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Role of Tyrosine Kinase in Cancer Cell Regulation: An Updated Review

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ABSTRACT

Tyrosine Kinase 3 belongs to TAM receptors family a unique subfamily of Receptor Tyrosine Kinase. These are membrane proteins which can be regulated by same ligand growth arrest specific 6, and protein S. This small family of RTKs regulates an intriguing mix of processes, including cell proliferation/survival, cell adhesion and migration, blood clot stabilization, and regulation of inflammatory cytokine release. Genetic or experimental alteration of TAM receptor function can contribute to a number of disease states, including coagulation, autoimmune disease, retinitis pigmentation, and cancer. Targeting TYRO3 could break the kinase signaling, stimulate antitumor immunity, reduce tumour cell survival, and regain drug sensitivity. To date, there is no specific TYRO3-targeted drug, the effectiveness of targeting TYRO3 in cancer is worthy of further investigations. In present review, an effort has been made to present an update on molecular biology of TYRO3, summarize the development of potential inhibitors of TAM family members, and provide new insights in TYRO3-targeted treatment.

INTRODUCTION

Cancer is the second leading cause of death globally and is responsible for an estimated 9.6 million deaths in 2018. Globally, about 1 in 6 deaths is due to cancer. The current treatments, including surgery with chemotherapy and/or radiation therapy, are not effective enough to provide full protection from cancer, which highlights the need for novel therapeutic approaches [1]. The TAM family (TYRO3, AXL, and MER), a subfamily of the receptor tyrosine kinases (RTKs), has been reported to regulate different cellular functions, including platelet aggregation, immune responses, and cell growth and differentiation. These receptors share common ligands such as growth arrest-specific gene 6 (Gas6) and protein S (Pros1). Among these RTKs, TYRO3 was first shown to express in tissues associated with myelination in the brain. However, emerging evidence has demonstrated the oncogenic effect of TYRO3 in promoting the survival, chemoresistance, tumorigenesis, and metastasis of cancer cells. This review summaries recent advance about the mechanisms regulated by the TYRO3 to promote oncogenesis. In addition, we will also discuss possible strategies of targeting TYRO3 as an anticancer regime[2]. Receptor tyrosine kinases (RTKs) are transmembrane proteins which transducer signals from the extracellular environment to the cytoplasm and nucleus. In this manner, RTKs regulate normal cellular processes, including survival, growth, differentiation, adhesion, and motility. Abnormal expression or activity of RTKs can render them transforming in cellular and animal models. Furthermore, increased RTK expression or activation has been directly implicated in the pathogenesis of myriad human cancers leading to intense interest in the development and testing of tyrosine kinase inhibitors as cancer therapeutics[3].

Molecular Biology of TAM Receptors

TYRO3 belongs to the TAM family of RTKs. Structurally, TYRO3 exhibits two fibronectin type III domains and two immunoglobulin (Ig)-like domains in the extracellular portion, a transmembrane portion, and a kinase domain in the cytoplasm (Figure 1(a)).

