed p85α and p85β subunits of PI3K and PLCγ as binding partners for the Axl intracellular domain (32). Gas 6/Axl signaling promotes growth, survival, and proliferation of numerous cell types by activation of the RAS/RAF/MAPK/ERK1/2 and PI3K signaling pathways (23, 33). The RAS/ERK pathway is essential for Gas 6–induced mitogenesis of NIH3T3 cells, which may be activated by multiple TAM receptors. The MAPK/ERK pathway results in Axl-mediated proliferation and Axl binding to and activation of PI3K linked to multiple downstream pathways, leading to increased cell survival. One such pathway is the classical PI3K stimulation of Akt and S6 kinase (56K; ref. 25). Downstream survival pathways activated by Gas 6/Axl signaling via PI3K/Akt also include phosphorylation of NF-kB, increased expression of antiapoptotic proteins such as B-cell lymphoma gene 2 (Bcl-2) and antiapoptotic proteins such as caspase 3 (34). Suppressor of cytokine signaling (SOCS-1) was originally identified as a negative regulator of cytokine signaling and could serve a similar role in Axl signaling. The noncatalytic region of tyrosine kinase adaptor protein 2 (Nck2) is an adapter protein composed of 3 tandem SH3 domains and 1 SH2 domain, which may serve to tether Axl to other signaling complexes (35). The Axl-Nck2 interaction connects Axl to a ternary complex consisting of the particularly interesting new cysteine-histidine–rich protein (PINCH) and integrin-linked kinase (ILK), which is a signaling platform at focal adhesions regulating cytoskeleton dynamics and downstream signaling pathways. Gas 6/Axl signaling has also been linked to neuronal cell migration. Studies of GnRH neurons suggest that Axl directs migration of these cells via a signaling pathway involving PI3K, RAS, RAC, p38 MAPK, MAPKAP kinase 2, and HSP25, resulting in actin reorganization (36).

Soluble Axl and Mer

Membrane-bound receptors generate soluble ligand-binding domains either by proteolytic cleavage of the extracellular domain or alternative mRNA splicing, yielding a secreted protein (Fig. 1B). Axl exists as a transmembrane protein and as a soluble molecule, which was recently shown in mouse serum (37). Constitutive and phorbol 12-myristate 13-acetate–induced generation of soluble Axl (sAxl) involves the activity of disintegrin-like metalloproteinase 10 (ADAM10). Spontaneous and inducible Axl cleavage is inhibited by the broad-spectrum metalloproteinase inhibitor GM6001 and by hydroxamate GW280264 (Fig. 2), which is capable of blocking ADAM10. Experimental evidence exists that supports a role for immobilized sAxl in promoting cell migration and activation of membrane-bound full-length
Axl and its downstream target, PI3K. A dynamic equilibrium between sAxl and Gas 6 levels in biological fluids may have an important regulatory role and affect Gas 6 function. sAxl may increase the bioavailability of Gas 6 by prolonging its half-life and slowing ligand release, thereby resulting in local or systemic effects of this protein and the nature and/or duration of the signaling event. A potential ability of sAxl to serve
as a natural antagonist of Gas 6 could have clinical relevance. Similarly, the membrane-bound Mer protein is cleaved in the extracellular domain via a metalloproteinase (38). Further studies are needed to establish sAxl and sMer as important biomarkers for correlation with disease stage and predicting prognosis.

Role of TAM Receptors in Cancer

Proto-oncogenes can be activated by a variety of mechanisms, including gene amplification and mutations, proteolytic cleavage, and altered protein expression. To date, no activating TAM receptor mutations have been associated with the development of cancer; however, aberrant regulation of these signaling pathways and cellular processes play an important role in oncogenic transformation. Therefore, overexpression and ligand-induced activation represent the primary mechanisms of activation in a wide array of human cancers (Table 1).

TAM receptors activate prosurvival signaling pathways in both normal and cancer cells. In some cases, they prevent apoptosis without stimulating proliferation (30), whereas in others they increase proliferation without inhibiting apoptosis (26). Finally, some TAM receptors simultaneously promote both survival and proliferation. An example of Mer-mediated cell survival is by activation of the Ack1 and downregulation of the tumor suppressor Wwox, as discussed above. Expression of the constitutively active Ack1 in human prostate adenocarcinoma cells induced anchorage-independent growth and increased tumor growth in an ectopic xenograft model (31). A 4- to 5-fold increase in phosphorylated Ack1 and 6-fold decrease in Wwox proteins were detected in patients with advanced stage prostate cancer compared with normal prostate. Mer is not expressed in normal human lymphocytes, but is ectopically expressed in T-cell leukemias and E2A-PBX1-positive B-cell leukemias (39, 40). Lymphocytes from a Mer transgenic mouse model exhibited a functional survival advantage in vitro compared with wild-type lymphocytes when treated with glucocorticoids, a standard leukemia therapy.

