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TAM receptor tyrosine kinases: Expression, disease and oncogenesis in the central nervous system

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Abstract

Receptor tyrosine kinases (RTKs) are cell surface proteins that tightly regulate a variety of downstream intra-cellular processes; ligand-receptor interactions result in cascades of signaling events leading to growth, proliferation, differentiation and migration. There are 58 described RTKs, which are further categorized into 20 different RTK families. When dysregulated or overexpressed, these RTKs are implicated in disordered growth, development, and oncogenesis. The TAM family of RTKs, consisting of Tyro3, Axl, and MerTK, is prominently expressed during the development and function of the central nervous system (CNS). Aberrant expression and dysregulated activation of TAM family members has been demonstrated in a variety of CNS-related disorders and diseases, including the most common but least treatable brain cancer in children and adults: glioblastoma multiforme.

Keywords

Cell signaling; Alzheimer's disease; Glioblastoma; Multiple sclerosis; Developmental neurology

1. Isolation and nomenclature of Tyro3, Axl, and MerTK

The prototypic TAM family member Axl was originally described as an unknown transforming gene present in the DNA of two patients with chronic myelogenous leukemia (Liu et al., 1988; O'Bryan et al., 1991). The authors subsequently cloned and characterized a receptor tyrosine kinase and named it Axl, from the Greek work anexelekto, or "uncontrolled" (O'Bryan et al., 1991). The DNA sequence predicted a protein of 894 amino acids and revealed for the first time the hallmark features of all TAM family members: an extracellular domain consisting of two fibronectin type III (FNIII) repeats and two immunoglobulin-like (Ig-like) repeats. These features immediately distinguished Axl from previously described protein tyrosine kinases. O'Bryan et al. prepared polyclonal anti-Axl antiserum and were able to detect 140- and 120-kDa proteins in Axl-transformed mammalian (3T3) cell lines, corresponding to fully and partially processed/glycosylated forms of the protein (O'Bryan et al., 1991).

Meanwhile, another group was exploring receptor tyrosine kinase genes in developing nervous system of the rat (Lai and Lemke, 1991). In 1991, Lai et al. published a report identifying partial sequences from 13 novel protein tyrosine kinase genes, three of which more closely resembled each other than any previously described kinase. The proteins encoded by the genes, originally designated Tyro3, Tyro-7, and Tyro-12, later came to be known as Tyro3, Axl and MerTK respectively, and comprise the TAM family of RTKs (Lai and Lemke, 1991). Interestingly, all were detected in the rat embryonic brain and, albeit with more restricted distribution, in the adult rat brain.

The same year, two additional groups independently isolated Axl. Rescigno et al. cloned a murine protein tyrosine kinase based on homology to the Bek FGF receptor and recognized two Ig-like domains and two FNIII domains; their find was dubbed Ark (adhesion-related kinase) (Rescigno and Mansukhani, 1991). Janssen et al. (1991) detected transforming activity derived from a patient with chronic myeloproliferative disorder and isolated the corresponding oncogene and named it UFO.

Soon after the description of Axl, Lai et al. published the isolation of Tyro3 in the mouse, and predicted, based on sequence analysis, the two Ig-like domains and two FNIII repeats in the extracellular region that later became recognized as hallmarks of this family (Lai et al., 1994). Tyro3 was independently described by a variety of labs and has been identified as Brt, recovered from fetal mouse brain (Fujimoto and Yamamoto, 1994); Sky, cloned from a human hepatoma cDNA library (Ohashi et al., 1994); Rse, from human and mouse brain (Mark et al., 1994); Etk2, from mice (Biesecker et al., 1993); and Dtk, from mice (Lewis et al., 1996).

Graham et al. isolated the last novel TAM family member in 1994 from a screen of a human B-lymphoblastoid expression library with anti-phosphotyrosine antibodies and designated it c-Mer, for its expression in macrophages and tissues of epithelial and reproductive origin (in the original report mononuclear cells were isolated from human peripheral blood based on their ability to adhere to plastic and found to express c-Mer; these cells are now defined to be macrophages) (Graham et al., 1994, 1995). c-Mer contained the now-characteristic two Ig and two FNIII coding regions in the extracellular portion of the protein. Sequence analysis suggested that c-Mer was the putative proto-oncogene corresponding to an oncogenic avian retrovirus (*v-eyk/v-ryk*) published in 1992 (Graham et al., 1995; Jia et al., 1992; Jia and Hanafusa, 1994) and associated with UFO/Axl/Ark by virtue of its structure (Jia and Hanafusa, 1994). Initial characterization demonstrated that c-Mer protein was found in prostate, testis, ovary, placenta, lung, liver, and kidney, but not in normal human brain (Graham et al., 1994). MerTK, as it came to be known (Weier et al., 1999), was also independently isolated from a cDNA clone derived from a human glioblastoma expression library and labeled Nyk (Ling and Kung, 1995b).

2. TAM protein structure, ligands, and tissue expression

2.1. Structure

The TAM family of RTKs has been amply reviewed (Binder and Kilpatrick, 2009; Lemke and Rothlin, 2008; Linger et al., 2008; Verma et al., 2011). As typical receptor tyrosine

kinases, Tyro3, Axl, and MerTK contain an extracellular domain, a transmembrane domain, and a conserved intracellular kinase domain. Three features distinguish TAMs from other RTKs: a conserved KW(I/L)A(I/L)ES sequence (in the kinase domain); two Ig domains (near the N-terminal of the extracellular domain); and two FNIII domains (between the N-terminal of the extra-cellular domain and the trans-membrane region). The TAM extracellular domain is recognizable by antibodies on non-permeabilized cells in assays such as flow cytometry, indicative of cell surface expression; accordingly, Ig and FNIII domains are thought to be important in cell-to-cell contacts and adhesion to extracellular matrix. Both Ig domains are required for Gas6 binding to Axl in vitro and may facilitate other TAM receptor/ligand interactions (Sasaki et al., 2006). Recent studies have revealed that full-length TAMs contain putative nuclear localization and export signals and MerTK can be also found within the nucleus (Migdall-Wilson et al., 2012).

The observed molecular weights of Axl, Tyro3 and MerTK vary with tissue and cell type and may be considerably larger than their predicted weights due to posttranslational modifications including glycosylation, phosphorylation, and ubiquitination. For example, Axl and Tyro3 both range from 108 to 140 kDa, while MerTK ranges from 165 to 205 kDa; multiple forms are frequently found in the same cell types (Linger et al., 2008). At least one study has shown that Axl glycosylation does not affect the affinity of Gas6 binding (Sasaki et al., 2006). After extended periods of ligand stimulation, a less-glycosylated, nuclear soluble form of MerTK was found to be the predominant form, while surface expression decreased by roughly 25% (Migdall-Wilson et al., 2012).

