



The Role of the TAM Family of Receptor Tyrosine Kinases in Neural Development and Disorders

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Abstract

Tyrosine kinase receptor 3 (Tyro3), Axl, and Mer constitute the TAM family of receptor tyrosine kinases. These receptor tyrosine kinases are characterized by a conserved sequence within the kinase domain and adhesion molecule-like extracellular domains. The TAM family and their shared ligands, growth arrest-specific gene-6 (Gas6) and protein S, are differentially expressed in the developing and adult nervous systems. The TAM family regulates normal cellular processes and plays an important role in nervous system development and diseases, including cell proliferation/survival, cell adhesion and migration, neuronal differentiation, cell apoptosis, myelination, degeneration, and immune regulation. This review summarizes the developing expression of the TAM family and ligands and their function in neural development and nervous system diseases. Further research will be necessary to fully elucidate the function of the TAM family and to evaluate the clinical implications of TAM family expression and activation in nervous system diseases.

Keywords

TAM, Tyro3, Axl, Mer, Nervous system

Introduction

Receptor tyrosine kinases (RTKs) are high-affinity cell surface receptors that transduce signals from the extracellular environment to the cytoplasm and nucleus [1]. RTKs are key regulators of normal cellular processes, and these receptors also play a critical role in the development and progression of many nervous system diseases [2-4]. RTKs include 20 families that are distinguished by amino acid sequence identity within the kinase domain and exhibit structural similarities within their extracellular regions [5]. This review focuses on the TAM family, which is one of the latest to evolve and last to be identified [2,6].

The TAM family includes three receptors, tyrosine kinase receptor 3 (Tyro3), Axl, and Mer, which share the vitamin K-dependent

ligands, growth arrest-specific protein 6 (Gas6) and protein S. Gas6 binds all three TAM receptors, and protein S binds only Tyro3 and Mer [2,6]. The most prevalent functions of the TAM family and their ligands were investigated in tumour genesis [2,6]. Tyro3, Axl, and Mer are overexpressed in numerous cancers, including myeloid and lymphoblastic leukaemias, melanoma, breast, lung, colon, liver, gastric, kidney, ovarian, uterine, and brain cancers [2,6]. TAM receptor induction in tumour cells predominately promotes survival, chemoresistance and motility [2,6]. Inhibition of TAM kinases stimulates antitumour immunity, reduces tumour cell survival, enhances chemosensitivity and diminishes metastatic potential [2,6]. However, the function of the TAM family and ligands is not well documented in the nervous system. This review summarizes

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the developmental expression of TAM receptors and ligands in the nervous system. We also highlight their role in neural development and the mechanisms in some nervous system diseases. We discuss possible means of targeted inhibition of the TAM family in the treatment of these diseases and future directions for the investigation of this RTK pathway.

TAM Family: Structure, Ligands and Signalling Pathways

The TAM family generally includes Tyro3, Axl, and Mer. These three receptors contain an adhesion molecule-like extracellular domain, a single pass α -helical transmembrane domain, and a conserved intracellular tyrosine kinase domain [2]. Two immunoglobulin-like (Ig) domains and two fibronectin-type III (FNIII) domains are the major structures of the extracellular domain [2]. The TAM family is distinguished from other RTKs by a conserved sequence, KW (I/L)A(I/L)ES, within the kinase domain and adhesion molecule-like domains in the extracellular region [2]. The functional form of the receptor is a dimer, which is similar to other RTKs, and the receptors form hetero- and homodimers [2,6].

The full-length Tyro-3, Axl, and Mer proteins were fully cloned and contain 890, 894, and 999 amino acids, respectively. The predicted protein sizes are 97, 98, and 110 kDa for Tyro-3, Axl, and Mer, respectively [2,5]. However, the actual molecular weights range from 140 to 100 kDa for Tyro3 and Axl and 165–205 kDa for Mer due to posttranslational modifications, including glycosylation, phosphorylation, and ubiquitination [2,5,7].

