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**Gas6 /TAM system: physiological insights and
diseases**

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Abstract:

Growth arrest specific 6 (Gas6) is a multimodular circulating protein, the actions are mediated by the interaction with three transmembrane tyrosine kinase receptors: Tyro3, Axl, and MerTK, named TAM. Their regulatory roles are prominent in the mature immune, reproductive, hematopoietic, vascular, and nervous systems. Gas6 /TAM system plays a role in the regulation of inflammation, coagulation, cell growth, and clearance of apoptotic bodies. Deficiencies in TAM signaling are thought to contribute to chronic inflammatory and autoimmune disease in humans, and aberrantly elevated TAM signaling is strongly associated with cancer progression, and metastasis. We review the function of the Gas6/TAM system and the current evidence supporting its potential role in the pathogenesis of different diseases.

Key words: Growth arrest specific 6, tyrosine kinase receptors, TAM.

The Gas6/TAM Receptors System

Gas6 is a member of vitamin K–dependent family of proteins (VKD) which includes the procoagulant factors II, VII, IX, and X, and the anticoagulant factors, protein C and S, as well as protein Z [1]. Its concentration in plasma is around 20-50 ng/mL (0.25 nmol/L). In contrast to the VKD proteins of the blood coagulation cascade, Gas6 is not primarily synthesized in the liver, but is widely expressed and has been found in the lung, heart, kidney, intestine, endothelial cell, bone marrow, vascular smooth muscle cell, monocytes and at very low levels in the liver [2].

The gene coding for Gas6 was first discovered in 1988 through the screening of genes whose expression was upregulated in growth arrest embryonic mouse fibroblast so its name derives from its discover. Six genes were found and they were named *gas1 to gas6*. In 1993, the gene was sequenced and found to be similar to plasma anticoagulant protein S sharing 44% homology with it [3]. Despite this high degree of structural similarity, they are functionally different [4].

Gas6 is the ligand for the TAM family of receptors, which is composed of 3 members: Tyro3, Axl, and Mer. Gas6 binds the TAM receptors with different affinities: $Axl \geq Tyro3 \geq Mer$ [3].

Axl was first discovered in 1991 and was named Axl from the Greek word “anexelekto,” meaning uncontrolled based on the initial observations of its function as it was a product of a transforming gene in a T-cell leukemia cell line [5].

The human genome encodes 58 receptor tyrosine kinases (RTKs), which are grouped in 20 families based on homology [6]. Axl is a member of TAM subfamily of receptors which belong to the large family of type I transmembrane receptor tyrosine kinase [7]. Axl is expressed in most

human cells originating from hematopoietic, epithelial, and mesenchymal sources. Tyro3 is mostly found in the central nervous system, kidneys, ovaries, and testes. Mer is predominantly expressed in ovaries, testes, prostate, lungs, and kidneys and to a lesser extent in the thymus, spleen, liver, small intestine colon, and placenta [2]. Membrane-bound Axl can be shed from the cell membrane as a result of proteolysis, and Axl is therefore present in the circulation in a soluble form (sAxl) that consists of the extracellular region of the protein [8]. The levels of sAxl (0.6nmol/L) are normally in excess of Gas6 (0.25 nmol/L) and that all Gas6 is bound to sAxl in normal human blood [9]. The soluble receptors can remove the ligand from cell bound receptors and thus inhibit signaling [10].

Structure of Gas6 and TAM receptor

Gas6 is a multidomain protein (Figure-1A). Its molecular weight is 75 kDa. It contains an amino terminal γ carboxyglutamic acid (Gla domain) which gives VKD proteins the ability to bind to anionic phospholipids at the cell surface. Gla domain is followed by a loop maintained by a disulfide bridge followed by 4 epidermal growth factor-like domains ending with the carboxyterminal (C-terminal), consisting of 2 laminin G (LG) repeats that together comprise the sex hormone-binding globulin domain (SHBG) interacting with the TAM receptors [2].

Axl is a 140-kDa protein. The N-terminal starts with extracellular domain composed of 2 Ig-like domains followed by 2 fibronectin type 3 domains. Then a single-pass transmembrane domain (TM) followed by the cytoplasmic tail (intracellular domain) which contains a protein tyrosine kinase (TK) domain at the C-terminal (Figure -1B) [11].

