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REVIEW

Regulation of phagocytosis by *TAM* receptors and their ligands

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Abstract The *TAM* family of receptors is preferentially expressed by professional and non-professional phagocytes, including macrophages, dendritic cells and natural killer cells in the immune system, osteoclasts in bone, *Sertoli* cells in testis, and retinal pigmental epithelium cells in the retina. Mutations in the Mertk single gene or in different combinations of the double or triple gene mutations in the same cell cause complete or partial impairment in phagocytosis of their preys; and as a result, either the normal apoptotic cells cannot be efficiently removed or the tissue neighbor cells die by apoptosis. This scenario of *TAM* regulation represents a widely adapted model system used by phagocytes in all different tissues. The present review will summarize current known functional roles of *TAM* receptors and their ligands, Gas 6 and protein S, in the regulation of phagocytosis.

Keywords *TAM* family (Tyro3, Axl and Mertk), ligands, growth-arrest specific gene 6 (Gas 6), protein S, regulation, phagocytosis

1 Introduction

Receptor protein tyrosine kinases (RPTKs) are characterized by an extracellular ligand binding domain that recognizes ligands, one or more transmembrane domains, and a cytoplasmic domain with intrinsic protein-tyrosine kinase activity, followed by a short carboxyl-terminal tail housing a few tyrosine residues that are phosphorylated when the receptors are activated (Schlessinger, 2000). They transmit signals for multiple cellular functions including growth control, differentiation, proliferation, motility, cell-cell interaction and death. Generally, RPTKs exist as monomers on the cytoplasmic membrane and binding of ligands leads to their dimerization and subsequent trans-autophosphorylation of tyrosine residues in their kinase domains (Hubbard, 1998). These phosphotyrosine residues provide docking sites for downstream signaling molecules that contain modular domains such as src homology 2 (SH2) or phosphotyrosine binding (PTB) domains (Hubbard and Till, 2000). There are many adaptor molecules possessing SH2 or PTB domains, and the specificity of one specific signal transduction event is usually determined by a consensus amino acid sequence flanking the phosphotyrosine residue for a specific downstream adaptor protein and the availability of both receptor and such adaptor protein in the responding cells (Songyang et al., 1993).

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2 *TAM* receptors belong to a subfamily of receptor type protein tyrosine kinases

There are 58 members of the receptor PTKs in the human and mouse genomes, which are divided into 20 receptor PTK families based on their primary DNA sequences, particularly on the similarity of their kinase domains and biological functions (Manning et al., 2002). The *TAM* family is comprised of three members: (1) Tyro 3 (also named Rse, Sky, Brt, Tif, Dtk and Etk-2) (Lai et al., 1994; Ohashi et al., 1994), (2) Axl (other names are Ark, Ufo, and Tyro 7) (Janssen et al., 1991; O'Bryan et al., 1991; Rescigno et al., 1991), and (3) Mertk (also referred to as Eyk, Nyk, Tyro 12 and mer) (Graham et al., 1994; Jia and Hanafusa, 1994). These receptors share a distinct structure with an extracellular domain containing two immunoglobulin (Ig)-related domains, followed by two fibronectin type III (FN-III)-related repeats, a single transmembrane domain, and a cytoplasmic tyrosine kinase moiety

followed by a short C-terminal tail housing a few tyrosine residues (Fig. 1) (Robinson, 2000). When the receptor is activated, those tyrosines are phosphorylated and the newly phosphorylated tyrosine residues in turn provide docking sites for downstream signaling components. The unique arrangement of the Ig and FN-III domains in the *TAM* receptors determines their specific binding of their common ligands, Gas 6 and protein S (Mark et al., 1996).