The Tyro3 protein was first isolated from rat in 1991, then from mouse, human and chicken [8]. This protein was named Dtk, Brt, Rse and Tyro3 in mice, Sky, Tif or Rse in human and Rek in chicken [2]. *Axl* was first detected in 1988 as an unidentified transforming gene in two patients with chronic myelogenous leukaemia (CML) [9]. The gene *axl* was named from the Greek word for uncontrolled, anexelekto [7]. Axl was also called UFO or Tyro7 [2]. The Mer protein was isolated from the chicken retrovirus RLP30 in 1992 [10]. Mer was named because it was found in monocytes, epithelial and reproductive tissues [11]. This protein was also called c-ryk, c-eyk, Nyk or Tyro-12 [2].

These three receptors share the vitamin K-dependent ligands Gas6 and Protein S. Gas6

and Protein S share 43% amino acid sequence identity and a similar domain structure [2]. Gas6 and Protein S possess 1 Gla domain, 4 epidermal growth factor (EGF)-like domains and 2 laminin-G (LG) domains at the N-terminus. Both ligands are dependent on vitamin K for post-translational modification of the Gla domain [12]. The Gla domain is a binding site for phospholipids on the surface of cell membranes. The LG domains mediate binding to the TAM receptors. *Gas6* was first identified as a gene that was upregulated in response to growth arrest in serum-starved NIH 3T3 fibroblast cells, from which its name arose. Gas6 activates each of these receptors. The relative affinity of Gas6 is Axl>Tyro3>Mer, and Gas6 is the sole ligand for Axl [13]. Protein S is a ligand for the Mer and Tyro3 receptors, but not the Axl receptor [12]. Other neurotrophic factors may also regulate TAM receptors. Nerve growth factor (NGF) upregulates the expression of Tyro3 and Axl on PC12 pheochromocytoma cells [14,15].

TAM activates several intracellular signalling pathways, including PI3K (phosphatidylinositol-3-kinase)-Akt, extracellular signal-regulated kinase 1/2 (ERK1/2), and/or P38 MAPK (mitogen-activated protein kinase) [16–21]. Gas6/Axl promotes cytoskeletal remodelling and the migration of GnRH neuronal cells via activation of P38 PI3K and Rho family GTPase Rac signalling. Axl phosphorylates the p85 subunit of PI3K and contributes to Rac activation, which is required for Gas6/Axl-induced neuron migration [16,17]. Gas6/Axl/PI3K-Akt promotes the survival of oligodendrocytes in human foetal spinal cord [20]. Protein S/Tyro3 also activates the PI3K-Akt pathway, which is required for Protein S-mediated protection from excitotoxic NMDA-mediated neuritic bead formation and apoptosis in mouse cortical neurons [18,21]. Gas6 phosphorylates Axl and Tyro-3 simultaneously, which results in ERK2 activation in primary cultured Schwann cells [19].

Axl and Mer have soluble forms in murine and human plasma. Soluble Axl and Mer are produced via the proteolytic cleavage and release of the ectodomain, which only contains the extracellular domain of the full-length receptor [22,23]. However, no extracellular soluble Tyro3 is detected in human plasma [23]. Soluble TAM receptors bind Gas6 and act as a ligand sink to inhibit the normal cellular functions of full-length receptors [22,23]. Therefore, soluble TAM receptors may function as inhibitors of

full-length receptors and exhibit therapeutic potential in pathological conditions.

Developmental Expression and Distribution of TAM and TAM Ligands

Several studies reported the developmental expression of TAM and TAM ligands Gas6 and protein S [8,13,24-27]. Northern blotting and mRNA in situ hybridization analyses were used in these studies, and a summary of expression patterns is provided below.

■ Expression pattern of TAM receptors

Tyro3, Axl, and Mer exhibit widespread distribution with overlapping but unique expression profiles. Tyro3 is highly expressed in the central nervous system (CNS), and Axl and Mer expression is more restricted and occurs at a lower level than Tyro3 [12].