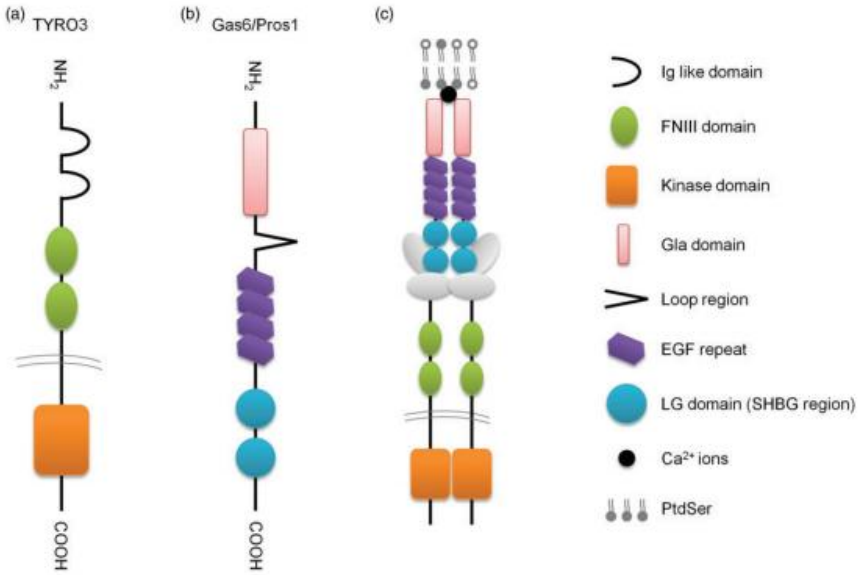

Figure 1. Schematic representation of TYRO3 receptor and ligands protein structure. (a) TYRO3 receptors carry two immunoglobulin (Ig)-like domains in their N-terminus, followed by two fibronectin type III repeats, a transmembrane region and a tyrosine kinase domain in the C-terminal intracellular region. (b)Growth-arrest-specific 6 (GAS6) and protein S (Pros1), TYRO3 ligands, carry a Gla domain in their N-terminus, followed by four EGF repeats and two laminin G (LG) domains in their C-terminus. (c) TYRO3 dimers bind to their ligands through interaction between the two N-terminal Ig-like domains of the receptors and the two C-terminal LG regions, which together make up the sex hormone binding globulin (SHBG) domain of the ligands. γ-carboxylation of glutamic acid residues in the Gla domain and calcium ions (Ca^{2+}), enable Gas6 and Pros1 to bind to phosphatidylserine (PtdSer). The two LG domains form a sex hormone binding globulin-like domain and trigger the activation of TYRO3. (A color version of this figure is available in the online journal.)

Figure No. 1: Schematic representation of TYRO3 Receptors and Ligand Protein structure.

TYRO3 receptors bind to their ligands through the Ig-like domains. Gas6 and Pros1 which are established natural ligands for TYRO3. After ligand binding, tyrosine residues of TYRO3 are autophosphorylated and downstream signalling is activated. In some cases, high levels of cytoplasmic TYRO3 can be activated even in the absence of a ligand. Under this condition, it functions as a dimeric tyrosine kinase and transforms RatB1a fibroblasts. There are many aliases for Tyro3 gene as it was cloned from multiple species by different research groups. These data suggest that even in the absence of its ligand, TYRO3 retains all the properties of the full-length TYRO3 kinase similarly, 1991, Tyro1 to Tyro13 were found from rat brain[4]. Tyro3, Tyro7, and Tyro12 were grouped into a subfamily based on the unique amino acid sequences found in their kinase domains. Afterwards, it was found that Tyro7 and Tyro12 are the same genes as Axl and Mer, respectively, while Tyro3 became the third member of the TAM family. In 1993, fragments of murine Tyro3 were found and named Etk2. In 1994, several TAM family genes were cloned from mouse and human by different groups. The murine gene was named Dtk, 5 Brt, 6 Rse, 7 or Tyro3,8 while the human gene was called Sky, 9 Tif, 10 or Rse 7. Two years later, it was learned that Dtk and Brt were encoded by the same gene with alternative splicing[5]. There are three splicing variants for TYRO3 that contain exons 2A, 2B,

and 2C, respectively. These exons encode different signalling peptide sequences, indicating that the expression of these alternative splicing variants may affect the subcellular localization and thus the function of TYRO3[6].

Ligands and Crystal Structures

The vitamin K-dependent protein Gas6 was first identified as a ligand for Axl in 1995. The related vitamin K-dependent anticoagulation factor, Protein S, was described as a ligand for Tyro-3 [7]. Although numerous subsequent studies confirmed that Gas6 binds to and activates all three members of the TAM receptor family, the validity of Protein S as a ligand for any of the TAM receptors became subject to extensive debate[8]. At the heart of the dispute was the issue of physiological relevance as the initial study used human Protein S to activate murine Tyro-3. Further studies were unable to demonstrate that Protein S could activate a TAM receptor of the same species, possibly due to the need for additional cofactor(s) or modification of the Protein S ligand. However, it was recently determined that purified recombinant murine Protein S does bind to and activate both endogenous murine Mer and heterologously expressed murine Tyro-3[9]. There is currently no evidence that Protein S activates Axl. A large number of additional studies have investigated the interspecies affinities of Gas6 and Protein S for TAM receptors. Studies which evaluated the Kd values for human Gas6 binding to each of the three human TAM receptors in vitro suggest that Axl and Tyro-3 bind Gas6 with roughly equal affinity while Mer affinity for Gas6 is 3–10-fold lower[8]. Gas6 was originally identified based on its dramatic up regulation after growth arrest with unknown function [10]. In 1995, it was reported that Gas6 could bind and activate AXL. Shortly thereafter, Gas6 was found to activate all TAM receptors [11]. Since the secretion signal and the c-carboxyglutamic acid domain are highly conserved in human, mouse, and bovine, Gas6 subfamily members are 74–81% homologous to each other and moderately homologous to human and bovine Pros1.16 The glutamic acid residue is required for the binding of TYRO3 to the phosphatidylserine of the cell membrane in a calcium-dependent manner [12], especially when it is c-carboxylated [13]. The two laminin G motifs within the C-terminal sex hormone-binding globulin domain are required for the binding to TYRO3 and the activation of downstream signalling pathways including phosphatidylinositol 3-kinase (PI3K)/AKT, ERK, and PLC-c (Figure 1(c) [14]. The functional importance of other domains of GAS6 and Pros1 awaits further characterization. Two potential TYRO3 ligands, tubby-like protein (Tulp) 1 and Tulp2, were identified recently and linked to phagocytosis [15]. By co-immunoprecipitation, Tulp1 was found to interact with MER, AXL,