3 Ligands for *TAM* family receptors

Two related proteins, Gas 6 (growth-arrest-specific 6) and protein S, have been identified as ligands of *TAM* family receptors (Stitt et al., 1995). Gas 6 is named for its original identification as a gene specifically induced upon growth arrest of cultured mouse fibroblasts (Schneider et al., 1988). It is a member of the vitamin K-dependent proteins and is structurally closely related to protein S (Manfioletti et al., 1993). Protein S belongs to the protein C anticoagulation cascade; it acts as a cofactor in the degradation of blood coagulation factors Va and VIIIa (Dahlback, 1991). Heterozygous or, in very rare cases, homozygous patients with severe protein S deficiency have a high risk for venous thrombosis (Garcia de Frutos et al., 2007; D'Angelo and Vigano D'Angelo, 2008). Null mutation of protein S in conditional knockout mice leads to embryonic lethality due to massive coagulopathy and hemorrhage (Burstyn-Cohen et al., 2009; Saller et al., 2009). Gas 6 and protein S are secreted proteins with a 44% amino acid identity. Both proteins are the only ligands for *TAM* family as currently known and function redundantly in the regulation of *TAM*-mediated phagocytosis, since single knockout of either protein showed no obvious phagocytic defects (Prasad, 2006; Burstyn-Cohen, et al., 2009).

Gas 6 and protein S share a distinctive structural arrangement with characteristics of an amino-terminal γ-carboxyglutamic acid (Gla) domain, followed by a loop region, four epidermal growth factor (EGF)-like repeats, and a carboxy-terminal region containing two tandem globular (G) domains which show a similarity to the sex hormone-binding protein (SHBP) (Fig. 1) (Lundwall, 1986; Manfioletti et al., 1993). The Gla domain results from a vitamin K-dependent γ-carboxylation modification of the glutamic acid residues and is required for calcium-dependent phospholipid binding and biological activities (Sugo, et al., 1986; Nakano et al., 1997; Huang et al., 2003; Hasanbasic et al., 2005). The C-terminal G-domains are sufficient for receptor binding and activation (Mark et al., 1996; Sasaki et al., 2002; Sasaki et al., 2006). Binding of the Gla domains on the ligands to the phosphorylserines on apoptotic cells or spent rod outer segments (OS) and of the G-domains to receptors has been postulated to bridge apoptotic cells or spent outer segments to phagocytes for phagocytic clearance of the target prey cells (Nagata et al., 1996; Ishimoto et al., 2000; Hall et al., 2001, 2002, 2005; Anderson et al., 2003; Hasanbasic et al., 2005).