Previous studies demonstrated that Tyro3 was most abundantly expressed and widely distributed in the nervous system of adult mice [8,26]. High levels of tyro3 mRNA are expressed in neurons of the cerebral cortex, lateral septum, hippocampus, olfactory bulb, and cerebellum [8,26]. Tyro3 protein is detected in most cells of all cortical layers. Tyro-3 is detected in granule neurons and Bergmann glia in cerebellar Purkinje cells. Tyro3 exhibits a punctate pattern in the soma and dendrites of brain pyramidal neurons [27], and it was identified in the axons and growth cones of immature neurons [25]. Tyro3 is also found in ovary, testis, breast, lung, kidney, osteoclasts, and retina [2,8,26]. Tyro3 is expressed in haematopoietic cell lines, including monocytes/macrophages and platelets [2]. Tyro3 expression levels are low at embryonic stages, increase dramatically during the second postnatal week, reach the highest level by P30, and remain at that level in the adult [27]. Tyro expression in the hippocampus begins at P6, and it is expressed at high levels in CA1 pyramidal neurons and lower levels in the CA3. Tyro3 is not detected in dentate granule neurons [27].

Axl and Mer are expressed at low, but relatively constant, levels throughout development [24]. Axl is expressed in the hippocampus, cerebellum, monocytes/macrophages, oligodendrocytes, Schwann cells, platelets, endothelial cells, heart, skeletal muscle, liver, kidney, and testis during development [2,24]. Axl RNA levels are low in the spinal cord [24]. Axl is expressed in non-neuronal cells, but not neurons, in dorsal root ganglia [19,24]. Mer exhibits low level expression

in brain, heart, and skeletal muscle, but high levels of expression in ovary, prostate, testis, lung, retina, and kidney. Mer is also expressed in monocytes/macrophages, dendritic cells, natural killer cells, natural killer T cells, megakaryocytes, and platelets [2].

■ Expression pattern of Gas6 and Protein S

Gas6 is detected as a single 85-kDa protein that is secreted by neurons and endothelial cells in the nervous system [12,20]. Gas6 concentrates along the plasma membrane in resting endothelial cells [20]. Expression levels of Gas6 are low during embryonic stages, but gradually increase in late embryonic stages and reach the highest level in adulthood [28,29]. Gas6 expression in the rat embryo is primarily confined to non-neuronal tissues at early stages of development, but it is strongly expressed in neurons in the adult [30]. Gas6 is detected in the heart, blood vessels, testes, choroid plexus, and ventral spinal cord at embryonic day 14. Gas6 is initially expressed at embryonic day 17 in rat brain and gradually increases thereafter [29]. Gas6 is widely expressed in numerous brain regions, including several cortical regions, hippocampus, midbrain and cerebellum, from the day of birth. Gas6 is expressed in the cerebral cortex, predominantly layer V, the piriform cortex, and hippocampal areas CA1, CA3 and the dentate gyrus in the adult. It is also expressed in thalamic and hypothalamic structures, the midbrain, and a subset of motor and trigeminal nuclei [29]. Gas6 is expressed in Purkinje neurons and deep cerebellar nuclei in the cerebellum [29]. Gas6 mRNA is detectable in spinal motor neurons and larger dorsal root ganglia neurons [19].

Protein S is only detected at low levels in the CNS [29]. Protein S is expressed in the locus coeruleus, choroid plexus, astrocytes and retinal pigment epithelial cells. Protein S mRNA and protein are detectable in pyramidal neurons of the deep layer of the cortex, hippocampus, and the dentate fascia neurons of the hippocampus in rabbit [12].

TAM Receptor Function in the Nervous System

TAM signalling pathways play important roles in neural development and nervous system diseases.