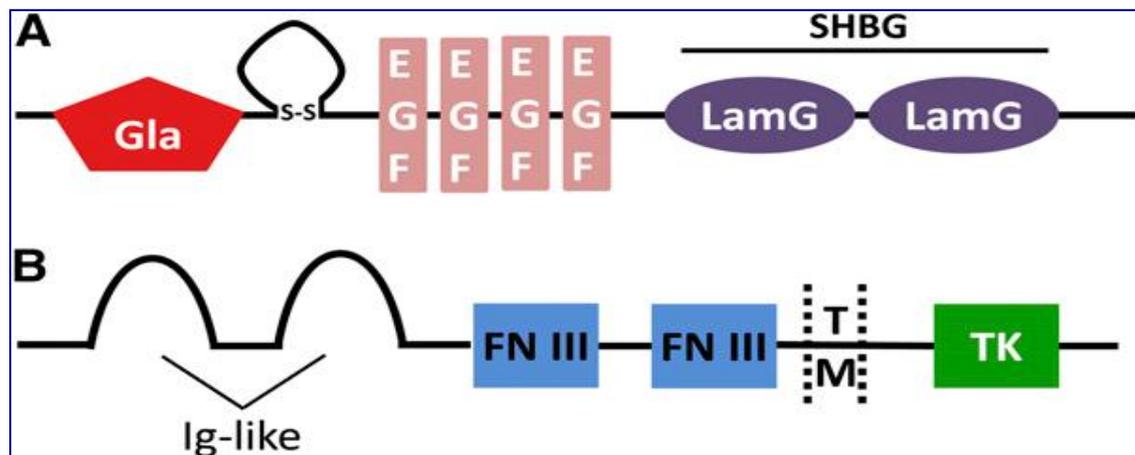


Figure (1): Gas6 and TAM receptor structure [3]

(A) Gas6 is composed of, from N to C terminus, a Gla domain, a loop maintained by a disulfide bridge, 4 EGF domains, and 2 LamG subdomains containing the SHBG domain.

(B) TAM receptors are composed of 2 Ig-like domains, 2 FN III domains, a TM, and a TK domain. EGF, epidermal growth factor; FN III, fibronectin type III like; SHBG, sex hormone binding globulin; TAM, Tyro3, Axl, and Mer; TK, tyrosine kinase; TM, transmembrane domain.

Gas6-receptor interaction and downstream consequences

When the ligand Gas6 binds TAM receptor, the receptors assemble into dimers, bringing the intracellular domains in close proximity. The intracellular domains will phosphorylate each other at specific tyrosine residues, enabling adaptor proteins to bind to the now activated receptor dimer. The adaptor proteins will transmit the signal into the cell, leading to altered cell behavior (Figure-2) [12].

Gas6/Axl binding followed by

1) Activation of phosphatidylinositol 3-kinase (PI3K) and its downstream target, serine/threonine protein kinase (Akt), which is a

central step in Axl-dependent signal transduction. The Gas6/Axl/PI3K/Akt pathway is required for the antiapoptotic function of gas6 in several cell types such as endothelial cells (EC), vascular smooth muscle cells (VSMC), fibroblasts, chondrocytes, oligodendrocytes, neurons, and several cancer cells [13].

2) Activation of Akt leads to the inactivation of pro-apoptotic caspase 3, phosphorylation of BCL2-associated agonist of cell death (Bad), a proapoptotic mediator, and to an increase of the antiapoptotic protein B-cell lymphoma 2 (Bcl-2) by an nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) dependent mechanism [14].

3) Axl provides a binding site for the adaptor protein Grb2 (growth factor receptor-bound protein 2), which might be involved in the activation of the mitogen-activated protein (MAP) kinases (extracellular-signal-regulated kinase (ERK), mitogen-activated protein kinase (p38), c-Jun N-terminal kinase (JNK)). The Ras/ERK1/2 pathway is essential for mediating Gas6 mitogenic activity [15]. Src (non-receptor tyrosine kinase) is also involved in Gas6-mediated survival or mitogenic effect and a binding site has been identified for Src on Axl. Activation of p38 and phosphorylation of heat shock protein 25 (HSP25), a regulator of actin remodeling, is downstream of Axl [3].

4) Gas6/Axl pathway can inhibit vascular endothelial growth factor receptor (VEGFR) 2 in endothelial cell morphogenesis through activation of the tyrosine phosphatase SHP-2 [16].

5) Phospholipase C (PLC γ) interacts with TAM, in the process of efferocytosis, inducing cytoskeletal rearrangements [17].

6) Gas6/Axl pathway is an inhibitory mechanism for toll-like and cytokine receptor signaling in innate immune cells. Gas6/Axl activates interferon- α/β receptor (INFA β)/signal transducer and activator of transcription (STAT1) pathway which increases expression of

suppressors of pro-inflammatory signals such as transcription factor twist homolog 1 (Twist1), suppressor of cytokine signaling (SOCS1 and SOCS3) [18].