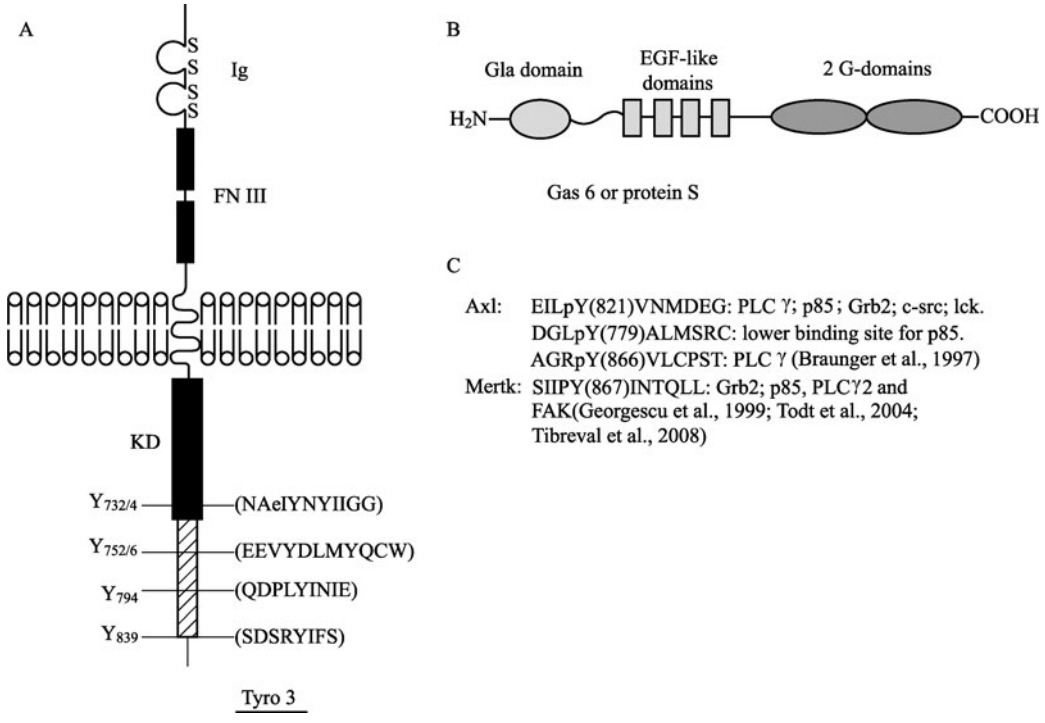

Fig. 1 Domain structure of Tyro 3, Axl and Mertk family receptors (A) and ligands, Gas 6 and protein S (B). The tyrosine (Y) residues and the flanking sequences listed in (A) represent the mouse Tyro 3; and those important for phagocytosis regulation on Axl and Mertk are listed in (C). See text for details.

4 *TAM* downstream signal transduction pathways that affect phagocytosis

Although Tyro3, Axl, and Mertk receptor tyrosine kinases share similarities in substrate binding and intracellular signaling pathways, they have distinct expression profiles, allowing for a diversity of functions (Hafizi and Dahlback, 2006). Each of the receptors has multiple tyrosine residues on the intracellular domain, which provides docking sites for downstream signaling molecules when phosphorylated. Association of the p85 subunit of PI3K, PLC-γ, Src, Vav and FAK has been demonstrated to be important for Mertk mediated phagocytosis. PI3K is a prone signaling pathway to regulate phagocytosis (Araki et al., 1996; Hall et al., 2003; Shankar et al., 2003, 2006; Keating et al., 2006; Kamen et al., 2007; Zheng et al., 2009). The tyrosine residue at Mertk-Y867 flanked by a Grb2 consensus binding site, YVNM, is a multi-substrate docking site, potentially interacting with Grb2, PI3K, PLC-γ2, c-Src, Lck and FAK (Braunger et al., 1997; Georgescu et al., 1999; Todt et al., 2004; Tibrewal et al., 2008). The phosphorylated Mertk was found to recruit and activate PLC-γ2, which is responsible, if not all at least partially, for the phagocytosis of apoptotic thymocytes by macrophages (Todt et al., 2004). The guanine nucleotide-exchange factor Vav1 binds to Mertk in a constitutive and phosphotyrosine-independent manner (Mahajan and Earp, 2003). Activation of Mertk results in tyrosine phosphorylation of Vav1 and release from Mertk. The released Vav1 subsequently activates the Rho A family of small GTPases, e.g. Rac1, Cdc42 and Rho A, which causes cytoskeleton rearrangement during phagocytosis. Mertk activation of the $p130^{cas}$/CrkII/Dock180 complex has been independently studied and signals through different signaling pathways. The activated Mertk was shown to induce Src-mediated tyrosine phosphorylation of focal adhesion kinase (FAK) on Tyr861 as well as its recruitment to the αvβ5 integrin, which further causes formation of the $p130^{cas}$/CrkII/Dock180 in a human cell line (Wu et al., 2005). Interestingly, in studies on the cultured RPE cells or the β5 integrin knockout mouse retina, Mertk has been shown to function downstream of the integrin signaling. The β5 integrin-deficient retina loses synchronized activation of FAK and Mertk and shows no response of Mertk phosphorylation to the rod outer segment binding (Nandrot et al., 2004). Mertk activation by αvβ5 integrin depends on the presence of FAK for engulfment of the rod outer segment (Finnemann, 2003; Nandrot et al., 2006). Those studies suggest a bidirectional, phosphorylation-dependent interaction between αvβ5 integrin and Mertk, which optimally activates Rac1 and thus the actin-mediated phagocytosis; and PKC may play a crucial role in integrating the Mertk and αvβ5 integrin signaling pathways (Finnemann and Rodriguez-Boulan, 1999; Tibrewal et al., 2008).