■ TAM receptors in neurogenesis

Neurogenesis is the process by which neurons are generated from neural stem cells (NSCs) and

progenitor cells. Neurogenesis occurs during embryogenesis and the adult central nervous system in many vertebrates [31]. Multipotent NSCs are located in the subgranular zone of the hippocampal dentate gyrus and the subventricular zone of the lateral ventricles in adult brain [31]. Neurogenesis is a dynamic process that is modulated by a variety of intrinsic and extrinsic factors, including growth factors, cell surface receptors, signal transduction molecules, transcriptional factors and cytokines/chemokines [32,33].

TAM receptors may play an important role in the regulation of NSC survival, proliferation, and differentiation [33]. All three TAM receptors are expressed in cultured primary NSCs. Cultured primary NSC lacking TAM receptors exhibit slower growth, reduced proliferation, increased apoptosis and delayed neuronal differentiation and maturation. A significant reduction in the expression of nerve growth factor and brain-derived neurotrophic factor, accompanied by compensational increases in the expression of the TrkA, TrkB, TrkC and p75 receptors, were observed in cultured primary NSCs without TAM receptors [33]. TAM triple knockout (TKO) impeded neural stem cell proliferation and differentiation in mice. These studies indicate that TAM receptors support NSC survival, proliferation and differentiation and may be regulated by neurotrophins.

TAM receptor function is also important for the differentiation of cortical neural progenitor cells [34]. Cortical neural progenitors were dissociated from E15.5 mice cortices. All three TAM receptors and Protein S were selectively enriched in neural progenitor cells. Axl silencing using a dominant negative Axl or soluble Axl receptor induced greater cortical cell translocation from the ventricular zone into the intermediate zone and cortical plate, which indicates differentiation of the affected neural progenitor cells [34]. The Axl/Mer double-knockout mutant brains exhibited an early differentiation of neural progenitor cells due to deletion of the Axl and Mer genes [34].

TAM receptors play pivotal roles in adult hippocampal neurogenesis. Mice lacking TAM receptors exhibit impaired adult hippocampal neurogenesis. TAM receptors negatively affect microglia and peripheral antigen-presenting cells, inhibit MAPK and NF- κ B activation and prevent the overproduction of pro-inflammatory cytokines. Therefore, the loss of these receptors

disinhibits these processes and comprises neurogenesis in the dentate gyrus of the adult hippocampus, which leads to NSC proliferation, differentiation, and survival [32,33]. IL-6 is a major downstream neurotoxic mediator that is under the homeostatic regulation of TAM receptors in microglia [32]. Gas6 activates TAM receptors in adult neurogenesis, and knockout of Gas6 reduces the number of NSCs in the subventricular zone of the lateral ventricles. .

NGF triggers PC12 differentiation via the TrkA receptor [35,36]. TAM receptors may play a similar role in PC12 cell neuronal differentiation, instead of NGF [15]. Tyro3 and Axl are expressed on the surface of PC12 cells and colocalize with TrkA receptors [15]. NGF induces Tyro3 and Axl expression, which interact functionally on the cell surface with TrkA to signal neuronal differentiation. Gas6 may replace NGF to support PC12 growth and differentiation via activation of PI3K and MAPK, which induces neuronal differentiation and neurite outgrowth [15].

Gas6 and Axl proteins are also involved in glioblastoma growth and migration [37]. Gas6 and Axl are frequently overexpressed in human gliomas and predict a poor prognosis in patients with glioblastoma multiforme [30]. Immunohistochemical studies demonstrated that Axl primarily localized in glioma cells and reactive astrocytes, and coexpression of Axl and Gas6 was observed in glioma cells and tumour vessels [30].