TAM receptor signaling pathways have been implicated in the regulation of the actin cytoskeleton, which results in changes in cellular morphology. Axl is overexpressed in glioblastoma cells, and an experiment with a dominant negative form of Axl resulted in reduced motility, altered morphology with loss of filopodia, and loss of cell-to-cell interactions, eliciting its role in oncogenesis (12). In breast cancer models, ectopic expression of Axl conferred a highly invasive phenotype to weakly invasive MCF7 cells. Inhibition of Axl signaling by short hairpin (shRNA) knockdown or an anti-Axl antibody decreased the motility and invasiveness of highly invasive breast cancer cells (41).

The extracellular domains of the TAM receptors contain adhesion molecule–like motifs and may be involved in cell–cell contacts. Overexpression of murine Axl has been shown to result in cell aggregation. Experiments have shown that Axl-expressing cells bind to immobilized Axl extracellular domain, mediated by hemophilic binding. Studies have shown that cell aggregation is the result of a Gas 6–mediated interaction between Axl and Mer.

Table 1. Upregulation of Axl/Mer/Gas 6 in cancer

<table>
<thead>
<tr>
<th>Number</th>
<th>Cancer type</th>
<th>Upregulation of Axl/Mer/Gas 6</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acute leukemia (ALL, AML)</td>
<td>Axl, Mer</td>
<td>(15, 39, 40, 49, 60, 68)</td>
</tr>
<tr>
<td>2</td>
<td>Astrocytoma</td>
<td>Axl, Mer, Gas 6</td>
<td>(12, 51, 53)</td>
</tr>
<tr>
<td>3</td>
<td>Breast cancer</td>
<td>Axl, Mer, Gas 6</td>
<td>(9, 22, 54–56, 58)</td>
</tr>
<tr>
<td>4</td>
<td>Colorectal carcinoma</td>
<td>Axl</td>
<td>(6)</td>
</tr>
<tr>
<td>5</td>
<td>Esophageal adenocarcinoma</td>
<td>Axl</td>
<td>(7, 69)</td>
</tr>
<tr>
<td>6</td>
<td>Gastrointestinal stromal tumors</td>
<td>Axl</td>
<td>(70)</td>
</tr>
<tr>
<td>7</td>
<td>Gastric cancer</td>
<td>Axl, Mer, Gas 6</td>
<td>(71)</td>
</tr>
<tr>
<td>8</td>
<td>Hepatocellular carcinoma</td>
<td>Axl</td>
<td>(11, 61)</td>
</tr>
<tr>
<td>9</td>
<td>Kaposi sarcoma</td>
<td>Axl</td>
<td>(72)</td>
</tr>
<tr>
<td>10</td>
<td>Lung cancer</td>
<td>Axl</td>
<td>(10)</td>
</tr>
<tr>
<td>11</td>
<td>Mantle cell lymphoma</td>
<td>Mer</td>
<td>(16)</td>
</tr>
<tr>
<td>12</td>
<td>Melanoma</td>
<td>Axl, Mer</td>
<td>(19)</td>
</tr>
<tr>
<td>13</td>
<td>Ovarian cancer</td>
<td>Axl, Gas 6</td>
<td>(67)</td>
</tr>
<tr>
<td>14</td>
<td>Osteosarcoma</td>
<td>Axl</td>
<td>(44)</td>
</tr>
<tr>
<td>15</td>
<td>Pancreatic ductal adenocarcinoma</td>
<td>Axl, Gas 6</td>
<td>(46, 73)</td>
</tr>
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<td>16</td>
<td>Renal cell carcinoma</td>
<td>Axl, Gas 6</td>
<td>(74)</td>
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<td>17</td>
<td>Prostate cancer</td>
<td>Axl, Mer</td>
<td>(20, 26, 31)</td>
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<tr>
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<td>Thyroid cancer</td>
<td>Axl, Gas 6</td>
<td>(8)</td>
</tr>
<tr>
<td>19</td>
<td>Uterine endometrial cancer</td>
<td>Axl, Gas 6</td>
<td>(75)</td>
</tr>
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</table>
neighboring cells. Axl expression correlates with the adherence of lung cancer cells (42). sAxl bound to the extracellular matrix may constitute a chemoattractant for Axl-mediated migration, as scratch tests revealed that immobilized Axl-Fc promotes migration of primary fibroblasts prepared from Axl wild-type mice (37).