Soluble forms of Axl and MerTK derived from full length transcripts circulate in human and mouse plasma; the transmembrane and intracellular domains may be deleted by alternative splicing or by proteolytic cleavage of the full length protein (O'Bryan et al., 1991, 1995; Sather et al., 2007). This process may regulate tyrosine kinase activation and/or sequester ligand.

2.2. Ligands

Axl, Tyro3, and MerTK were considered orphan receptors until 1995, when two laboratories independently purified Growth-arrest-specific gene 6 (Gas6) by screening for factors that could bind and activate Axl (Stitt et al., 1995; Varnum et al., 1995). Gas6, originally identified based on its dramatic upregulation after growth arrest, had no known function at that time (Manfioletti et al., 1993; Schneider et al., 1988). Isolation of a Tyro3 phosphorylating activity resulted in the purification of Protein S, a protein known to play a critical role in anticoagulation and complement cascades by directly or indirectly regulating the activity of various clotting factors (Stitt et al., 1995). Shortly thereafter, Gas6 was found to bind to MerTK (Nagata et al., 1996).

Gas6 and Protein S are structurally related vitamin K-dependent proteins. Human, mouse, and bovine Gas6 are highly homologous to each other (ranging from 74 to 81% homology) and moderately homologous to human and bovine Protein S (ranging from 42 to 47% homology); the similarity is particularly strong in the γ -carboxyglutamic acid (Gla) domain and in regions that signal for secretion (Manfioletti et al., 1993; Stitt et al., 1995). The regions of Protein S that are most divergent from Gas6 are those that are responsible for

interactions with clotting and complement factors. Full receptor binding and activation ability is contingent upon a posttranslational modification mediated by γ -glutamyl carboxylase in a reaction that requires vitamin K as a cofactor. The addition of carboxy groups to glutamic acid residues confers an affinity for negatively charged membrane phospholipids to the Gla domain, a feature that proves useful in TAM's role in phagocytosis and clearance of apoptotic cells [reviewed in (Hafizi and Dahlback, 2006)]. Eight different variants of the Gas6 gene have been identified; one has been associated with stroke patients although the mechanism is not known (Munoz et al., 2004).

Three other ligands were recently isolated from mice; their physiological significance has yet to be determined and may be limited to phagocytosis. Tubby and Tubby-like protein (Tulp1) were identified with a novel functional assay based on cell binding and phagocytosis (Caberoy et al., 2009). Tulp1 interacted with Axl, Tyro3 and MerTK in co-immunoprecipitation experiments, while Tubby interacted with MerTK only (Caberoy and Zhou, 2010). A similar assay based on phagocytosis and MerTK binding ability resulted in the isolation of Galectin-3 (Gal-3), a previously known protein with suspected links to phagocytosis. Co-immunoprecipitation experiments confirmed that Gal-3 associates with MerTK, but the affinity of Gal-3 for either Axl or Tyro3 has not been reported (Caberoy et al., 2012).

2.3. Ligand binding and TAM activation

Interactions between TAM RTKs and ligands have been investigated by various labs and it is now generally accepted that Gas6 activates Axl, Tyro3, and MerTK, and that Protein S activates MerTK and Tyro3 (Burstyn-Cohen et al., 2012; Prasad et al., 2006). Gas6 binds directly to the extracellular domain of a TAM family member and stimulates phosphorylation of the tyrosine domain. The affinity of Gas6 for Axl is significantly stronger than its affinity for either Tyro3 or MerTK: the calculated dissociation constant K_d for interactions between rat Gas6 and receptor-Fc fusion proteins of Axl, Tyro3, and MerTK were 0.4 nM, 2.7 nM, and 29.0 nM respectively (Nagata et al., 1996). Similar results were obtained by other labs (Chen et al., 1997), suggesting that Axl may be the most relevant of the three receptors for Gas6 in many tissues. Tyro3 has somewhat lower affinity, but may be the relevant receptor in the brain since it is so highly expressed there. MerTK has the weakest affinity and the highest dissociation rate for Gas6, but may nevertheless be a relevant receptor in certain tissues and conditions (Chen et al., 1997; Nagata et al., 1996). Protein S is less well studied, perhaps because its contribution to physiological TAM binding was questioned for some time.

It is now established, however, that Protein S can stimulate MerTK and Tyro3 activation as it was able to substitute for Gas6 function in the retina of mice lacking Gas6 (Prasad et al., 2006). The concentration of Protein S in human plasma is quite high, suggesting that it is ubiquitously available, while the plasma concentration of Gas6 is much lower and its availability is more likely to be regulated locally [reviewed in (Hafizi and Dahlback, 2006)].

Human Gas6 and Axl associate in a 2:2 stoichiometry, with no direct contacts between the two Axl molecules or the two Gas6 molecules. Gas6 and Axl first form a receptor/ligand complex; then two complexes dimerize (Sasaki et al., 2006). Dimerization activates RTKs

by causing the autophosphorylation of tyrosine residues in the cytoplasmic domain, leading to the recruitment of downstream signaling proteins [reviewed in (Schlessinger, 2000)]. Signaling pathways do not operate in isolation, however: they are part of a complex network regulated by cell types, extracellular events, and microenvironments (Linger et al., 2008; Schlessinger, 2000). Sasaki et al. (2006) noted that overexpression of Axl in cell lines resulted in constitutive receptor activation in the absence of Gas6, and Tyro3 has been shown to dimerize and autophosphorylate independent of ligand, a property that might be important in cell adhesion (Heiring et al., 2004; Taylor et al., 1995a). Disruption of signaling through one TAM receptor by knockdown or knockout can affect the expression and therefore the signaling of another TAM receptor (Prasad et al., 2006). TAMs may crosstalk with other RTKs – for example, Axl is required for VEGF activation of PI3K/Akt activation in endothelial cells (Ruan and Kazlauskas, 2012) and Axl (but not Tyro3 or MerTK) directly interacts with MET in an Axl-positive GnRH neuronal cell line (Salian-Mehta et al., 2013). MerTK has been shown to crosstalk with $\alpha v \beta 5$ integrin in the phagocytosis of apoptotic cells (Wu et al., 2005). Furthermore, TAM receptors potentially heterodimerize: Axl and Tyro3 have been shown to interact and can modulate Gas6-initiated signaling (Brown et al., 2012; Pierce et al., 2008). Finally, while Gas6 is recognized as the primary ligand for TAM receptors, Protein S can and does act as a legitimate ligand and can maintain TAM function in a Gas6 knockout mouse model of retinal pigment epithelium (Prasad et al., 2006), protecting Axl- and MerTK-deficient (but not Tyro3-deficient) neurons and mice from apoptosis (Zhong et al., 2010).