■ TAM receptors in GnRH neuronal development

Gonadotropin-releasing hormone (GnRH) neurons are brain cells that control reproduction via secretion of GnRH from the pituitary into the hypophyseal portal capillary bloodstream. GnRH neurons migrate into the brain along olfactory axon fibres from the nose during development [38,39]. All three TAM receptors promote GnRH neuronal cell migration and survival and affect normal female reproductive function [40-42]. Patients with idiopathic hypogonadotropic hypogonadism exhibit functional consequences of Axl sequence variants, which indicates the importance of Axl in reproductive development [42]. The loss of GnRH neurons in Axl/Tyro3 null mice impairs the sex hormone-induced gonadotropin surge and results in estrous cycle abnormalities [40]. Axl/Tyro3 null mice also exhibit reproductive malfunctions [40-42]. These reproductive abnormalities in Axl/

Tyro3 null mice are due to GnRH neuron malfunction in hypothalamic, but not pituitary or ovarian, defects [40-42]. The anti-apoptotic effect of Tyro3 or Axl plays a role in mediating the survival and appropriate targeting of GnRH neurons to the ventral forebrain, which contributes to normal reproductive function and cyclicity in the female [40]. Gas6 null adults and embryos exhibit a diminished GnRH neuronal population, which delays vaginal opening and sexual maturation in mice [42].

Axl activates several cellular signalling pathways in GnRH neuronal development. Axl activation of the PI3K-Ras pathway and P38 MAPK is involved in GnRH neuronal cell migration [16,43] and hepatocyte growth factor/scatter factor-activated MET signalling is required for GnRH neuronal survival [44]. ERK and PI3K/Akt pathways mediate Gas6/Axl signalling, which protects GnRH neurons from programmed cell death across neuronal migration [17]. Axl suppresses GnRH gene expression via the coordinated activation of a Rac 3 ERK signalling pathway and a distinct MEF2-dependent mechanism [17]. In a summary, different cellular pathways mediate the various functions of Axl in GnRH development.

■ TAM in demyelination and remyelination

Demyelinating diseases include any disease of the nervous system in which the myelin sheath of neurons is damaged [45]. This damage impairs signal conduction in the affected nerves. This reduction in conduction ability produces deficiencies in sensation, movement, cognition, or other functions, depending on the nerves involved. Toxic, chemical or autoimmune substances and degeneration may damage the myelin sheath of neurons [46]. Oligodendrocytes are the myelin-producing cell of the CNS, and Schwann cells supply the myelin for the peripheral nervous system. The loss of oligodendrocytes or Schwann cells causes demyelination. A reduced efficiency in the phagocytosis of apoptotic cells and myelin debris inhibits remyelination. Immune hyperactivation, including microglia activation and proliferation and some infiltration of peripheral macrophages, were demonstrated in these diseases [12].

Gas6-dependent TAM receptor signalling is an important modulator of oligodendrocyte survival and microglial activation in vitro and in vivo [47]. The significant upregulation of Gas6 concentrations in the cerebrospinal

fluid of patients with chronic inflammatory demyelinating polyneuropathy provides a strong basis for the investigation of a potential link between TAM receptor signalling and chronic demyelinating diseases [12]. Human oligodendrocytes growing on a monolayer of NIH 3T3 cells stably expressing Gas6 exhibited more primary processes and arborizations than cells plated on NIH 3T3 cells lacking Gas6 expression. Recombinant human Gas6 (rhGas6) administration to oligodendrocytes reduced oligodendrocyte apoptosis, which was mediated by the PI3K pathway, but not MEK/ERK [20]. Gas6 knockout affected myelination and microglial activation in cuprizone-induced demyelination in mice. Gas6 knockout mice exhibit fewer oligodendrocytes in the rostral and caudal corpus callosum than wild-type mice. This loss of oligodendrocytes was accompanied by a reduction in overall myelination. Gas6 knockout mice exhibit a more profound increase in microglial activation than wild-type mice [47]. Administration of rhGas6 protein into the CNS resulted in more efficient repair of demyelination following cuprizone-induced injury and improved recovery following cuprizone withdrawal [48]. A similar protect effect of Gas6 delivery to the CNS was observed in a mouse experimental autoimmune encephalomyelitis model [49].