5 *TAM* knockout mice exhibit a broad spectrum of defective phenotypes

Soon after the three receptors had been identified, each gene was knocked-out by homologous recombination in mice (Bellosta et al., 1997; Camenisch et al., 1999; Lu et al., 1999). Three single gene knockout mice were initially found no overt or minor developmental defects. To overcome the functional redundancy, three single knockout mouse lines were bred to make different combinations of double and triple mutant mice (Lu et al., 1999). To our surprise, even the triple knockout mice displayed no overt defects during embryonic and early stages of postnatal development. However, those mice displayed massive tissue degeneration approximately three weeks after birth (Lu et al., 1999). Although the Mertk single knockout mice showed increased TNF-α production and lipopolysaccharide-induced endotoxic shock (Camenisch et al., 1999), mice lacking the three receptors exhibited an even broader spectrum of autoimmune disorders including high-circulating autoantibody, lymphocyte infiltration of all tissues, and massive splenomegaly (Lu and Lemke, 2001; Lemke and Lu, 2003), largely due to loss of *TAM* inhibition of Toll-like receptors (TLR) and cytokine signaling pathway (Rothlin et al., 2007; Lemke and Rothlin, 2008). Detailed discussion on *TAM* regulation of immune response is beyond this review, in which I will focus mainly on the functional roles of this family of receptors as well as their ligands in the regulation of phagocytosis.

6 Phagocytosis and *TAM* regulation

Phagocytosis is a form of endocytosis wherein large particles are enveloped by the cell membrane of specialized cells called phagocytes. Our knowledge about phagocytosis comes mainly from studies of professional phagocytes such as macrophages and neutrophils, which serve to fight infection and remove self waste tissue debris. However, in many cases, tissue homeostasis is primarily balanced by the resident, non-professional phagocytes, whereas the neighboring cells phagocytose their adjacent apoptotic cells or spent cell debris. There are two well-known examples of these phenomena; one is the massive cell debris from daily differentiating germinal cells phagocytosed and cleared by the supporting *Sertoli* cells in testis, and the other is the daily shed photoreceptor distal tips phagocytosed and cleared by the neighboring RPE cells in the retina.

Phagocytosis is triggered by the recognition of ligands on targets with corresponding receptors on the phagocytes and takes place by surrounding the target particles with pseudopods, a specialized organelle formed from plasma membrane. It is thought that the local reorganization of actin beneath the phagosome and the contractile motors are

the driving forces for further extension around the phagosome (Swanson et al., 1999; May and Machesky, 2001; Niedergang and Chavrier, 2004). As phagocytosis progresses, the circular margin of the phagosome becomes a narrower aperture and finally closes the phagocytic cup completely. The resulting phagosome becomes merged with lysosomes containing digestive enzymes, forming a phagolysosome, where the particles will then be digested and the released nutrients diffused or transported into the cytosol for use in other metabolic processes.

Rapid and efficient phagocytic clearance of the apoptotic and cellular debris is a mechanism to maintain tissue and cellular homeostasis. Failure to do so can lead to severe states of cellular degeneration and death. In the *TAM* receptor mutants, there are abundant examples of this phenomenon, suggesting a role for the receptors in phagocytosis (Lu et al., 1999; Lu and Lemke, 2001).

Three members of the *TAM* family receptors are preferentially expressed by professional and non-professional phagocytes, including macrophages, dendritic cells and natural killer cells in immune system, osteoclasts in bone, *Sertoli* cells in testis, and RPE cells in retina (Lu et al., 1999; Lu and Lemke, 2001; Lemke and Lu, 2003; Caraux et al., 2006; Prasad et al., 2006). Mutation in the Mertk single gene (in RPE) or in different combinations of double or triple *TAM* mutations in the same cell caused complete or partial impairment in phagocytosis of their neighboring preys; as a result, the neighbor cells died by apoptosis. This scenario in *TAM* mutants represents a widely adapted model system used by the phagocytes in all different tissues.

7 All the three members of the *TAM* family are required to support *Sertoli* cell phagocytic activity in testis

Despite the normal development of all reproductive cell types in testis, the *TAM* triple mutants exhibited inhibition of spermatogenesis in young adult males gradually, eventually leading to an almost complete depletion of differentiating germ cells in older animals (Lu et al., 1999). Spermatogenesis is a synchronized and highly regulated event of cellular differentiation that is highly dependent on communication and support from the adjacent *Sertoli* cells. The germ cell differentiation gradually transforms the large diploid spermatogenic cells into small and compact haploid spermatozoon. During this process, a large body of cytoplasm is abandoned and removed by *Sertoli* cells. Our studies showed that *Sertoli* cell was the only cell type contained inside the seminiferous tubule expressing the three receptors and two ligands, and the cells most dramatically affected in the triple knockout mice did not express any of the receptors (Lu et al., 1999). A major function of the *Sertoli* cells is phagocytic clearance of apoptotic germ cells and extruded cytoplasm during spermatogenic differentiation (Miething, 1992). Failure to eliminate apoptotic germ cells, as well as cytoplasm and organelles abandoned from developing spermatids, leads to dramatic impairment in spermatogenesis (Maeda et al., 2002).