2.4. Expression in tissues

Tissues high in Tyro3 expression include the nervous system, ovaries, testis, breast, lung, kidney, osteoclasts, retina, monocytes/macrophages, and platelets [reviewed in (Linger et al., 2008)]. Tyro3 is highly expressed in the adult rat CNS (Prieto et al., 2000). Axl is expressed in a wide variety of tissues and organs including hippocampus, cerebellum, heart, skeletal muscle, liver, kidney, testis, brain, monocytes, macrophages, platelets, endothelial cells and dendritic cells; cDNA has been recovered from cell lines of epithelial, mesenchymal, and some hematopoietic origins [reviewed in (Linger et al., 2008)]. It is expressed in many normal, nontransformed cell lines, and is often overexpressed in lines of malignant origin. Axl is considered the key TAM receptor in human and mouse vascular cells but is only minimally expressed in certain cells of the brain (Fig. 1). MerTK expression is found in the ovary, prostate, testis, lung, retina, and kidney, and macrophages, dendritic cells, NK cells, NKT cells, megakaryocytes and platelets; low levels are found in the heart and skeletal cells [reviewed in (Linger et al., 2008)]. The normal brain expresses very low to undetectable levels of MerTK protein (Fig. 1).

Surprisingly, mice deficient in the three known TAM family members are viable and survive for over a year, but display multiple severe organ defects and neurological abnormalities (Lu et al., 1999). Mice that lack any one or two TAM receptors exhibit more subtle defects in function and/or morphology. Affected organs include brain, prostate, liver, spleen, lymph nodes, blood vessels, and gonads. Adult triple knockouts are blind and adult males infertile. The immune system is profoundly affected, with lymphoproliferative disorders, impaired immune function, and autoimmunity (Li et al., 2013; Lu et al., 1999; Lu and Lemke, 2001).

Interestingly, the cell types most severely affected by loss of TAM receptors (spermatids, spermatocytes, B cells and T cells) do not express Tyro3, Axl, or MerTK, suggesting that targeted inhibition of TAM receptors may have unexpected consequences in a variety of tissues.

3. General functions

3.1. Survival, growth and migration

Axl utilizes two major signaling pathways as it promotes survival and growth. The MAPK/ERK1/2 pathway is critical to Axl-mediated proliferation in numerous cell types, but signals survival in migratory GnRH neurons (which do not have significant proliferative responses to mitogen) (Allen et al., 1999; Linger et al., 2008). Axl promotes cell survival by signaling through the PI3K/Akt and PI3K/Rac/Rho pathway in numerous cells including migratory GnRH neurons (Allen et al., 1999; Linger et al., 2008). Bellosta et al., 1997 showed that ectopic expression of Gas6 in cells that do not spontaneously express it protected those cells from serum deprivation-induced apoptosis in an Axl-dependent manner, but the cells did not become transformed or proliferate.

MerTK is strongly linked to survival: a MerTK/EGFR chimera dramatically reduced apoptosis induced by IL-3 withdrawal in murine 32D cells (Guttridge et al., 2002), and MerTK protected primary macrophages from oxidative stress-induced apoptosis (Anwar et al., 2009). In addition, MerTK and its ligand Tulp1 were linked to survival and liver fibrosis progression in a study of HCV-infected patients (Patin et al., 2012). MerTK is known to signal through the PI3K/Akt pathway and the MAPK/ERK1/2 pathway via Ras/Raf to promote survival (Anwar et al., 2009; Linger et al., 2008). MerTK is less strongly associated with proliferation: a chimera stimulated a proliferative response through PI3K and induced anchorage-independence growth of NIH3T3 cells (Ling and Kung, 1995b) but not in the murine 32D cells described above (Guttridge et al., 2002). MerTK can also signal migration and actin cytoskeleton rearrangement through the PLC, FAK and Rho (Linger et al., 2008; Rogers et al., 2011).

The role of Tyro3 in survival and growth is less well defined: experiments using a Tyro3/EGFR chimeric showed that the cytoplasmic domain of Tyro3 associated with and phosphorylated PI3K and led to transformation of NIH3T3 cells (Lan et al., 2000). Recent work describing the role of Tyro3 in survival of neurons will be described later.

3.2. Clearance of apoptotic cells, immunomodulation and autoimmune disease

MerTK appears to have a critical role in the recognition, phagocytosis, and clearance of apoptotic cells in a variety of tissues. A search for the retinal dystrophy locus of the RCS rat, a Royal College of Surgeons model of retinal degeneration, revealed that the rats express a nonfunctional MerTK protein in retinal pigment epithelial (RPE) cells (D'Cruz et al., 2000); mutated MerTK was also linked to retinitis pigmentosa in humans (Gal et al., 2000). MerTK is involved in phagocytosing the distal ends of photoreceptor outer segments and preventing photoreceptor degeneration in mice, rats, and humans (Carr et al., 2009; D'Cruz et al., 2000; Duncan et al., 2003). While mice lacking functional MerTK have postnatal degeneration of photoreceptors and blindness, mice lacking Gas6 are normal, leading to the conclusion that

Protein S and Gas6 (or other MerTK ligands) stimulate MerTK in RPE cells (Burstyn-Cohen et al., 2012; Prasad et al., 2006). Additional roles for MerTK in mediating phagocytosis and clearance of apoptotic cells have been described in MerTK^{-/-} macrophages (Scott et al., 2001) and TAM^{-/-} Sertoli cells (Chen et al., 2009; Xiong et al., 2008).

The inability of macrophages from MerTK knockdown mice to efficiently clear apoptotic cells has been associated with production of nuclear autoantibodies, suggesting that TAM proteins may protect against inflammation and auto-immune diseases such as systemic lupus erythematosus (Rothlin and Lemke, 2010; Scott et al., 2001). Interestingly, TAM receptors actively limit inflammatory responses in a cytokine-dependent negative feedback loop, in which the initial pro-inflammatory activation of the innate immune response induces Axl expression. Axl then induces the Suppressor of Cytokine Signaling (SOCS) proteins, which inhibit Toll-Like Receptors and cytokine-receptor signaling in dendritic cells and limit inflammation (Rothlin et al., 2007). Protein S expressed by mouse and human activated T cells in response to dendritic stimulation acts as a negative feedback mechanism dampening the stimulatory activity of dendritic cells, dependent on their expression of Axl and MerTK (Carrera Silva et al., 2013). In a mouse model, treatment with either Gas6 or Protein S reduced the symptoms of arthritis by limiting inflammatory signaling (van den Brand et al., 2013). Additionally, MerTK signaling in macrophages negatively regulates LPS-dependent TNF- α transcription by blocking production of NF- κ B and thus a broad proinflammatory cytokine response (TNF- α , IL-1, IL-6, and others) (Camenisch et al., 1999).