The above evidence demonstrates a beneficial role of Gas on myelination in peripheral and central nervous systems. Gas6 is a growth factor for human Schwann cells, and it also exhibits anti-apoptotic effects on these cells [12]. The expression and role of TAM receptors was also identified in human and rodent oligodendrocytes in vitro and in vivo. The three TAM receptors are upregulated in white matter areas before and during myelination in development [12]. However, Tyro3 is dominant in the transduction of the pro-myelinating effect of Gas6 in oligodendrocytes. The loss of Tyro3 on oligodendrocytes abolished the pro-myelinating effect of Gas6, delayed developmental myelination and resulted in thinner myelin production [50]. Decreased Tyro3 expression and oligodendrocyte loss were observed in the corpus callosum in the cuprizone-induced demyelination model, and Axl, Mer, Gas6 mRNA expression increased [47]. Axl exhibits a less profound effect on demyelination because Axl knockout mice exhibit no significant loss of oligodendrocytes or microglia activation after 6 weeks of a cuprizone challenge [12]. However,

the absence of Axl delayed phagocytosis and the removal of myelin debris, which inhibited remyelination and prolonged axonal damage and recovery from cuprizone toxicity [13]. Axl also played a protective role in experimental autoimmune encephalomyelitis, which is a demyelinating mouse model. Significant induction of Gas6, Axl, and Mer, but not Pros1 or Tyro3, mRNA was observed in mice with experimental autoimmune encephalomyelitis [49]. Loss of the receptor tyrosine kinase Axl in Axl^{-/-} mice enhanced inflammation in the central nervous system and delayed the removal of myelin debris during experimental autoimmune encephalomyelitis [51]. Gas6/Axl or Mer signalling also plays a role in oligodendrocyte survival during a period of active myelination in human foetal spinal cord development. High Axl and Mer mRNA expression in oligodendrocytes was observed from the second trimester in human foetal spinal cord. Axl inhibition using Axl-Fc abolished the effect of Gas6 on oligodendrocyte survival [20]. Axl also plays a protective role in fibroblast survival. Axl^{-/-} mouse fibroblasts exhibited higher levels of serum deprivation-induced apoptosis, and Gas6 did not rescue serum deprivation-induced apoptosis in these cells [13]. Only one study investigated the regulation of Axl and Mer genes. Transcriptional factor Nrf regulated Axl and Mer in microglial phagocytosis and inflammatory gene expression. Nrf2^{-/-} microglia significantly decreased mRNA levels of Axl and Mer, but not Tyro3 [52].

■ TAM signalling in degeneration following central or peripheral injury

Neurodegeneration is the progressive loss of neuronal structure or function, including neuronal death. Many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease (HD), are the result of neurodegenerative processes. Central or peripheral injury also causes neurodegeneration. Nerve injury often triggers Wallerian degeneration in the peripheral nervous system. TAM knockout mice provided data on the relationship of TAM receptors and neurodegeneration. Triple TAM knockout mice exhibit cellular degeneration in the neocortex, hippocampus, cerebellum, and rods and cones in the retina [3,12,53].

Tyro3 in the hippocampus is involved in amyloidogenic APP processing and beta-amyloid deposition in AD models [54]. Overexpression

of the Tyro3 receptor in cell models significantly decreased A β generation and down-regulated the expression of β -site amyloid precursor protein cleaving enzyme. In contrast, Tyro3 knockdown in an AD transgenic mouse model significantly increased the number of amyloid plaques in the hippocampus [54].

Anorexia nervosa (ANR) is an eating disorder that often accompanies neurodegenerative disorders, including ALS, AD and HD [55]. Tyro3 in the hypothalamus is related to ANR, and it improves weight and survival in an anorexia mouse model. Tyro3 is one of the few factors that sustains appetite regulatory circuitry. The Tyro3 gene exhibits prosurvival roles by enhancing lifespan and neuropeptide Y (Npy) neuron survival in the mouse anorexia (anx) mutation model [55].

The pathogenesis of PD is largely unknown. However, α -synuclein (α -SYN) is closely associated with neurodegeneration. α -SYN accumulates in Lewy bodies and dystrophic neuritis in idiopathic PD, which may underlie the pathology of neurodegeneration and progression of clinical symptoms. The transcriptional factor Nrf2 cooperates with α -SYN on protein aggregation, neuroinflammation and neuronal death in early stage PD. Nrf regulates Axl and Mer in microglial phagocytosis and inflammatory gene expression, and Axl and Mer may play a role in PD development [52].