A completely inhibited spermatogenesis in the triple mutant testis is largely due to the incapability of *Sertoli* cells to phagocytose the abandoned residual bodies from differentiating germ cells as well as normal apoptotic germ cells. This hypothesis was further demonstrated in the cultured *Sertoli* cells isolated from *TAM* triple knockout mice (Xiong et al., 2008). Normally, after ingestion of apoptotic germ cell and residual bodies, *Sertoli* cells retain multiple spots of lipid droplets inside the cytoplasm for more than 48 h of post-phagocytosis (Wang et al., 2006). Specific blockage of germ cell phagocytosis prevents Oil Red-O positive droplet formation in the *Sertoli* cells. This technique was used to examine phagocytic ability of the *TAM* triple mutant *Sertoli* cells in culture and it showed that there were almost no lipid droplets formed in the triple mutant *Sertoli* cells after fed with apoptotic germ cells, suggesting that ingestion of apoptotic spermatogenic cells by the triple knockout *Sertoli* cells was significantly abrogated (Xiong et al., 2008).

Interestingly, different from other systems discussed in the following sections of this review, the *Sertoli* cell expresses all the three members of *TAM* receptors and is able to maintain its functional support for developing germ cells, even when five copies of all the six *TAM* alleles are eliminated. All single and different combinations of double knockouts as well as those with a phenotype heterozygous in Axl and homozygous in both Tyro3 and Mertk ($Axl^{+/-}$, $Tyro3^{-/-}$ and $Mertk^{-/-}$) are fertile and exhibit normal spermatogenesis (Lu et al., 1999). *Sertoli* cell maintains its six fortresses to defend one population from complete elimination by nature.

8 Macrophage and dendritic cells differentially use Mertk, Axl and Tyro 3 receptors for phagocytic clearance of apoptotic cells

Among its many functions, one of macrophage's essential tasks is to scavenge and clear apoptotic cells from the tissue. Detailed studies on phagocytic clearance of apoptotic cells in the lymph system show that all the three receptors contribute collectively to regulate macrophage phagocytosis; however, Mertk plays a predominant role in macrophage and thymus. Mice with a mutation in a single *TAM* receptor, Mertk, were unable to efficiently clear apoptotic cells (Scott et al., 2001). This deficiency is attributed to macrophages as demonstrated through *in vitro* phagocytosis assay as well as the chimaric bone marrow reconstitution experiments (Scott et al., 2001). Such defects are clearly more dramatic and severer in triple mutant macrophages (Lu and Lemke, 2001). Elimination

of Tyro 3, Axl or both reduced phagocytic activity in macrophage by approximately half, but almost completely abolished apoptotic cell clearance by dendritic cells (Seitz et al., 2007; Sen et al. 2007).

Mertk regulation of phagocytosis is specific to apoptotic cells. Mertk single or *TAM* triple gene deficiency displayed equal efficiency in phagocytosis of Gram-negative *E. coli*, Gram-positive *S. aureus* bioparticles, latex beads and opsonized particles as wild type cells (Scott et al., 2001; Williams et al., 2009). Accumulation of apoptotic cell debris provides a plentiful supply of autoantigens to stimulate production of autoantibodies, and it has been suggested that the clearance impairment leads to the development of autoimmunity (Taylor et al., 2000). Accordingly, both Mertk single and *TAM* triple mutants exhibit elevated levels of circulating autoantibodies and all develop autoimmunity (Lu and Lemke, 2001; Scott et al., 2001).

Such defective Mertk signaling in macrophages has been shown to promote accumulation of apoptotic cells and proinflammatory immune response in atherosclerotic lesion sites and accelerates atherosclerosis (Ait-Oufella et al., 2008; Thorp et al., 2008; Thorp et al., 2009).