3.3. Mitogenesis, proliferation, and cancer

From its identification, Axl was recognized as having oncogenic potential. It was originally isolated as a transforming gene from two patients with chronic myelogenous leukemia (Janssen et al., 1991; Liu et al., 1988). NIH 3T3 cells transfected with expression vectors containing full length Axl formed colonies, suggesting that Axl was necessary but not sufficient for transformation (O'Bryan et al., 1991). Early experiments demonstrated that chimeric EGFR/Axl receptors could induce proliferation through Ras/Raf1/MEK-1/ERK1/2 (Fridell et al., 1996) and Gas6 stimulation of endogenous Axl was mitogenic in fibroblasts (Fridell et al., 1996; Goruppi et al., 1996). Clearly, TAM function is cell type dependent, because Bellosta et al. showed that ectopic expression of Axl did not cause proliferation or transformation in some cells (Bellosta et al., 1997). The ability to drive both proliferation and survival suggest a fundamental role for Axl in tumor progression. Indeed, inhibition of Axl signaling in ectopic and orthotopic glioma models impaired tumor cell proliferation as well as migration, invasion, and cell-to-cell interactions (Vajkoczy et al., 2006). It is important to note that many studies, particularly the early investigations, did not fully characterize TAM expression in their experimental systems. Considering the ability of TAM receptors to dimerize, autophosphorylate, and cross-talk, interpretation requires an understanding of all TAM members present.

Similarly, MerTK was derived from a human lymphoblastoid expression library; expression was found in neoplastic B- and T-cell lines but not in normal B- and T-lymphocytes (Graham et al., 1994). A chimeric Fms-Nyk (Fms-MerTK) receptor was shown to confer strong mitogenic and transforming potential to NIH 3T3 cells (Ling and Kung, 1995a).

Furthermore, transgenic mice with exogenous MerTK expression targeted to the hematopoietic lineage developed T-cell lymphoblastic leukemia/lymphoma at a much higher rate than WT mice (Keating et al., 2006) and MerTK expression was found in blood and bone marrow samples from human patients with T-cell acute lymphoblastic leukemia (Graham et al., 2006). However, a MerTK chimera consisting of the EGFR extracellular and transmembrane domains and the MerTK cytoplasmic domain did not stimulate proliferation; it blocked apoptosis instead (Guttridge et al., 2002).

Tyro3 was cloned from a human hepatoma library (Ohashi et al., 1994), and experiments showed that Rat-2 fibroblasts transfected with Tyro3 could grow in soft agar, suggesting that Tyro3 was oncogenic (Lai et al., 1994). In addition, Li et al. demonstrated that Gas6 treatment of human Schwann cell cultures caused phosphorylation of both Axl and Tyro3 and acted synergistically with heregulin and forskolin to stimulate Schwann cell proliferation, but the roles of Axl and Tyro3 were not separated (Li et al., 1996). A mutant Tyro3 that is overexpressed in the cytoplasm (and therefore presumably in a ligand-free environment) can dimerize and transform rat fibroblasts (Taylor et al., 1995a); Tyro3 transcripts have been associated with human and mouse mammary tumors and rat fibroblasts transduced with Tyro3 become transformed and can form tumors in nude mice (Taylor et al., 1995b).

Overexpression of one or more TAM receptors has been found in cancers of many tissues, including leukemias, lymphomas, multiple myeloma, glioblastoma, and cancers of the colon, lung, breast, liver, and kidney (Table 1). Ectopic expression or overexpression of TAM family members, rather than mutation, appears to be the predominant mode of action, affecting many factors involved in tumor development including cell morphology, cell-to-cell interactions, adherence, proliferation, motility and migration, tissue invasion, angiogenesis, and survival [reviewed in (Linger et al., 2008)]. Axl overexpression is associated with a poor clinical prognosis in NSCLC, acute myeloid leukemia, breast cancer, and pancreatic cancer. Glioblastoma, the most common and aggressive tumor of the brain, exhibits dramatic overexpression of Axl and MerTK (Table 1) (Linger et al., 2010). Prognosis is dismal and treatment options are limited for brain cancer patients, so clearly, TAM receptors are attractive potential therapeutic targets.

3.4. TAM expression in the CNS: Brain and neuronal Subtypes

The expression of TAM receptors in the CNS was reported in 1991, when Lai and Lemke conducted a search for receptor tyrosine kinases in the nervous system of the rat (Lai and Lemke, 1991). They identified 27 receptor tyrosine kinase genes that are transcribed in peripheral nerve and brain, including Tyro3, Axl (previously described as tyro-7) and MerTK (described as tyro-12). Several of the RTKs, including Tyro3, had relatively high transcript levels and were expressed preferentially in the developing nervous system. In situ hybridization experiments pinpointed Tyro3 mRNA to specific locations such as the CA1 field of the rat hippocampus, where Tyro3 expression is so pronounced that it can be considered a marker for the CA1/CA2 transition. Tyro3 is expressed strongly throughout the neocortex and in the granule cells of the cerebellum, while Axl and MerTK transcript expression in the brain is relatively low (Lai and Lemke, 1991).

A follow-up study of TAM family member expression and localization in the rat CNS showed that Tyro3 is highly expressed in the adult rat CNS at more sites and at higher levels than either Axl or MerTK (Table 2) and is thought to be the major Gas6 signal transducer in the CNS (Prieto et al., 2000). Tyro3-rich regions of the brain (by in situ hybridization) include the olfactory bulbs, cerebral cortex, piriform cortex (ventrolateral edge of the cerebral hemispheres), all cortical layers of the cerebellum, the amygdala, hippocampus and the cerebellum (Funakoshi et al., 2002; Prieto et al., 2000). Human and mouse brain endothelial cells both express Tyro3, Axl, and MerTK (Zhu et al., 2010). Oligodendrocytes exhibit high levels of all three receptors before and during myelination, but only Tyro3 persists in the adult (Prieto et al., 2000).

Axl and MerTK are expressed at low levels in selected cellular subsets of the adult rodent brain; neuronal expression is mostly restricted to the Purkinje cell layer of the cerebellum (Prieto et al., 2000), although earlier reports had found abundant Axl mRNA in the pyramidal layer of the hippocampus and the granule layer of the dentate gyrus of the mouse (Table 2) (Bellosta et al., 1995). Non-neuronal cells of the CNS, such as endothelial cells, glia, oligodendrocytes, Schwann cells, and astrocytes, express Axl and MerTK to variable degrees (Table 2) (Cahoy et al., 2008). An important site of Axl expression in the brain is the vasculature (Fig. 1) (Zhu et al., 2010). Axl expression in certain cells of the mouse brain changes markedly during development (discussed later).

The shared TAM ligand Gas6 is widely expressed in the rat CNS as determined by in situ hybridization and immunohistochemical methods, and appears to be present during late embryonic development as well as postnatally (Prieto et al., 1999). In the brain, Gas6 is expressed throughout several anatomical locations, including areas within the olfactory bulb, cerebellum, hippocampus, and the cerebral cortex, with high concentrations within the large pyramidal neurons. Additionally, Gas6 hybridization is strong within the choroid plexus, indicating that Gas6 may be made and secreted with the cerebrospinal fluid (Funakoshi et al., 2002; Prieto et al., 1999). Interestingly, Gas6 mRNA and Tyro3 mRNA tend to colocalize in the brain – particularly in the cerebral cortex, cerebellum, hippocampus, and hypothalamus (Funakoshi et al., 2002). A similar relationship was not seen with Protein S mRNA levels.