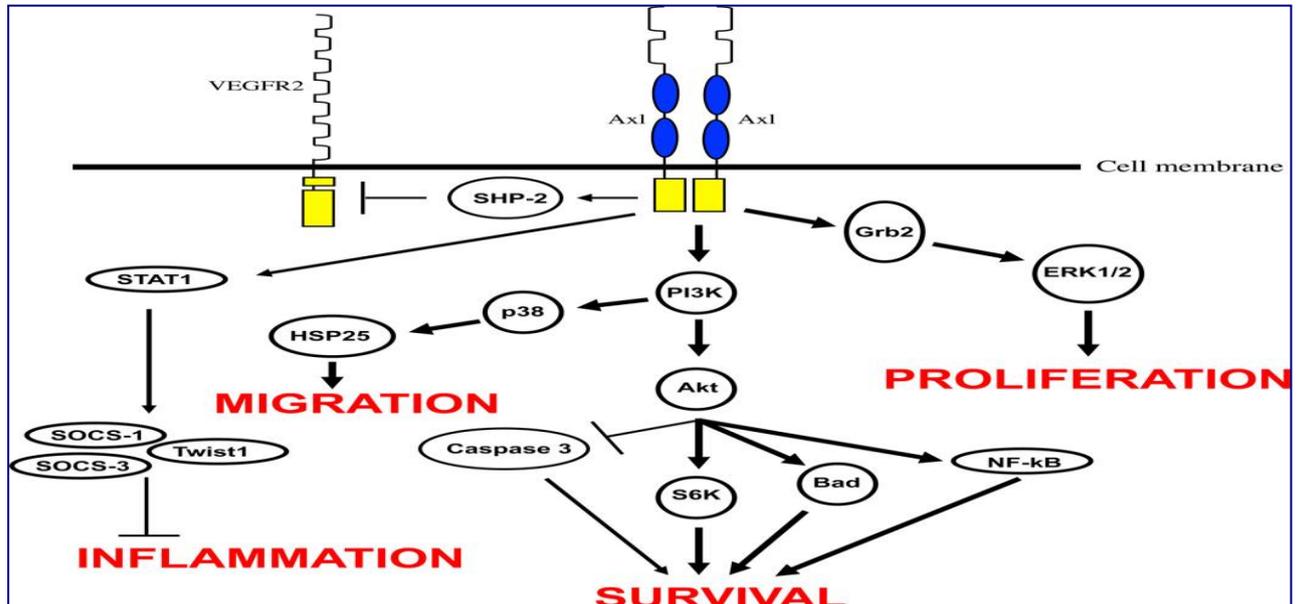


Figure (2): Axl receptor signal transduction [15]

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.

Function of Gas6/TAM system

The Gas6/TAM system regulates multiple biological processes, including cell survival and proliferation, cell adhesion and migration, thrombus stabilization, and inflammatory cytokine release [19]. Therefore, the role of this system has been found to be important in inflammation, hemostasis, autoimmune disease, nervous, reproductive and vascular systems and cancer [14].

- **Anti-apoptotic and mitogenesis**

Several studies have been presented on the antiapoptotic and mitogenic effects of Gas6 signaling. These effects have been documented in fibroblasts [20], EC [21], VSMC [22], oligodendrocytes [23], Schwann

cells [24], lens epithelial cells [25], neurons [26] and liver cells [27]. Gas6 decrease apoptosis after serum starvation [20] and TNF α - treatment in several cell types [25].

Up-regulation of Gas6 and the TAM receptors have been observed in many malignancies, including leukemia, cancer of the thyroid, lung, uterus, endometrium, ovary, prostate, gastric cancer, breast cancer, Kaposi's sarcoma, malignant gliomas and renal cell carcinoma [3].

Presence of Gas6 and the TAM receptors have clinical implications for cancer patients. Low expression of Axl mRNA indicates a good prognosis in patients with renal cell carcinoma. High Axl expression in breast cancer, pancreatic adenocarcinoma, malignant glioma and esophageal adenocarcinoma is a negative prognostic factor [28].

Gas6/Axl pathway regulates tumor genesis via several mechanisms which include tumor cell survival and growth, anti-apoptosis, increased migration, immune cell activation and angiogenesis [29].

Inhibition of RTKs has been shown to be a viable way of treating several cancers. Inhibiting Axl with antibodies leads to decreased proliferation and invasiveness in animal models [30].