and TYRO3, while Tulp2 can beco-precipitated with AXL and TYRO3, but not with MER. These results suggested that Tulp1 and Tulp2 have distinct binding specificities to TYRO3. Unlike Gas6 and Pros1, Tulp ligands lack the signature laminin G motifs for receptor binding but contain minimal phagocytic determinant (MPD) as a new type of TAM-binding motif. It is suggested that the five MPDs of mouse Tuip1 may cause home and/or hetero-dimerization of TAM receptors, though it is unclear whether one or multiple receptors will be bound[16]. Interestingly, Tulp proteins lack signal peptide and have been identified as intracellular proteins by immune histochemistry [17]. The next query is regarding how does intracellular Tulps interact with plasma membrane receptors to facilitate phagocytosis One explanation for Tulp1 functions as phagocytosis ligand is via active secretion through a non-classical pathway coined unconventional secretion. Similar mechanism has been reported for a number of proteins without a classical signal peptide [18]. Indeed, Caberoy and Li32 had demonstrated that Tulp1 can be secreted to extracellular space, which cannot be blocked by brefeldin A and monensin, inhibitors that block protein transport via the endoplasmic reticulum-Golgi pathway. This finding supports the notion that Tulp proteins can function as TAM receptor ligands; nevertheless, their functions other than facilitating phagocytosis remain to be characterized [19].

TAM Receptor Signaling Pathways

The first hint towards understanding TAM receptor signalling came from studies of FMS–Mer receptor chimera by Ling and Kung in 1995. Around the same time, studies of EGF–Axl receptor chimera were published by an independent group [20]. When the studies began, the ligand for TAM receptors was unknown, necessitating the use of receptor chimera composed of, in the latter report, the EGFR receptor ectodomain and transmembrane domain fused to the intracellular kinase domain of Axl. During the course of the studies, Gas6 was discovered as a ligand for Axl and Tyro-3 and additional work was conducted with the native Axl receptor. Two important findings came out of this seminal work. First, signalling pathway(s) downstream from the Mer and Axl kinase domains were determined to include PI3K, Ras, and ERK. Second, studies of the Axl receptor chimera compared to the native Axl RTK demonstrated that variation in the extracellular domain has a significant impact on downstream signalling.

In the 12 years since an abundance of research has been conducted with the goal of outlining signalling pathways downstream of TAM receptors. Most of these experiments utilize Gas6 to stimulate TAM receptor function but discuss relevance to only one TAM receptor, usually Axl.

It should be noted that Gas6 will also activate other TAM receptors endogenously expressed by the cells under investigation. For example, all three TAM receptors are expressed in platelets and are required for normal function of these cells. The downstream signalling pathway whereby TAM receptors mediate platelet aggregation likely involves cross-talk with the integrin family of receptors as platelets from TAM receptor knockouts exhibit impaired spreading after adhesion to fibrinogen. Indeed, Gas6 stimulates phosphorylation of β3 integrin, PI3K, and Akt in resting platelets from WT, but not TAM receptor knockout mice [21]. Importantly, the specific contributions of each TAM receptor to this signalling pathway have yet to be clarified.

To avoid uncertainty regarding which TAM receptor is responsible for the observed effects, some studies have continued to use the receptor chimera approach, fusing a TAM receptor intracellular kinase domain to an extracellular receptor kinase domain not normally expressed in the cells being studied. Although the use of chimeric receptors allows for determination of signalling pathways downstream from a single TAM receptor kinase, data from such experiments must be interpreted conservatively, given evidence provided by suggesting that the extracellular domain impacts downstream signalling. This issue along with inducible expression of TAM receptors in various cell types and unknown variables such as hetero-dimerization has made characterization of TAM receptor signalling pathways a complex task [22].