Protein S transcripts in the rat were found in multiple brain regions, but were much more plentiful in non-neural tissues such as the liver and uterus. High levels were found in cultured Schwann cells and astrocytes from the rat embryo, and expression of Protein S is upregulated in reactive Schwann cells and astrocytes, suggesting that Protein S may be involved in injury response (Stitt et al., 1995).

3.5. Hippocampal and cortical neurons

Some insight into the function of Tyro3 in the brain was gained when neuronal cells harvested from E17-18 rat hippocampal and cortical tissues were immunostained (Prieto et al., 2007). Tyro3 protein was detected over the surface of the cell body, dendrites, and axon. Staining in the dendritic compartment appeared punctate, suggesting that Tyro3 was localized to the synapses but did not appear to localize to either the postsynaptic compartment or at presynaptic terminals. The dendritic region is important for integrating

synaptic inputs, though, and Tyro3 may modulate signaling in neurons. Indeed, Prieto et al. found that Gas6 phosphorylates Tyro3 and activates MAPK and PI(3)K signaling pathways in cortical neurons (Fig. 2) (Prieto et al., 2007). While the downstream targets of these signaling pathways have not yet been identified, Tyro3 likely modulates strength of synaptic connection.

Tyro3 appears to promote survival in neurons. Tyro3 protein (but not Axl) is expressed at high levels in the adult rat hippocampus, and treatment of cultured primary hippocampal neurons with Gas6 (10 to 30 ng/ml) protected against cell death in a serum deprivation model (Funakoshi et al., 2002). In mouse cortical neurons, Protein S was shown to protect against excitotoxic injury by activating Tyro3 and signaling through PI3K and Akt (Fig. 2) (Zhong et al., 2010). Bad and Mdm2 were phosphorylated, Bcl-2 and Bcl-X levels increased, and p53 and Bax levels decreased. In a mouse model of NMDA-induced excitotoxic lesions, Protein S reduced lesion volume in control mice and protected mice lacking Axl and MerTK but not Tyro3 from injury (Zhong et al., 2010).

3.6. GnRH neurons

TAM expression can change throughout development; for example, Tyro3 levels are relatively low during embryogenesis and increase during early postnatal stages (possibly reflecting synaptogenesis), while Axl and MerTK expression remains steady and at much more modest levels in cell subsets (Prieto et al., 2000). Mouse GnRH neurons undergo a radical switch in behavior and protein expression during development. They migrate along olfactory nerves from their origin in the olfactory placode to the cribriform plate, where they turn and follow vomeronasal nerve fibers into the forebrain. An unsuccessful migration process results in defective sexual function, so cell survival and motility are critical. Axl was identified as a factor involved in GnRH neuronal migration in a screen of two cell lines representing consecutive stages in GnRH neuronal development (Allen et al., 1999). Gn10 cells were derived from a mouse that developed a GnRH neuronal tumor in the olfactory area, through which GnRH neurons migrate during their embryonic journey. Gn10 cells are migratory and express high levels of Axl and Tyro3. In contrast, GT1-7 cells were derived from a GnRH neuronal tumor that developed in the forebrain, are non-migratory and express MerTK and Tyro3 but very low or undetectable levels of Axl (Allen et al., 1999).

Initial studies in GnRH neurons focused on survival during growth factor withdrawal, because Axl was already known to protect fibroblasts from programmed cell death. Gn10 neurons are more resistant to growth factor withdrawal-induced apoptosis than GT1-7 cells, and while ERK protein in serumfed Gn10 cells were highly phosphorylated, ERK in GT107 cells was not, correlating to ERK's known role of ERK in antiapoptotic signaling (Fig. 2). Furthermore, Gas6 (400 ng/ml) alone stimulated ERK phosphorylation in Gn10 cells, and MAP/ERK Kinase (MEK) inhibitor PD98059 blocked ERK activation by Gas6 (Allen et al., 1999). NLT cells, like Gn10 cells, were derived from a mouse olfactory region of the brain and are migratory, express high levels of Axl and Tyro3 and low levels of MerTK, and show increased apoptosis in serum-deprived conditions when Axl and/or Tyro3 are silenced (Pierce et al., 2008).

Since the previous work had demonstrated that NLT cells but not GT1-7 cells migrated in response to Gas6, NLT cells with Axl and/or Tyro3 silenced were tested for migration and results showed conclusively that both Axl and Tyro3 contributed to Gas6-dependent migration (Fig. 2). Work with Axl^{-/-}Tyro3^{-/-} (double knockout, or DKO) mice demonstrated that adult mice lacking both Axl and Tyro3 had significantly fewer immunoreactive GnRH neurons overall, higher rates of apoptosis, and a shift in distribution of GnRH neurons suggestive of a defect in migration. While female DKO mice were fertile, estrus cycles were markedly irregular, indicative of a defect in sexual development and GnRH neuron function (Pierce et al., 2008). Moreover, DKO mice were found to have an impaired LH surge mechanism (Pierce et al., 2011).

4. TAM RTKs and disease in the CNS

4.1. Alzheimer's disease

Alzheimer's disease is characterized by accumulation of tangles comprised of hyperphosphorylated tau and plaques containing amyloid β protein (A β) [reviewed in (Ramachandran and Udgaonkar, 2013)]. Yagami et al. (2002) published a report describing the role of Gas6 in A β -induced apoptosis. Alzheimer's disease is characterized by accumulation in the brain of A β . A β (10 uM) has neurotoxic properties on neuronal cells linked with increased Ca²⁺ influx induction of apoptosis. Treatment of cells with Gas6 derived from conditioned media caused a significant dose-dependent reduction in Ca²⁺ uptake and a decrease in A β -induced apoptosis (Yagami et al., 2002). The authors concluded that since Tyro3 is highly expressed in the CNS, including the cerebral cortex, Gas6 protected cortical neurons from apoptosis via Tyro3. They did not, however, characterize TAM expression on their cells. Zheng et al. found that Tyro3 overexpression in HEK293 cells stably expressing a mutant amyloid precursor protein was linked to decreased A β accumulation and Tyro3 partial knockdown in vivo was associated with increased amyloid plaque formation, but they found that Gas6 inhibited rather than enhanced the effects (Zheng et al., 2012).