- **Phagocytosis & Migration**

Gas6 can act as a bridging molecule with the Gla domain binding negatively charged phospholipids on the surface of the apoptotic cell that will be engulfed, coincident with LG domains binding a TAM-bearing phagocytic cell. Phosphatidylserine (PS) normally resides in the inner leaflet of the cell membrane. Exposition of PS at the cell surface is a feature of cell injury, activation, and apoptosis [31]. Without proper removal of apoptotic cells, secondary necrosis occurs and leads to inflammation. Gas6 binds PS in microtiter plates, and monocytes bind PS-coated microtiter plates when Gas6 is present, but not in its absence [32].

Gas6 can induce migration in Axl expressing cells, including VSMC, neurons, and dendritic cells, but is reported to inhibit migration in mouse fibrosarcoma cells, renal carcinoma cells, and to inhibit chemotaxis of endothelial cells, showing that the migration is highly dependent on cell type [33].

- **Regulation of inflammation**

Axl stimulation by Gas6 can inhibit release of proinflammatory cytokines from human macrophages, dendritic cell, sertoli cells, and glial cells, thus limiting the immune response [34]. In bone marrow derived dendritic cells, Gas6 induces upregulation of SOCS proteins, known for their suppression of cytokine signaling [18].

- **Gas6/Axl in innate immunity**

TAM receptors protect innate immune cells (macrophages, dendritic and NK cells) from apoptosis and are involved in phagocytosis of apoptotic bodies [35]. The protective role for Axl receptor in chronic immune disorders such as rheumatoid arthritis, systemic lupus erythematosus has been demonstrated in TAM knockout mice. These mice develop a lupus-like syndrome and present high levels of circulating auto-antibodies against DNA, collagen, and phospholipids. They also show an abnormal growth of peripheral lymphoid organs such as the spleen and lymph nodes and demonstrate a delayed clearance of apoptotic cells [36].

- **Gas6 and TAM in the vasculature**

Strong evidence suggested that Gas6/Axl signaling is important in the vasculature. Axl and Gas6 are expressed by numerous cell types in the vascular wall, including endothelial cells, smooth muscle cells and fibroblasts [37]. Gas6/Axl pathways not only increase survival but also protect VSMCs from apoptosis and from calcium deposition in vitro [22]. Axl is involved in the integrity of the vasculature and its expression is

upregulated at the site of vascular injury, suggesting a role for Axl in vascular remodeling [3].

Animals deficient in Axl or Gas6 display impaired vessel integrity and have increased vessel leakage compared to their wild type littermates [38]. 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 [15].

- **Gas6 and mesangial cell proliferation**

Hyper proliferation of mesangial cells in the kidney is a hallmark of glomerular disease. When mesangial cells were treated with medium from Gas6-producing cells, they started to proliferate [39]. Gas6 and Axl were found to be upregulated in the mesangial cells in a mouse model of experimental glomerulonephritis [40]. Kidney expression of Gas6 is increased during chronic rejection of transplanted kidneys, lupus nephritis, glomerulonephritis and IgA Nephropathy [41].

Role of Gas6/ TAM system in some diseases:

- **Liver pathology**

Gas6 and Axl are mainly expressed in oval cells of the liver, and not in hepatocytes. Oval cells are precursors which differentiate and proliferate upon hepatic injury. In these cells, Gas6 acts as a survival factor that protects against apoptosis. Oval cells are the secondary response to hepatic injury, in the situation where hepatic stellate cells (HSCs) are unable to proliferate. HSCs are mature cells that are responsible for the liver's regenerative ability, and which accumulate at the site of injury and transform into cytokine-secreting myofibroblasts. Axl is also expressed in HSCs, and signals through the PI3K/Akt and NFκB pathways to protect against apoptosis. In liver pathologies, a hepatoprotective role for Gas6

has been reported in ischemia/ reperfusion-induced damage, and in the wound healing response to liver injury [11].

Gas6/Axl is a profibrogenic route that is activated in patients with chronic liver disease. The role of Gas6/Axl pathway in liver fibrosis is by participating in the activation of HSC. Therefore, small molecule inhibitors against Axl, that effectively eliminate HSC activation and reduce experimental fibrosis progression, may be interesting therapeutic tool for future clinical trials [42].

Axl is found to be upregulated in HCC tumors compared to normal hepatocytes and seems to be more associated with lymph node metastasis [43].

[44] reported that the increase of Gas6, sAxl and Gas6/sAxl molar ratio were correlated with the progression and poor prognosis of HCC, so it could be used as useful biomarkers for HCC.