1. MER SIGNALLING—Much of the evidence delineating Mer signalling pathways is provided by studies of chimeric receptors. This approach originated out of necessity as the ligand for Mer was unknown when many of the studies began. Three well-known signalling pathways, those downstream from PI3K/Akt, PLCγ, and MAPK/ERK, were linked to Mer tyrosine kinase activation by early studies of chimeric Mer receptors expressed in NIH3T3 fibroblasts. In this context, ligand stimulation of Mer kinase led to cellular transformation exemplified by increased proliferation and DNA synthesis. Additional experiments indicated that activation of the MAPK/ERK pathway correlated with activation of Raf and p90RSK kinases as well as phosphorylation of Shc and association of Grb2 with Mer [23]. Later studies identified Gas6 as a ligand for Mer and confirmed that ligand-dependent activation of endogenous Mer stimulates phosphorylation of ERK1/2[8]. Future interaction studies revealed that Phosphorylation and activation of PLCγ may occur through direct binding of one of its SH2 domains to endogenous phospho-Mer [24]. Similarly, there is evidence to suggest that PI3K

may interact with Mer via an SH2 domain [25]. However, the co-immunoprecipitation experiments of the previous studies do not demonstrate direct binding and it is possible that association of PI3K and PLCγ with Mer is mediated by interaction of Mer tyrosine 872 with additional adapter proteins such as Grb. The ultimate downstream targets of the PI3K/Akt, PLCγ, and MAPK/ERK pathways may differ according to several factors, including cell type and the tissue microenvironment. In some cells, the PI3K/Akt and MAPK/ERK pathways may act in parallel. In leukaemia cells, for example, ligand-dependent activation of an EGFR–Mer chimeric receptor stimulated phosphorylation of Akt, ERK1/2, and p38 MAPK resulting in reduced apoptosis without a change in proliferation [26]. The presence of multiple Mer signalling pathways which converge on the same pro-survival outcome gives these cells a strong advantage over noncancerous lymphocytes. In other instances, the PI3K/Akt and MEK/Erk pathways may act in opposition. Similar to the study of leukemia cells discussed earlier, the PI3K/Akt and MAPK/ERK pathways were activated by ligand stimulation of an FMS–Mer chimeric receptor in prostate cancer cells. Additional experiments demonstrated that the Raf and p90RSK kinases act upstream and downstream, respectively, of MAPK/ERK, leading to transcriptional activation of IL-8 via c-Fos/c-Jun binding to the AP-1 promoter region [27]. Preincubation with a MEK inhibitor produced the expected result of decreased IL-8 production. However, preincubation with a PI3K inhibitor increased IL-8 production. The authors therefore speculated that the PI3K/Akt pathway may attenuate the effects of the MAPK/ERK pathway by phosphorylating and inhibiting Raf. In this case, activation of Mer may both stimulate and reduce proinflammatory cytokine production. It should be noted that other studies have suggested that Mer reduces production of proinflammatory cytokines in noncancerous cells [28]. Ectopic expression of Mer in prostate cancer cells may therefore result in activation of altered downstream signalling pathways. The tonic strength of normal versus aberrant signalling may therefore determine the oncogenic potential of Mer activation and the ultimate phenotypic fate of the tissue. Yet another possibility exists whereby activation of Mer stimulates a unique complement of signalling events under specific conditions, thus altering the downstream effect(s) of each individual pathway. For example, some studies of Mer signalling suggest that the PI3K/Akt pathway activates NFκB while others suggest that NFκB is inhibited by the PI3K/Akt pathway. Expression of a constitutively active CD8–Mer chimera in pro-B cells resulted in transcriptional activation of NFκB via PI3K/Akt [26]. Additional signalling pathways activated by CD8–Mer included p38/MAPK and MEK1. These cells were protected from apoptosis and became IL-3-independent. Conversely, pretreatment of dendritic cells with apoptotic cells prior to LPS exposure induces Mer-mediated stimulation of