9 Mice lacking Mertk receptor undergo rapid postnatal retinal degeneration

The retina is another example of a phagocytosis system in which the *TAM* family plays an essential role. All the three receptors are expressed in retina. However, only Mertk and Tyro3 are expressed by the retinal pigment epithelium (RPE), a highly polarized pigmented cell type that is intimately associated with photoreceptor cells by extending their apical microvilli deep into the photoreceptor layer to sheathe the outer segments. The specialized functions of the RPE cell depend on a highly polarized organization of organelles and a molecular asymmetry of the membrane, the establishment and maintenance of which is dependent on the cytoskeleton (Mays et al., 1994). Among many vital roles the RPE plays (Bernstein et al., 1987), the phagocytic clearance of the spent photoreceptor outer segments (OS) is indispensable for the survival of the photoreceptors. Photoreceptors are specialized photosensitive nerve cells located on the back of eyeball, sensing and receiving light by a specialized stack of membrane-enclosed disks. The enormous number of photons absorbed by these disks demand constant regeneration of those membrane organelles, and the newly-synthesized membrane disks are indeed added to outer segments constitutively (Young, 1967; LaVail, 1973). To maintain a constant outer segment length, the spent distal portion of the outer segments is removed by RPE (Young and Bok, 1969; LaVail, 1976). Failure of the RPE to phagocytose rod OS leads to progressive photoreceptor death, a retinal dystrophy called *retinitis pigmentosa* (RP) in human (Bok and Hall, 1971; Mullen and LaVail, 1976; Edwards and Szamier, 1977). The role of the RPE in rod OS turnover has been extensively studied since the discovery of the Royal College of Surgeons (RCS) rat model, which displays an RP phenotype (Dowling and Sidman, 1962). The RCS rat displayed progressive photoreceptor degeneration due to an autosomal recessive mutation in the RPE cells which carried an inherited defect in rod OS phagocytosis (Bok and Hall, 1971; Edwards and Szamier, 1977).

The function of the *TAM* receptors in retina was first studied due to availability of the *TAM* triple knockout mice, in which the retina displayed a progressive degeneration of photoreceptors (Lu et al., 1999). Unlike the other model systems discussed above wherein the three receptors collectively play a role in many regulatory functions including phagocytosis control (Lu et al., 1999), inactivation of Mertk single gene is sufficient for the retinal phenotype (Duncan et al., 2003a; Prasad et al., 2006). Mertk mutant retina accumulated spent OS debris soon after the photoreceptor fully differentiated *in vivo* (Prasad et al., 2006); and ingestion of the fed OS particles by the cultured mutant RPE was significantly impaired (Feng et al., 2002; Duncan et al., 2003a, b; Prasad et al., 2006). Those Mertk mutants, even the *TAM* triple knockout mice, produce every normal retinal cell types that are properly differentiated during embryonic development as well as within the first two weeks after birth. However, at approximately three weeks of age, the photoreceptors begin to undergo apoptotic cell death. The photoreceptor death continues over the next four weeks and the entire photoreceptor layer is almost completely deleted by the age of 8 weeks. The photoreceptor degeneration observed in the triple mutant was entirely due to mutation of the Mertk gene. Mertk single gene knockouts exhibited the same degeneration pattern and cell death time-course as those found in the triple knockouts (Fig. 2).

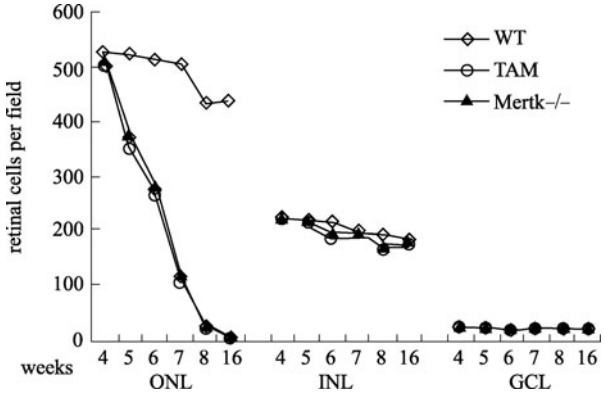

Fig. 2 Cell number per 200-μm of segment at 200 μm temporally to optic nerve in a horizontal section of the retina in WT, Mertk$^{-/-}$ and *TAM* triple mutant mice. Counts were performed from the outer nuclear layer (ONL; i.e., photoreceptor layer), inner nuclear layer (INL), and ganglion cell layer (GCL).