Angiogenesis is the formation of new blood vessels and commonly promotes tumor growth and transformation. Proliferation and migration of vascular smooth muscle cells (VSMC) are required for angiogenesis. VSMCs express Gas 6, and exogenous application of Gas 6 promotes proliferation and migration of VSMCs (27). Genetic silencing of Axl or Gas 6 significantly reduced migration of human umbilical vein endothelial cells (HUVEC), whereas overexpression of Axl increased HUVEC growth and tube formation (43). Axl knockdown in HUVECs led to upregulation of Ang-2, the angiopoietin signaling system that plays a key role in the regulation of angiogenesis, vascular homeostasis, and vascular regression. Ang-1 is an agonist that supports EC survival and endothelium integrity through the PI3K/Akt signaling pathway. In the presence of VEGF, Ang-2 provides an important angiogenic stimulus. Thus, Axl knockdown is additive with anti-VEGF agents in inhibiting endothelial tube formation (12).

Axl kinase activity is important for the regulation of EC growth and regulates tube formation in a kinase-independent manner. Elevated Axl correlates with adhesion, motility, and invasiveness of osteosarcoma cell lines selected for their high metastatic ability in an in vitro model of lung metastasis (44). Axl expression also correlates with invasiveness of lung cancer lines in vitro and in patients with adenocarcinoma (10). In a recent study, an orthotopic breast cancer model was used to investigate the functional significance of Axl in metastasis, in which Axl expression was required for MDA-MB-231 cells to establish metastatic foci in the lung. Axl knockdown by shRNA completely abolished the ability of cells emerging from the primary tumors to colonize the lungs. This study implicated Axl in the early stages of the MDA-MB-231 cancer cell metastasis and provided the first in vivo evidence that directly links Axl to metastasis (45). Recently, an experiment showed that downregulation of numerous transcripts associated with the phenomenon of cancer cell invasion, including significant reduction in levels of snail, slug, and twist mRNA, whose products regulate the process of epithelial-mesenchymal-transition (EMT; ref. 46). EMT is required for vascular intravasation and metastatic seeding by tumor cells, and the snail/slug/twist family of transcriptional repressors plays an important role in phenotypic switch. This group is the first to describe an association between transcription factors facilitating EMT and Axl and, hence, mechanistic basis for the observed blockade in invasion and/or migration upon knockdown of this RTK in MIA PaCa-2 cells.

Roles for Axl or Mer kinases have been reported in a variety of tumor types and have been extensively reviewed elsewhere (47). This review focuses on the cancers reported to be the most closely associated with Axl and Mer function. In addition to playing a role in cancer initiation and progression, Axl and Mer have been strongly associated with other disease physiology, such as the immune system and autoimmune disease. This topic has also been recently reviewed in detail (48).

Axl in Pancreatic Carcinoma

Ductal adenocarcinoma of the pancreas is a uniformly lethal malignancy. Immunohistochemical (IHC) analysis for Axl expression was done on a panel of 99 pancreatic cancers using anti-Axl–specific antibody, and Axl labeling was observed in 55% of cases (46). Axl protein expression was significantly associated with lymph node metastasis, and patients harboring Axl-positive tumors had a significantly shorter median survival of 12 months, compared with Axl-negative cancers of 18 months. This finding was reaffirmed by the same group in MIA PaCa-2 pancreatic cancer cells with genetic knockdown of endogenous Axl protein, which resulted in profound loss of invasive and migratory capabilities, accompanied by near-complete loss of filopodial extensions. Axl promotes the growth and survival of neoplastic and nonneoplastic cells through activation of MAPK and the PI3K/Akt signaling pathways, and both effector arms are inhibited in MIA PaCa-2 cells upon Axl knockdown, as evidenced by the decreased phosphorylation of the ERK1/2, PDK1, and Akt (33, 49). Recently, an association between transcription factors facilitating EMT and Axl and, hence, a mechanistic basis for the observed blockade in invasion and/or migration upon knockdown of this RTK in MIA PaCa-2 cells has been described. Matrix metalloproteinase-9 (MMP-9), a type IV collagenase involved in basement membrane proteolysis and in tumor angiogenesis, is expressed in the tumor microenvironment promoting invasion and metastasis. MMP-9 is reported to be overexpressed in pancreatic cancers, and recently, it has been documented that Axl in cancer cells can upregulate MMP-9 and render the cells more invasive (50).