PI3K/Akt. Under these experimental conditions, the p38/MAPK, MEK1, and JNK signalling pathways were active but unaffected by Mer stimulation. The phenotypic result in this case was reduced production of the proinflammatory cytokine, TNFα, following exposure to LPS [25]. Additional experiments in the same study demonstrated that PI3K/Akt negatively regulates NFκB by inhibiting IKK activity and thus preventing degradation of IκB. As is observed with Axl-mediated survival PI3K/Akt is classically thought to phosphorylate and activate IκB kinase (IKK), leading to phosphorylation and degradation of inhibitor of κB (IκB) releasing NFκB from the inhibitory complex. However, different isoforms of IKK have been discovered that are differentially phosphorylated by Akt[29]. Thus, there are many factors that define the downstream effects of TAM signalling pathways, including the isoforms of numerous kinases involved and the concomitant activity of additional signalling pathways. Clearly, further investigation is needed to elucidate the myriad signalling pathways activated by Mer kinase. In addition to the well-known pathways mediated by PI3K/Akt, PLCγ, and MAPK/ERK, some atypical signalling pathways have been proposed as a link between Mer and the actin cytoskeleton. Yeast two-hybrid experiments revealed Mer interactions with Grb2, SHC, and Vav1, the latter is a guanine nucleotide-exchange factor regulating Rac and cdc42 GDP to GTP exchange. Surprisingly, the Mer interaction with Vav1 involved the Vav1 SH2 domain but was constitutive and phosphotyrosine-independent. Subsequent Mer activation induced both Vav1 tyrosine phosphorylation and release of Vav1 from Mer. GDP/GTP exchange on Rac1 and cdc42 followed. These small G proteins are commonly recognized as regulators of the actin cytoskeleton. The initial experiments cited earlier were conducted using an EGFR–Mer chimera expressed in 32D cells. Further study, however, demonstrated that Gas6 stimulation of endogenous Mer in human macrophages also results in Vav1 release and subsequent Rac1 and cdc42 GTP loading [30]. These data suggest a potential mechanism whereby activation of Mer may induce spatially focused regulation of the actin cytoskeleton, thus providing a model whereby Mer may mediate changes in cellular morphology necessary for phagocytosis of apoptotic cells bound at specific sites on the macrophage surface. Interestingly, the site of Vav1 interaction was mapped to amino acids 697–754 of Mer. This region contains the three putative Mer autophosphorylation sites. As tyrosine phosphorylation of Vav1 was not sufficient for release from Mer, it is enticing to speculate that another SH2 domain-containing protein, perhaps with higher affinity for phosphorylated Mer, is required to release Vav1 and initiate cytoskeletal rearrangement. However, to our knowledge no other proteins have been suggested to interact with Mer in this region. Another study suggests that Mer regulates the actin cytoskeleton via PLCγ2 and Src. Upon exposure of macrophages to apoptotic cells, PLCγ2

associates with Mer and becomes phosphorylated. PLC can activate classical protein kinase Cs (PKCs) such as PKC βII, which is required for PS receptor-dependent phagocytosis in macrophages [31]. In addition, the Gas6–Mer system may also cooperate with the soluble bridging molecule milk fat globule-EGF factor 8 protein (MFG-E8) and its receptor αvβ5 integrin to stimulate the lamellipodia formation necessary for phagocytic engulfment of apoptotic cells. Studies utilizing constitutively active Mer chimera and kinase dead mutant Mer demonstrated that Mer stimulates Src-mediated phosphorylation of FAK and p130CAS/ CrkII/Dock180 complex activation of Rac1 in an αvβ5 integrin-dependent manner [32]. This pathway may also involve PLCγ2 as FAK association with αvβ5 integrin is dependent on PKC [33].

Mer activation has also been linked to cell survival via atypical signalling pathways. Gas6 stimulation of a human prostate adenocarcinoma cell line resulted in phosphorylation of a 120-kDa protein that was identified as Cdc42-associated kinase (Ack1) by mass spectrometry [30]. Constitutive association of Mer and Ack1 could be detected by coimmunoprecipitation of the endogenous proteins. Experiments with constitutively active and kinase dead mutant constructs of Ack1 demonstrated that Ack1 is not a direct Mer substrate, but that Ack1 autophosphorylation (and presumably activation) is facilitated by ligand activation of cell surface Mer. Continued Ack1 kinase activity required the chaperone activity of heat shock protein 90β (Hsp90β). Additional mass spectrometry sequencing of constitutively active Ack immune precipitates identified the tumor suppressor Wwox as an Ack1-interacting protein. Further investigation suggests that Ack1 induces phosphorylation, ubiquitination, and degradation of Wwox. Downregulation of this proapoptotic tumor suppressor may be one mechanism by which Ack1 and perhaps Mer relay survival signals in cancer cells. Since the physiologic function of the high levels of Mer expressed in normal prostate is not known, it is difficult to assess the normal role of the Mer–Ack axis.