4.2. Multiple sclerosis

Oligodendrocytes are the myelin-producing cells of the CNS, providing support and insulation to the axons. Oligodendrocyte apoptosis with microglial infiltration is thought to be an early event in the pathology of multiple sclerosis, a complex demyelinating disease that involves interactions between many cell types (Binder and Kilpatrick, 2009). A cDNA micro-array analysis of human fetal oligodendrocytes performed to identify oligodendrocyte growth factors singled out TAM family members, leading to the hypothesis that Gas6 acts as an important survival factor (Shankar et al., 2003). Studies showed that rhGas6 at concentrations as low as 25 ng/ml reduced the number of apoptotic cells (compared with untreated cells) and that the effects were blocked by cotreating cells with Axl-Fc and Akt inhibitors LY294006 and wort-mannin (Fig. 2) (Shankar et al., 2003). Subsequent studies demonstrated that rhGas6 increases survival after insulin withdrawal or tumor necrosis factor α (TNF α) treatment as well (Shankar et al., 2006).

Because Gas6 is a survival factor for oligodendrocytes, Hoehn et al. predicted that in the absence of Axl, oligodendrocytes would not recover from cuprizone treatment. Cuprizone (0.2% w/w) fed to WT mice induces oligodendrocyte death, active phagocytosis, and demyelination in the corpus callosum in 3 weeks. Axl^{-/-} mice displayed significantly more apoptotic oligodendrocytes and damaged axons but fewer microglia and less phagocytosis when compared with WT and Tyro3^{-/-} mice after 4 weeks of cuprizone but reversed after 6 weeks, suggesting a delay in the clearance of apoptotic debris (Hoehn et al., 2008). Withdrawal of cuprizone restores the mature oligodendrocyte population within 3 weeks in WT mice but not in Axl^{-/-} mice. Axl^{-/-} mice did, however, show a nonsignificant trend towards increased progenitor oligodendrocytes, fewer mature oligodendrocytes, and less remyelination than in WT and Tyro3^{-/-} mice, hinting at a delay in recovery. In a separate study, Gas6^{-/-} mice showed a more severe phenotype than WT mice after cuprizone challenge: increased demyelination and reduced oligodendrocyte number with *increased* microglial number and activity, supporting a role for Gas6 in oligodendrocyte survival and consistent with the ability of Gas6 to modulate microglial activation. This study correlated microglial activity with increased demyelination. Cuprizone withdrawal was not studied (Binder et al., 2008). Administration of recombinant human Gas6 directly into the CNS of mice after cuprizone challenge and withdrawal resulted in increased oligodendrocyte maturation, debris clearance, remyelination, and axonal survival compared with PBS controls (Tsiperson et al., 2010).

Microglia, CNS immune cells of monocytic lineage, express Axl and MerTK protein; treatment with Gas6 stimulates phagocytosis (Fig. 2) while suppressing inflammatory responses (Grommes et al., 2008). Experimental Autoimmune Encephalomyelitis (EAE) is an auto-immune disease of the CNS that has been useful as a model of MS. Mice are immunized with myelin oligodendrocyte glycoprotein (MOG) peptide and then injected with pertussis toxin, resulting in an immune-mediated inflammatory disease of the CNS that features an influx of inflammatory cells, myelin loss, accumulation of myelin debris, and axonal damage. Clearance of apoptotic cells and debris by microglia is critical to the process of remyelination. Although WT and mice with a global Axl deletion had no difference in the number of microglia in the brain and spinal cord, lesions in Axl^{-/-} mice failed to recruit activated microglia and macrophages to the affected area and were characterized by an accumulation of myelin debris and inflammation (Weinger et al., 2011). The data are consistent with that from the cuprizone model of MS and suggest that Axl and Gas6 are involved in migration of microglia and macrophages to sites of tissue damage and the clearance of myelin debris.

MerTK may also play a role in the pathogenesis of MS. Polymorphisms in the MerTK gene were recently associated with MS susceptibility (Ma et al., 2011). High levels of the soluble forms of both MerTK and Axl, which are known to act as decoy receptors and sequester Gas6, are found in established MS lesions and correlate with low levels of Gas6 (Weinger et al., 2009). In this study, MerTK expression was elevated on astrocytes and Axl expression was elevated on astrocytes and oligodendrocytes in chronic active lesions.

Astrocytes (astroglial cells) comprise nearly half of human brain cells but remain uncharacterized. Isolation of astrocytes (and other glial cells) is technically challenging;

furthermore, cultured astroglia and in vivo astrocytes have fundamentally different gene expression profiles (Cahoy et al., 2008). Astrocytes hold roles in functional or metabolic support of neurons and oligodendrocytes, regulation of inflammation, and synapse formation and regulation; in culture, astroglia are phagocytic. Their role in MS has been argued; functions both exacerbating and attenuating the disease process have been attributed to astrocytes [reviewed in (Williams et al., 2007)]. A recent study using mice with inducible astrocyte ablation in a cuprizone assay found that astrocytes are required for the recruitment of microglia and subsequent clearance of myelin debris following oligodendrocyte apoptosis, a phenomenon dependent on the upregulation of CXCL10, a cytokine associated with microglial recruitment and activation (Skrupuletz et al., 2013). As discussed earlier, mouse astrocytes express low levels of Axl and MerTK and reactive astrocytes express significant amounts of Protein S. The requirement for Axl in microglial recruitment and apoptotic clearance/phagocytosis in the cuprizone and EAE experiments described above may implicate astrocyte involvement.

4.3. Brain tumors and TAMs

Gliomas are the most common primary CNS tumor; they have an extremely high morbidity and mortality. The origin is uncertain but is probably either a neural stem cell or glial progenitor cell, self-renewing cells in the adult brain capable of producing cells in astrocytic and oligodendrocytic cell lineages (Walker et al., 2011). Human brain tumors are histologically heterogeneous and include a variety of subtypes, including astrocytomas, oligodendrogliomas, ependymomas, gangliogliomas and gangliocytomas. The identification of a brain tumor stem cell (BTSC) that expressed cell surface marker CD133 and exhibited stem cell activity was reported in 2003 (Singh et al., 2003). This CD133+ cell lacked neural differentiation markers but was capable of differentiating in vitro in to cell phenotypes identical to the primary tumor, raising hopes for understanding the mechanisms of brain tumorigenesis and developing therapies.

After Axl overexpression was associated with a variety of non-CNS tumors, Vajkoczy et al. (2006) looked at Axl and Gas6 mRNA and protein expression on glioma cell lines and found a positive correlation. They used an Axl dominant negative construct to inhibit Axl signaling and found that migration and tumor growth were both suppressed (expression levels of other TAMs, particularly MerTK, were not determined). Examination of human glioma specimens WHO grades 2 to 4, including astrocytic, oligodendroglial, and ependymal origin, revealed that Axl and Gas6 are frequently overexpressed; in GBM WHO grade 4, 55% were positive for Axl protein (Hutterer et al., 2008). Keating et al. (2010) found increased transcript and protein levels of MerTK and Axl in astrocytoma patient samples; high Gas6 expression correlated with both high MerTK expression and high Axl expression. Of 19 patient samples, 18 expressed MerTK, 18 expressed Axl, and 17 expressed both Axl and MerTK. Normal human brain shows very limited MerTK and Axl protein expression. Inhibition of MerTK and Axl RTKs in vitro with silencing shRNA constructs resulted in increased apoptosis and autophagy, decreased non-adherent colony formation, and increased chemosensitivity to temozolomide, carboplatin, and vincristine (Keating et al., 2010). In vitro studies demonstrated that inhibition of MerTK but not Axl with constitutive or inducible shRNA or monoclonal antibody inhibition decreased migration; reintroduction of

MerTK expression rescued the defect (Rogers et al., 2011). Wang et al. (2012) found MerTK function critical for the migratory and locally invasive properties of GBM cells and confirmed that knockdown of MerTK sensitized cells to chemotherapy (etoposide). Furthermore, MerTK and Axl are highly expressed in GBM neurospheres, thought to more realistically reflect tumors and human disease than cell lines grown in serum-replete media.