Axl, Gas 6, and Mer in Human Gliomas

Studies of fresh-frozen tumor samples and IHC studies of paraffin-embedded tumor tissue showed that Axl and Gas 6 are frequently overexpressed in World Health Organization (WHO) grade 2 and 4 gliomas, including those of astrocytic, oligodendroglial, and ependymal origins (51). Glial fibrillary acidic protein (GFAP), an astrocyte-specific intermediate filament controlling cell shape and cell movement, is commonly detectable in glioma cells. In these studies, vessels typically surrounded by Axl and GFAP–positive tumor cells represented necrotic areas and pseudopalisades caused by intravascular thrombosis. Both Axl and Gas 6 were strongly expressed in ECs of microvascular hyperplasia. Tumor cells adjacent to microvascular hyperplasia.
showed a pronounced Axl staining. A comparative IHC study showed that Axl and GFAP are coexpressed in glioma cells of pseudopalisades, thus showing a functional association of Axl and GFAP during glioma cell migration. Inhibition of Axl signaling almost completely suppressed glioma cell migration into fetal rat brain spheroids and into brain tissue of a mouse xenograft model (12). Cell death due to apoptosis and coagulative necrosis is fundamental to glioblastoma multiforme (GBM), and studies have shown Gas 6/Axl signaling to attenuate neuronal cell death caused by serum starvation, secretory phospholipase A2, and amyloid β protein (52). A strong coexpression of Axl/Gas 6 is found in reactive astrocytes and astrocytic end feet, which are in close proximity to microvessel walls and modulate the blood–brain barrier function. Axl may also have a comparable function in modulating tumor angiogenesis and blood–brain barrier function similar to VEGF and platelet-derived growth factor receptors. Recently, Mer and Axl overexpression has been shown in a majority of GBM cell lines and patient samples. Recent findings also show that inhibition of Mer significantly reduces migration of GBM cells in vitro (53).

Axl in Breast Cancer

Axl is overexpressed in human breast cancer cell lines and patient samples and correlates with advanced tumor stage (9, 54). Axl and Mer are both upregulated in metastasis relative to primary tumors and are associated with poor survival (22, 55). Axl expression is found in estrogen receptor–positive patients, whereas Gas 6 is upregulated in progesterone-positive patients. Overexpression of Axl correlates with poor prognosis, and upregulation of Gas 6 mRNA levels correlates with favorable prognosis (54, 56). Studies have shown Axl inhibition reduces migration and invasion of breast cancer cells by Axl-dependent expression of MMP-9 (50). Recent studies have shown a role for Axl in EMT-associated transcription factors twist, snail, and slug and metastasis of breast cancer.Murine breast tumors treated with an Axl inhibitor exhibit reduced snail expression in vivo (57). There is evidence that Axl also has a role in chemoresistance development, as shown when chronic exposure of an Axl-negative breast cancer cell line to lapatinib resulted in lapatinib resistance, de novo expression of Axl, and increased expression of estrogen receptor (58). Lapatinib sensitivity was restored by inhibition of either Axl or estrogen receptors.

Axl and Mer in Non–Small Cell Lung Cancer

High levels of Axl, Mer, and their ligands Gas 6 and protein S have been found in more than 50% of non–small cell lung cancer (NSCLC) cell lines (42, 47). Axl overexpression was also shown in 48.3% of patient samples of lung adenocarcinoma and correlated with lymph node metastasis and higher clinical stage, indicating it to be a poor prognostic factor (10). RNA interference (RNAi)–mediated silencing of Axl reduces the viability of NSCLC cells in vitro and inhibits tumor growth in xenograft models (45). Axl expression correlates with NSCLC cell invasiveness and migration, an effect that is mediated by Axl-dependent upregulation of MMP-9 expression (50).

Axl and Mer in Acute Leukemia

One common chromosomal rearrangement found in pre-B–acute lymphoblastic leukemia (ALL) is the t(1:19), resulting in fusion of 2 transcription factors, E2A and PBX1. Historically, E2A–PBX1 positivity is a poor prognostic indicator (59). Survival rates for children with relapsed ALL remain poor with contemporary therapy protocols. A recent study showed that Mer expression is associated with poor outcome in the E2A–PBX1 cytogenetic subgroup in newly diagnosed pre-B–ALL and also in various cytogenetic subtypes in chemoresistant or recurrent pre-B–ALL (40). Previous studies had identified ERK1/2 and Akt as downstream targets of Mer, and a recent study showed a link between Mer and mTOR signaling. ERK1/2 protects cancer cells from apoptosis, and in adults with ALL, it is associated with poor prognosis. In AML, Axl overexpression correlates with worse progression-free and overall survival (60).