Retinal degeneration was exclusively limited to photoreceptors at those ages. RPE cell death was not observed until 12 weeks of age. Consistent with those observations, the evidence of apoptotic cell death (as stained by a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay) is dramatically elevated in the photoreceptor resident area, the outer nuclear layer. The TUNEL labeled apoptotic nuclei are constantly shown within the first two months. A variety of experimental approaches, such as RNA *in situ* hybridization, immunohistochemistry, real-time qPCR and Western blotting, showed that RPE cells expressed both Mertk and Tyro 3, but not Axl (Prasad et al., 2006). However, the Tyro 3 single gene knockout retina showed no morphologic degeneration; and the Tyro 3/Mertk double knockout mice, on the other hand, showed no severer phenotype as compared with Mertk single knockout (Prasad et al., 2006). Whether or not the Tyro 3 in normal RPE cells plays a regulatory role in collaboration with Mertk could not be answered simply by studying of those gene knockout mice, since the Tyro 3 expression was dramatically abrogated in the Mertk mutant RPE cells (Prasad et al., 2006). To answer this question, a functional Tyro 3 gene driven by a non-native promoter should be introduced into Mertk mutated RPE cells and the resulting phenotype could be carefully compared with that of the Mertk single gene knockout RPE cells.

10 Mertk regulates RPE phagocytic clearance of the spent photoreceptor OS

Soon after the discovery that the Mertk deficiency caused photoreceptor degeneration in mice, the gene responsible for inherited retinitis pigmentosa degeneration in the RCS rat was identified as a Mertk gene mutation by positional-cloning (Nandrot et al., 2000; D'Cruz et al., 2009). RCS phenotype is due to a 409 bp deletion resulting in a frameshift and translation termination in the rat Mertk genes (Nandrot et al., 2000; D'Cruz et al., 2009). The cultured RPE cells isolated from either RCS rat or Mertk knockout mouse showed defective phagocytic functions (Bok and Hall, 1971; Edwards and Szamier, 1977; Hall et al., 2001, 2002, 2005; Duncan et al., 2003a; Prasad et al., 2006). Phagocytosis consists of a serial of distinctive steps, such as prey binding, ingestion, and phagosome formation and maturation. Mertk is responsible for ingestion of the prey particle since RCS RPE cells bind to but fail to uptake the photoreceptor outer segment particles (Hall and Abrams, 1987).

The human Mertk mutation, almost at the same time, was identified from RP patients, who suffered night blindness and poor vision in their childhood and lost vision gradually in the following decades (Gal et al., 2000; McHenry et al., 2004; Tschernutter et al., 2006). Mertk mutation contributes approximately one percent of the patients with various retinal dystrophies based on the first report on Mertk deficient patients (Gal et al., 2000; Tschernutter et al., 2006). Although those mutations were not the same mutation as that found in the RCS rat or they have not been tested in animal models, those mutations were postulated to yield a null Mertk protein responsible for defective RPE phagocytosis of the spent rod outer segments, and subsequently for photoreceptor death and vision loss. The photoreceptor degeneration and visual loss in patients with Mertk deficiency is conceivable to result from a defective phagocytic clearance of photoreceptor outer segments by RPE cells in a manner similar to that found in RCS rat and Mertk knockout mice, since the optical coherence tomography (OCT) showed some debris-like materials in the subneurosensory space (Charbel Issa et al., 2009).