While Keating et al. showed expressions, of Gas6 in astrocytoma patients that correlated with MerTK and Axl expressions, no evidence exists linking other TAM ligands to glioma. As discussed earlier, Tubby and Tulp1 appear to function mainly in the phagocytic activities of MerTK-positive cell. Protein S, now firmly established as a ligand of both MerTK and Tyro3, is ubiquitously available in human blood but its involvement in brain cancer is, at this time, not reported.

4.4. Blood brain barrier

Interestingly, Tyro3 has a role in maintaining the integrity of the blood brain integrity. Zhu, et al., found that Protein S binding to Tyro3 (but not Axl or MerTK) was required in human and mouse brain endothelial cells to protect against loss of barrier protection after O₂/glucose deprivation as determined by endothelial permeability (Zhu et al., 2010). In vivo, Tyro3^{+/+} mice in a middle cerebral artery occlusion model showed 100-fold more leakage of dextran from blood vessels than controls. Intravenous administration of Protein S rescued Tyro3^{+/+} mice but not mice lacking Tyro3. These findings have interesting implications for therapies targeting TAM-positive brain neoplasms.

5. Conclusion

TAM receptor tyrosine kinases are widely expressed in mammals, but expression profiles vary greatly with cell type and developmental stage. TAM members often hold different functions depending on cell type, and may utilize the same signaling pathways for different purposes. In the CNS of mice, rats and humans, multiple neural and non-neural cells express one or a combination of TAM members. Ligand may be expressed locally, activating receptors in an autocrine or paracrine fashion, or may circulate freely in the blood – or, in some instances, may even be superfluous. Physiological roles that TAMs play in the CNS generally reflect those seen in non-CNS tissues: foremost are protection against apoptosis (survival) and phagocytosis (clearance of apoptotic debris). Immunomodulation, proliferation, and migration in the CNS frequently involve the TAM family of receptors. Some novel roles have been described in the brain and nervous system, including synapse formation, protection from excitotoxic injury, and blood brain barrier integrity. With widespread or increased expression comes the potential for pathological involvement – aberrantly expressed TAMs are known or suspected to play a causative role in a variety of grave diseases and conditions. TAM levels correlate with prognosis and represent an attractive model for intervention in brain cancers, in particular, because of their association with survival, proliferation, and migration. However, it will be important to identify the variety of cells comprising human glioblastomas and characterize the “glioma-initiating cell” (or cells) before truly accurate models of human brain cancer can be created. Likewise, it will be critical to determine the effect of TAM inhibition on the blood brain barrier, but

the use of specific inhibitors against Axl and MerTK as therapeutics in the battle against brain cancer and other CNS disorders is an exciting and promising focus of investigation.

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Fig. 1.

TAM expression in the human brain. While Tyro3 is highly expressed in many regions of the adult human brain (Lemke and Rothlin, 2008; Mark et al., 1994), Axl and MerTK are present in fewer cell types and at lower levels.

By immunohistochemical analysis MerTK is expressed in less than 5% of the astrocytes (arrowheads; top middle panel) while Axl expression is limited to the endothelial cells of the vasculature (arrowheads; bottom middle panel).

Immunohistochemical staining of fixed adult human normal cerebrum was performed on normal brain obtained from University of Colorado Hospital. Paraffin embedded tissue blocks were cut into 5 μm sections, deparaffinized in xylene, and rehydrated through a descending concentration sequence of ethanol. Sections were subjected to antigen retrieval in citrate buffer (pH 6.0) for 10 min in a decloaking chamber. Endogenous peroxidase activity and nonspecific binding was blocked by 3% hydrogen peroxide block for 15 min and then 3% BSA protein block for 15 min. Immunohistochemical staining was performed using labeled streptavidin biotin reagents (LSAB) on a Dako Autostainer (Dako, Carpinteria, CA). The primary antibodies, 0.2 μg/mL rabbit anti-MerTK antibody (Epitomics, Burlingame, CA) and 0.5 μg/mL goat anti-human Axl antibody (R&D Systems, Minneapolis, MN), were incubated at room temperature for 60 min, followed by the biotinylated secondary antibody (LSAB+kit, Dako) for 30 min and streptavidin-horseradish peroxidase (LSAB+ kit, Dako) for 30 min. Localization of staining was performed with 3,3'-diaminobenzidine (Dako). Slides were counterstained with hematoxylin (Dako) and coverslipped using a xylene based mounting medium. Normal goat IgG (Santa Cruz Biotechnology, Santa Cruz, CA) and Rabbit Immunoglobulin (Dako) were used in place of the primary antibodies for negative staining controls. Human Mer/Fc Chimera (R&D Systems) and Human Axl/Fc Chimera (R&D Systems) were preincubated with the respective primary antibodies, in five-fold excess by weight, to determine specificity of antibody binding. Magnification bars provided represent 10 μm.

Fig. 2.

TAM signaling in cells of the CNS. The schematic represents a generalization of the important TAM signaling pathways within the CNS derived from a variety of studies. In many instances one or more receptors, or interactions between receptors, are responsible for the signaling pathways noted; this is a simplified summary representation. Refer to citations for specific details. (A) Protein S mediates survival of cortical and hippocampal neurons through Tyro3, regulating both the intrinsic and the extrinsic apoptotic pathways (Zhong et al., 2010). (B) GnRH NLT neurons, isolated from embryonic mouse brain, express no Tyro3 but abundant Axl and MerTK. Gas6 is the sole ligand recognized for Axl and stimulates both migration and survival through Axl in GnRH NLT cells (Allen et al., 1999; Nielsen-Preiss et al., 2007). (C) Gas6 does play a role in neuronal repair and synaptic plasticity: it binds Tyro3 in cortical and hippocampal neurons and upregulates components of transcriptional and translational machinery (Prieto et al., 2007). (D) Glial cells generally express much lower levels of Tyro3 than (adult) neurons. While oligodendrocytes express all three TAMs, Axl (not Tyro3) mediates survival signaling via Gas6, perhaps because Axl has greater affinity for Gas6 than Tyro3 does (Shankar et al., 2003; Weinger et al., 2008).