A study done by [45] showed that Plasma Gas6 concentration is a novel noninvasive biomarker of liver fibrosis but further studies are required to clarify its clinical and pathophysiological role in chronic liver diseases.

- ***Diabetes Mellitus***

According to the study done by [46], plasma Gas6 concentration was significantly lower among patients with type 2 diabetes and its value was inversely correlated with fasting glucose. Plasma Gas6 is associated with altered glucose tolerance, inflammation and endothelial dysfunction. It also may represent a risk factor of type 2 diabetes and a potential marker of inflammation and endothelial dysfunction.

Chronic inflammation and activation of the innate immune system are closely involved in the pathogenesis of type 2 diabetes. Gas6/TAM signaling resulted in inhibition of the inflammatory response in dendritic cells and macrophages so it has a role in controlling innate immunity and

inflammation processes so the inflammatory effects of high glucose may be mediated through low Gas6 levels as well as reduced TAM signaling and, consequently, activated innate immunity [47].

Autoimmune and Chronic Inflammatory Diseases

The TAM pathway has been implicated in various human chronic inflammatory and autoimmune diseases, including multiple sclerosis (MS), SLE, inflammatory bowel diseases, and rheumatoid arthritis [48]. Inefficient phagocytosis of apoptotic cells and membranes has been described in TAM knock-out (KO) mice. It is reported that the delayed clearance of apoptotic cells and the loss of regulation of the inflammatory response are associated with the development of a lupus-like syndrome in TAM KO mice [49].

Improved understanding of the specific immunological function of the TAM and their agonists is likely to pave the way for tailored therapeutic approaches in chronic inflammatory and autoimmune diseases [48].

Infectious Diseases

The role of TAM and their ligands in viral infectivity has been reported. It was found that, AXL favors filovirus infections. Furthermore, expression of TYRO3 and MERTK were similarly able to confer susceptibility to Ebola and Marburg viruses [50]. It was found that AXL could favor the infectivity of a wide array of viruses; vaccinia, Lassa, dengue, and West Nile. Although multiple studies have concurred on the ability of TAM to favor viral infection in vitro, the function of this AXL in viral infections in vivo remains controversial. When wild-type mice were infected with a lethal dose of the PR8 influenza virus strain, the systemic administration of an anti-AXL antibody significantly reduced mice mortality. This protective effect correlated with increased

expression of type I IFN and reduced lung pathology. Similarly, treatment with this anti-AXL antibody reduced the lung pathology upon respiratory syncytial virus infection in mice. Thus, the fundamental role of TAM as negative regulators of the immune response appears to have been exploited by viruses to dampen and bypass the host defense. The role of TAM in viral infections may be much more complex than their role in dampening type I IFN signaling [48].

Cancer

The discovery of TAM was encouraged by two major interests: their putative role in development and differentiation, and their potential function in transformation and carcinogenesis.

The predominant literature on TAM signaling in cancer focuses on its cell autonomous oncogenic function in tumor cells. One of the first evidences of a TAM signaling axis involving tumor cells and tumor-associated macrophages came from the experiments of [51], these authors demonstrated that tumor-infiltrating macrophages display higher levels of Gas6. This raises the possibility that factors predominant in the tumor microenvironment, such as IL-10 and M-CSF, lead to GAS6 upregulation in tumor-associated macrophages. Tumor-associated macrophages, in turn, use the upregulated Gas6 to engage TAM receptors in tumor cells. This TAM signaling promotes tumor cell proliferation.

A recent study described the therapeutic efficacy of a potent small molecule inhibitor of TAM in reducing cancer metastasis in mice to the inhibition of TAM signaling in NK cells and the subsequent enhancement of NK cell activation.

There are reports, in contrast, describe an anti-oncogenic role for TAM signaling in tumor-associated immune cells. Interestingly, these reports use a model of inflammation-induced colon carcinogenesis. Patients with

chronic intestinal inflammation and inflammatory bowel diseases are at a significantly increased risk of colorectal cancer. TAM signaling in colonic inflammation and colorectal cancers may consistent with an anti-inflammatory and antitumor function. Finally, the functional effect of TAM signaling during immune cell–cancer cell interaction may vary with the tumor type. The stage of the tumor—early stage, such as carcinogenesis and tumor initiation, versus late stage, such as tumor progression and metastasis—may also be crucial in determining how TAM function in tumor-associated immune cells can influence therapeutic outcomes. Thus, evidence-based targeting of the TAM may complement existing immunotherapy regimens to release the full power of the anticancer immune response [48].

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