11 Gas 6 and protein S functioning as *TAM* ligands stimulate phagocytosis

Both Gas 6 and protein S were identified as *TAM* ligands based on their binding affinity to Axl and Tyro 3 (Stitt et al., 1995). Both proteins display high similarity in domain structure and primary sequence with 44% amino acid identity between human Gas 6 and protein S (Manfioletti et al., 1993). While Gas 6 has been considered to be an authentic ligand for all the three receptors, whether or not protein S serves as a *TAM* ligand has been debated for more than a decade (Godowski et al., 1995; Nagata et al., 1996; Chen et al., 1997). However, the functional roles of both Gas 6 and protein S as *TAM* receptor ligands in the regulation of phagocytosis have been repeatedly tested on the cultured phagocytes including *Sertoli* cells (Xiong et al., 2008), macrophage (Ishimoto et al., 2000) and RPE cells (Hall et al., 2001, 2002, 2005; Prasad et al., 2006). Uptake of apoptotic cells by cultured macrophages is enhanced in the presence of Gas 6 (Ishimoto et al., 2000). Inhibition of Gas 6 by specific antibody or decoy soluble form of Mertk impaired macrophage engulfment of apoptotic cells (Sather et al., 2007). As cells undergo apoptosis, the negatively-charged phospholipid phosphatidylserine is exposed on the outer leaflet of the plasma membranes. The binding of phosphatidylserine is mediated by the Gla domains of Gas 6 and protein S (Nakano et al., 1997) and ligand activation has been shown to lead to Mertk phosphorylation (Prasad et al., 2006) and the recruitment of multiple signaling molecules including phospholipase-Cγ2, focal adhesion kinase, $\alpha v\beta 5$ integrin, and the cytoskeleton-associated GTPase Rac1 in macrophages and other cultured cells capable of phagocytosis (Todt et al., 2004; Wu et al., 2005).

The spent photoreceptor outer segments possess similar characters as apoptotic cells, in terms of exposure of the negatively-charged phosphatidylserine on the membrane surface (Miljanich et al., 1981). Phagocytosis of outer

segment by cultured RPE cells requires the presence of serum (Mayerson and Hall, 1986; Hall and Abrams, 1987) and this serum requirement can be completely replaced by the presence of recombinant Gas 6 or protein S (Hall et al., 2001, 2002, 2005; Prasad et al., 2006). The stimulatory effect of Gas 6 on phagocytosis depends on calcium-mediated interaction between the phosphatidylserine on the outer segment and the γ-carboxyl Gla domain on the N-terminus of the Gas 6 protein (Hall et al., 2002). However, complete elimination of Gas 6 in mouse imposed no effect on photoreceptor survival (Hall et al., 2005; Prasad et al., 2006), suggesting that protein S may compensate for the loss of Gas 6 in supporting RPE phagocytosis. This notion was further supported by *in vitro* activation of Mertk and phagocytosis in cultured RPE cells (Hall et al., 2005; Prasad et al., 2006). Similarly, protein S was also identified as the serum factor responsible for the *in vitro* stimulation of macrophage phagocytosis of apoptotic cells and binds exclusively to phosphatidylserine positive cells (Anderson et al., 2003). Whether or not the Gas 6 and protein S are of functional redundancy in the regulation of phagocytic activities for PRE cells, or other phagocytes, such as macrophage, dendritic cells or *Sertoli* cells, will be clearly demonstrated by the generation of double gene knockout mice since both Gas 6 and protein S conditional knockout mice are available (Angelillo-Scherrer et al., 2001; Burstyn-Cohen et al., 2009; Saller et al., 2009). Taken together, these studies demonstrate that the ligands for *TAM* family of receptor tyrosine kinases play an important role in the regulation of phagocytosis.

12 Perspective remarks

Many lines of evidence show that *TAM* receptors play a critical role in the regulation of phagocytic functions for several types of professional or non-professional phagocytes. Among the three members, Mertk is pivotal in supporting RPE phagocytic clearance of spent photoreceptor outer segments and Mertk deficiency eventually leads to almost complete loss of vision in RP patients. Clinical perspective view aims to cure such diseases by replacing the mutated receptor with a native functional receptor in affected patients. Experiments on RCS rat model showed that wild type Mertk could be delivered into RPE cells through subretinal injection of the viral vectors and able to prevent photoreceptor degeneration and preserve retinal function in the mutant rat (Vollrath et al., 2001; Smith et al., 2003; Tschernutter et al., 2005). The viral vector-based gene therapy has recently been successfully used to safely restore vision in patients with an inherited retinal degeneration called leber congenital amaurosis (LCA) (Maguire et al., 2008), which potentially creates a new therapeutic method for rescue of the photoreceptor loss in Mertk deficiency patients.

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