(E) Microglia express Axl and MerTK but have no detectable levels of Tyro3. Gas6 has been shown to bind both Axl and MerTK, limiting inflammatory responses and promoting phagocytosis in microglia, while Tubby reportedly binds MerTK and promotes phagocytosis in macrophages and retinal epithelial pigment cells (Caberoy et al., 2009; Caberoy and Zhou, 2010).

Table 1

Overexpression of TAM RTKs is seen in a wide variety of human cancers. T=Tyro3, A=Axl, M=MerTK.

System/organ Tissue/malignancy	TAM receptor implicated
Breast	
Carcinoma	TAM
CNS	
Glioma	TAM
Pituitary	M
Genitourinary	
Endometrium	A
Ovary	A
Uterine	TA
Prostate	TAM
GI	
Stomach	AM
Intestine/Colon	A
Liver	A
Pancreas	A
Hematopoietic	
ALL	M
AML	AM
CML	AM
Mantle cell lymphoma	M
Kidney	
Renal Cell Ca	A
Liver	
Hepatocellular Ca	TA
Musculoskeletal	
Skeletal muscle	M
Osteosarcoma	A
Skin	
Melanoma	TAM
Lung	
NSCLC	TAM
Thyroid	
Carcinoma	TA

Table 2

Reported expression of Tyro3, Axl, and MerTK transcript and/or protein in adult brain and CNS as determined by a variety of techniques including northern blot, immunoblot, microarray, immunohistochemistry, and others; emphasis is placed on protein expression. Tyro3 is the most widely expressed TAM member in the brain; expression is low in the embryo, but increases dramatically with synaptogenesis. Axl and MerTK expression is restricted to certain regions. +++=high; ++=moderate or present but not quantified;+= low; Ø=none detected.

Structure	Rat	Mouse	Human
Brain	+++ Tyro3, +Axl, +MerTK (Funakoshi et al., 2002; Lai and Lemke, 1991; Ohashi et al., 1995; Prieto et al., 2000)	+++ Tyro3 (Fujimoto and Yamamoto, 1994; Schulz et al., 1995)	+++ Tyro3 (Mark et al., 1994) +Ø Axl (Funakoshi et al., 2002; Hutterer et al., 2008)
Cerebral cortex	+++ Tyro3 (Funakoshi et al., 2002; Ohashi et al., 1995; Prieto et al., 2000; Prieto et al., 2007)	++/+++ Axl (Bellosta et al., 1995; Rescigno and Mansukhani, 1991; Weinger et al., 2008)	Ø MerTK (Graham et al., 1994; Wang et al., 2012)
Neocortex	+++ Tyro3, +Axl, +MerTK (Lai and Lemke, 1991)	+++ Tyro3 (Schulz et al., 1995)	
Hippocampus	+++ Tyro3 (CA1 region) (Funakoshi et al., 2002; Lai and Lemke, 1991; Ohashi et al., 1995; Prieto et al., 2000; Prieto et al., 2007)	+++ Tyro3 (Lai et al., 1994) +++ Axl (pyramidal layer) (Bellosta et al., 1995)	
Dentate gyrus	+ Axl, +MerTK (Prieto et al., 2000; Prieto et al., 2007)	+++ Tyro3 (Lai et al., 1994; Schulz et al., 1995)	
Amygdala	++ Tyro3 (Prieto et al., 2000)	+++ Axl (granular layer) (Bellosta et al., 1995)	
Cerebellum	+ Axl, +MerTK (Prieto et al., 2000)	+++ Tyro3 (Lai et al., 1994)	
	+++ Tyro3 (cortical, granule layers) (Funakoshi et al., 2002; Lai and Lemke, 1991; Ohashi et al., 1995; Prieto et al., 2000)	+++ Axl (Purkinje cells) (Bellosta et al., 1995)	
Olfactory bulb	ØAxl, +MerTK (granule layer) (Prieto et al., 2000) + Tyro3, +++ Axl, +++ MerTK (Purkinje cells) (Lai and Lemke, 1991; Prieto et al., 2000) + Tyro3 (Bergmann glia) (Lai and Lemke, 1991; Prieto et al., 2000) + Tyro3, Axl, MerTK (white matter) (Prieto et al., 2000) +++ Tyro3 (Ohashi et al., 1995; Prieto et al., 2000)	+++ Tyro3 (Schulz et al., 1995) See also GnRH neurons	

Structure	Rat	Mouse	Human
Priform cortex	+++ Tyro3 (Prieto et al., 2000)		
Hypothalamus/Thalamic nuclei	Ø Tyro3 (Prieto et al., 2000) +++Tyro3 (hypothalamus) (Funakoshi et al., 2002)		
Cell Type			
Neurons	+++ Tyro3 (cerebellar, cortical neurons) (Prieto et al., 2000) +++Tyro3 (hippocampal, cortical neurons) (Prieto et al., 2007) + Tyro3,+++ Axl, +++ MerTK (Purkinje neurons) (Lai and Lemke, 1991; Prieto et al., 2000)	++ Tyro3 (cortical neurons) (Guo et al., 2011; Zhong et al., 2010) ++Axl, ++MerTK (cortical neurons) (Zhong et al., 2010) +++ Axl (Purkinje cells) (Bellosta et al., 1995) Embryonic: +++ Tyro3, +++ Axl, +MerTK (olfactory region) (Allen et al., 1999; Pierce et al., 2008) Adult: +++ Tyro3, +Axl, +++ MerTK (forebrain) (Allen et al., 1999; Pierce et al., 2008) ++ Axl (Weinger et al., 2008)	++ Tyro3, ++ Axl, ++ MerTK (Shankar et al., 2003)
GnRH neurons			
Oligodendrocytes	++ Tyro3, +++ Axl, ++ MerTK (Hoehn et al., 2008; Kilpatrick et al., 2000; Prieto et al., 2000)		
Schwann cells (PNS)	++ Tyro3 (Prieto et al., 2000)	++ Axl, ++ Tyro3(Li et al., 1996)	
++ Tyro3, ++Axl, MerTK (Lai and Lemke, 1991; Li et al., 1996)			
Microglia	++Tyro3, ++ Axl (Funakoshi et al., 2002)	++ Axl, ++ MerTK (Grommes et al., 2008) ++ Axl (Weinger et al., 2008)	
Astrocytes	++ Tyro3 (Prieto et al., 2000; Prieto et al., 2007) + Tyro3 (Bergmann glia) (Prieto et al., 2000)	++ Axl, ++ MerTK mRNA (Cahoy et al., 2008)	+ Axl (Hutterer et al., 2008)
Endothelial	++ Axl (vasculature) (Prieto et al., 2000)	++ Axl (Weinger et al., 2008) ++ Tyro3, ++ Axl and ++ MerTK (microvessels) (Zhu et al., 2010)	+Ø Axl (vasculature) (Hutterer et al., 2008) ++ Tyro3, ++ Axl, +MerTK (brain endothelial cells) (Zhu et al., 2010)