Axl in Hepatocellular Carcinoma

A recent study using hepatocellular carcinoma (HCC) cells and cDNA microarray showed a 4.7-fold increase of Axl mRNA levels in Hca-F cells with high lymphatic metastasis potential, as compared with Hca-F cells with low lymphatic metastasis potential (61). The group also found that Hca-F cells with higher Axl levels had a proliferative advantage. siRNAs were used to inhibit Axl expression in Hca-F cells and showed that the silencing of Axl could impede Hca-F cell proliferation and anchorage-independent growth and resulted in decreased capacity to migrate and loss of capacity to invade the surrounding matrix in vitro. The study also showed that the angiogenic factor Cyr61, which belongs to a family of CCN proteins and promotes cell adhesion, proliferation, migration, and angiogenesis through cell-type–specific binding to different integrin receptors, was the principle gene affected by Gas 6 stimulation of Axl in the Hca-F cells. Elevated levels of Cyr61 correlated with poor prognosis, lymph-node involvement metastasis, and mortality and can be modulated by Gas 6. More studies need to be done to more fully show the role of the Axl/Gas 6 signaling pathway and any role for Mer in HCC.

Axl and Mer as Molecular Therapeutic Targets

Drug design

Axl/Gas 6 and Mer signaling pathways represent novel biologic targets and could interact synergistically
with standard chemotherapeutic agents, permitting dose reduction and toxicity. A crystal structure of Mer was recently published in complex with an ATP-competitive inhibitor (62), and this structure has allowed the construction of predictive homology models of Axl. In addition to the overall conserved structure of the kinase domain, the ATP-binding pockets of Axl and Mer possess features and establish molecular interactions with ATP that are common to other protein kinases. Understanding these interactions has proven useful in the design and identification of Axl and Mer ATP-competitive inhibitors (Fig. 2A).

In one example, the crystal structure of Mer was determined in complex with a small molecule ATP-competitive inhibitor (compound-52), on the basis of a trisubstituted purine scaffold (Fig. 2B). The cocrystal structure of the complex revealed the compound occupying the adenine and sugar pockets of Mer and making important hydrogen bonds, hydrophobic interactions, and van der Waals contacts with critical amino acid residues. The binding mode analysis of the Mer-compound-52 complex structure indicated that the compound binds within the ATP-binding pocket in such a way that the pyrimidine ring –N1 and –NH of aryl ring at 2-position are involved in hydrogen-bonding acceptor and/or donor interactions with hinge residue Met674 backbone –NH...N− and –C=O...HN− atoms. The substituent at the fifth position is involved in nonbonded interactions with gatekeeper Leu671. Additional substituents, such as the –CH3 functional group, was well tolerated into the gatekeeper region. Further, the 4-aminoaryl ring extended into the hydrophobic GXGXFG site. The N,N-dimethyl aryl-sulfone faces the pocket surrounded by Leu593 and Val601 and is positioned close to the DFG site involved in polar interactions with Asp741 residue. The 2-position of the aryl-piperazine ring moiety is exposed to the solvent accessible region, indicating that these positions should be able to accommodate a variety of substitutions to improve the potency and adjust physicochemical properties.

Despite the overall structural conservation, several unique features of the active site can be exploited for drug design. Unique to the TAM family of kinases is the presence of nonpolar residues, such as Phe622/673 and Pro621/672, located in the hinge region, which is adjacent to the gatekeeper site Leu620/671. These 2 unique residues and their location aid in the process of designing specific inhibitors. Sequence and structural similarities among all 3 TAM family members suggest that it will be a challenge to design ATP-competitive small molecules with absolute specificity for a single family member.

**Small molecule inhibitors**

Small molecule inhibitors of Axl and Mer have emerged and are in various stages of drug development (Fig. 2B). Axl has received more focus from drug discovery programs, although inhibitors of Mer are also beginning to surface; however, no compounds specifically designed to target Axl or Mer are in the clinical stages of development. It is likely that some of the agents described below will enter clinical trials in the near future.