TAM receptors, especially Mer, are involved in phagocytosis [56-58]. Phagocytosis is the process by which a cell engulfs a solid particle to form an internal compartment, known as a phagosome. Phagocytosis is the primary mechanism used to remove pathogens and cell debris. Mer deficiency inhibits phagocytosis in focal brain ischaemia and retinal pigment epithelial (RPE) cells [56,59]. Mer receptors were upregulated in activated microglia 3 days after injection of the vasoconstrictor endothelin-1 in a focal brain ischaemia rat model. Knockdown of Mer using Mer mutant mice inhibited microglial engulfment of neurons after ischaemia and produced a pronounced reduction of neuronal loss that strongly reduced brain atrophy and improved motor function in MerTK mutant rats [59]. However, the phagocytosis of the photoreceptor outer segment is critical to the normal functioning of the retina. RPE cells that lack Mer exhibit a severely compromised ability to phagocytose the distal ends of photoreceptor outer segments, which leads to the complete postnatal degeneration of photoreceptors and

blindness. TAM triple knockouts or Mer single knockout mice exhibited retinal degeneration and the loss of phagocytosis of photoreceptor outer segments [12,53]. Pre-incubation with antibodies against Mer blocked the phagocytosis of photoreceptor outer segments [56]. Protein S and Gas6 stimulate the phagocytosis of photoreceptor outer segment in cultured rat retinal pigment epithelial cells [57]. These studies indicate that Mer exhibits different effects on phagocytosis depending on the pathological condition.

In vitro and in vivo experiments demonstrated the role of Protein S/Tyro3 in N-methyl-D-aspartate (NMDA) receptor-induced excitotoxic neuronal injury [18,21]. Recombinant mouse protein S dose-dependently protected mouse cortical neurons from excitotoxic NMDA-mediated neuritic bead formation and apoptosis in vitro. Tyro3, but not Axl or Mer, was required for protein S-mediated protection. PI3K-Akt mediated the protective effect of Protein S/Tyro3 from excitotoxic injury [18,21]. Consistently, protein S dose-dependently reduced lesion volume in the striatum in an in vivo model of NMDA-induced excitotoxic lesions in control mice and protected Axl^{-/-} and Mer^{-/-} transgenic mice, but not Tyro3^{-/-} transgenic mice [21]. These data support that Tyro3, but not Axl or Mer, is required for protein S-mediated protection.

Gas6 and Tyro3 mRNA is expressed in larger dorsal root ganglia neurons at high levels [19,28]. Gas6 and Axl mRNA are also highly expressed in the intact sciatic nerve [28]. However, the mRNA level of Tyro3 in intact sciatic nerve is very low. Gas6 expression decreased six hours after sciatic nerve transection in the proximal and distal segments of the axotomized nerve, but Tyro3 level did not change [19,28]. Gas6 protein levels in the lesioned nerve progressively increased and attained control levels within 2 weeks after lesion generation [28]. Nerve injury increased Axl/Tyro3 in DRG Schwann cells, but no change was observed in DRG neurons [19]. This evidence suggests that Gas6/Axl function as trophic factors for Schwann cells and neurons, and these proteins are important in the maintenance of intact peripheral nerves. Optic nerve transection also increased Axl and Protein S in rat retinal ganglion cells [60].

■ TAM in immune regulation

TAM triple knockout (TKO) mice suggest that TAM receptors play a central role in immune

regulation. TKO mice are viable and fertile and develop apparently normally for the first few postnatal weeks. However, hyper-proliferation of B and T cells appears in most tissues from approximately 4 weeks of age in TKO mice. The B and T cells of the TKO mice are constitutively active, and these mice develop symptoms that are similar to a wide range of autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosis [12].