According to published data, R-428 is the furthest advanced Axl small molecule inhibitor in preclinical development (57). It is based on a trisubstituted triazole core and exhibits potent activity against Axl kinase (IC50 = 14 nmol/L) in both biochemical and cell-based experiments. In a kinase selectivity panel, R-428 showed good specificity toward Axl kinase with some cross-reactivity with Mer, Tyro3, VEGFR family members, Ret, Tie2, and Abl kinases. In cell-based models, R-428 inhibited Gas 6–stimulated phosphorylation of Axl and Akt (Ser473). R428 dose-dependently suppressed invasion of both human MDA-MB-231 and murine 4T1 breast cancer cells. In pharmacokinetic mouse studies, R-428 was shown to have good plasma stability (half life = 4–13 hours), and pharmacologically relevant doses were achievable by oral administration of the drug. Using additional animal models, R-428 was shown to inhibit breast cancer metastasis and suppress angiogenesis, and these activities correlated with inhibition of Akt and Erk phosphorylation. Furthermore, R-428 showed good synergistic activity with cisplatin in inhibiting liver and lung metastases in a breast cancer mouse model. Overall, the data surrounding R-428 offer a good rationale for its further development, particularly as an antimetastatic agent in breast cancer.

Other drug development groups have disclosed discovery programs focusing on Axl kinase, although the details surrounding these compounds are limited. A few years ago SGI-AXL-277, an Axl kinase inhibitor based on a pyrrolopyrimidine scaffold, was reported to be in lead optimization and biological testing phases of preclinical development (63). A series of Axl kinase inhibitors were shown to inhibit proliferation of a diverse panel of cell lines from hematologic malignancies (64).

CVO-102 is an inhibitor of Mer kinase in late preclinical development, although only a small amount of information is available about this therapeutic agent (65). It has been shown to have significant activity in preventing blood clotting and also shows promise as an anticancer agent, particularly in leukemias and lymphomas. The group developing this compound has reported the generation of some animal data to validate their approach and reportedly is continuing optimization of the lead agent.

**Biological therapeutics**

In addition to these small molecule Axl and Mer kinase inhibitors, biological therapeutics are also in preclinical development. Preclinical work was recently published using a monoclonal antibody (YW327.6S2) recognizing both human and murine forms of Axl (66). YW327.6S2 was shown to block Axl function by downregulation of the expression of Axl as well as through inhibition of Gas 6 binding, leading to inactivation of Axl and its
downstream signaling to Akt. As a single agent, YW327.652 showed significant activity against the growth of lung carcinoma cells (A549) in mouse xenograft models, along with the observed downregulation of Axl expression and induction of apoptosis in these cells. YW327.652 also targeted tumor stromal cells by enhancing the effects of an anti-VEGF antibody therapy on tumor vasculature, resulting in tumor stasis maintained for at least 4 weeks after treatment. Furthermore, YW327.652 sensitized wild-type EGFR lung carcinoma cells to erlotinib treatment, suggesting Axl inhibition may enhance the efficacy of ERGR inhibitors in tumors that are refractory to EGFR inhibition alone. Combination studies further showed that YW327.652 synergized with carboplatin/paclitaxel in the same lung cancer xenograft model. In the MDA-MB-231 breast cancer xenograft model, YW327.652 showed activity as a single agent and in combination with an anti-VEGF therapy. In this model, significant effects were observed on reducing tumor vascular density, tumor-inflammatory response, and MDA-MB-231 metastases to the bone. The advantages of a therapy based on a monoclonal antibody against the Axl receptor are clear, including the enhanced specificity and reduced off-target effects often achieved with biological therapies. An unexplored potential drawback associated with this approach is that the Axl antibody may be primarily binding to the sAxl in the bloodstream and, thereby, diminishing the effects of the antibody and requiring high doses of the antibody for effective treatment. An additional approach to targeting Gas 6/Axl signaling would be to use recombinant sAxl as a sponge to soak up free circulating Gas 6 (67). The bound Gas 6 would be unable to bind to the Axl receptor and initiate downstream signaling consequences. This approach would potentially have the advantage of using a biological agent with high specificity and limited toxicity, but the Gas 6-independent effects of Axl activation are not well understood and are likely to play a role in tumorigenesis, as using sAxl as a therapeutic agent would only inhibit the Gas 6–dependent function of the Axl kinase receptor.

Conclusion

Axl and Mer are novel RTKs and play a significant role in pathogenesis and progression of human cancers. They can serve as important biomarkers for disease status and prognosis. Ongoing studies are using their unique structure and role in cancer genesis and as a therapeutic target for drug development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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