TKO mice exhibit increased proinflammatory cytokine production, autoantibody deposition and autoreactive lymphocyte infiltration in a variety of tissues. This systemic autoimmunity in TKO mice causes inflammatory brain damage and cell death. TKO mice produce high levels of serum TNF- α , and specific autoantibodies are deposited on brain blood vessels. The brain-blood barrier is impaired in TKO mouse brains, which facilitates autoreactive T cells infiltration into all regions of the brain. A brain autoimmune disorder develops from the accumulation of the ubiquitin-reactive aggregates in the hippocampus, and early formation of autofluorescent lipofuscins is observed in neurons throughout the brain. Chronic neuroinflammation damages hippocampal mossy fibres and induces neuronal apoptotic death [61]. The Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway mediates the transduction of the anti-inflammatory effects of TAM receptors [12].

■ TAM receptors in hippocampal synaptic plasticity

The hippocampus plays an important role in learning and memory. Hippocampal long-term potentiation (LTP) underlies synaptic plasticity, which controls learning and memory [62,63]. Gas6 and Tyro3 are expressed in the adult rat hippocampus [26,29], which suggests a functional role for these proteins in hippocampus. Recombinant rat Gas6 exhibited an anti-apoptotic effect in cultured hippocampal neuronal cells during serum starvation [28]. Gas6 induced Tyro3 phosphorylation in cortical neurons in vitro and the recruitment of the MAPK and the PI3K signalling pathways. These pathways play critical roles in the induction of hippocampal LTP [25]. Tyro3 colocalizes with postsynaptic scaffolding proteins postsynaptic density protein-95 (PSD-95). PSD-95 is associated with the stabilization of synaptic enlargement, and it is an excellent marker for post-synaptic mechanisms [25]. These studies

suggest that Gas6/Tyro3 signalling influences hippocampal synaptic plasticity. However, further evidence is required.

■ Axl in Zika virus

Axl plays a role in Zika virus infection [64,65]. Zika virus is a mosquito-borne flavivirus that was first identified in monkeys in Uganda in 1947 [66]. The WHO has concluded in 2016 that Zika virus infection during pregnancy was a cause of congenital brain abnormalities, including microcephaly, which is a congenital malformation that results in smaller-than-normal head size for age and sex [67]. Axl is a glia-enriched putative viral entry receptor for Zika virus. Blockade of Axl expression or genetic knockdown of Axl reduced virus infection of astrocytes in vitro [65]. Axl facilitated the entry of Zika virus to human foetal neural progenitor cells (hNPCs) during the first trimester of pregnancy, which altered signalling and immune pathways in host cells [64].

Conclusion

In this review, we specifically summarized the localization of the TAM family of RTKs (Tyro3, Axl and Mer) in nervous system development and their function/role in neural development and nervous system diseases. TAM receptors share a similar structure that is characterized by a conserved sequence, but these receptors are differentially expressed in the nervous system and

dominate different nervous function. Research to develop possible drugs to target this signalling pathway and treat cancers is ongoing [6]. Small molecules that inhibit TAM RTKs and biological TAM inhibitors were developed. The small molecules specifically target one, two or three of the TAM receptors. Most of these small molecules and monoclonal antibodies exhibited antitumour activity and decreased tumour cell migration, invasion and growth. BGB324 is a specific Axl inhibitor [9] that is in Phase Ib trials in patients with AML [10] and non-small cell lung cancer (NSCLC) [6]. The data suggest that TAM is a potential target in cancer therapy. However, the function of the TAM family in the nervous system is largely unexplored. For example, the TAM response to peripheral nerve injury was demonstrated, but whether TAM receptors play a role in neuropathic pain is not known. TAM receptors clearly play a role in myelination, the response to peripheral nerve and central injuries and they contribute to immune regulation and degenerative diseases, however, whether these receptors may be targeted as an adjuvant to provide these patients with new options for durable responses is not known.

Acknowledgements

This work was supported by the Key Projects of National Health and Family Planning Commission of Tianjin, China(16KG157).

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