2. AXL SIGNALING—Gas6/Axl signalling promotes the growth and survival of numerous cell types, including cardiac fibroblasts [31]. These effects are likely mediated by Gas6/Axl-induced activation of the MAPK/ERK and PI3K signalling pathways. Early studies utilized a chimeric EGFR/Axl receptor expressed in a leukemic cell line. These experiments demonstrated that ligand stimulation of the chimeric receptor leads to cell proliferation via activation of Grb2, Ras, Raf1, MEK-1, and ERK1/2[32]. Interestingly, Grb2 can be activated either by direct binding to tyrosine 821 on Axl or by association with Shc, which is

phosphorylated upon ligand stimulation but does not associate with Axl. Later studies confirmed that the Ras/ERK pathway is essential for Gas6induced mitogenesis of NIH3T3 cells[33]. Importantly, NIH3T3 cells also express Tyro-3 and therefore this mitogenic pathway may be activated by multiple TAM receptors. Although more than one study has suggested that weak or partial activation of the Ras/ERK pathway contributes to Axl-mediated survival, more recent data indicate that Ras is dispensable for survival resulting from Gas6 stimulation of native TAM receptors in NIH3T3 cells[34]. However, the MAPK/ERK pathway may be important for Gas6/ TAM receptor-mediated survival in certain cell types, including GnRH neurons[35].

3. TYRO-3 SIGNALLING—The Tyro-3 receptor is the least studied of the TAM receptors and the signalling pathways downstream of Tyro-3 activation are poorly understood. Nonetheless, a handful of studies provided clues as to the molecules which mediate Tyro-3 signalling. Coimmunoprecipitation of Tyro-3 transiently expressed in COS cells revealed a potential interaction with a phosphorylated SFK[36]. Because of cross-reactivity of the antibody used, it remains unknown which SFK(s) (Src, Yes, and/or Fyn) interact with Tyro-3. Importantly, all three of these SFKs are highly expressed in tissues of the central nervous system where they are likely to be found co-localized with Tyro-3. Yeast two-hybrid studies identified a number of proteins that potentially interact with Tyro-3, including RanBPM, protein phosphatase 1 (PP1), and the p85 β-subunit of PI3K [37]. Sequencing of the DNAs encoding the interacting proteins demonstrated that PI3K binds Tyro-3 via one of its SH2 domains and the interaction was confirmed in vitro and in vivo by GST pull-down assay and co-immunoprecipitation, respectively. Furthermore, ligand stimulation of an EGFR/Tyro-3 chimera induces phosphorylation of Tyro-3, PI3K, and Akt resulting in a transformed phenotype. A MAPK signalling pathway has also been linked to Tyro-3 activation as phosphorylation of ERK1/2 was increased by Gas6 stimulation of NIH3T3 cells which express endogenous Tyro-3. Phosphorylation of ERK1/2 was also upregulated by Gas6 stimulation of endogenous Tyro-3 in mouse osteoclasts, resulting in bone resorption [38]. Importantly, phosphorylation of Tyro-3 at specific residues has not been described. Clearly, further investigation is necessary to elucidate the signalling pathways downstream of Tyro-3 activation.

Involvement of TAM receptors in cancer

There are many ways that protooncogenes such as TAM receptors can be activated, including gene amplification and mutations, proteolytic cleavage, and altered protein expression. These modifications have all been described for TAM receptors and each may result in generation of a constitutively active enzyme and/or over- or ectopically expressed proteins that are not subject to normal cellular regulation. Most of the TAM receptor gene mutations reported involve Mer and retinal degeneration [39]. To date, no activating TAM receptor human mutations have been associated with development of cancer. Although random retrovirus-induced mutations of Axl correlated with increased transformation of NIH3T3 cells, gene sequencing revealed that the mutations were silent and overexpression of Axl was determined to be a major contributor to cellular transformation. This idea is consistent with evidence discussed later, which suggests that the oncogenic potential of TAM receptors is related to aberrant regulation of the same signalling pathways and cellular processes in which these receptors normally play a role [40].

The oncogenic potential of the TAM receptor kinases was immediately evident as each family member was originally cloned from cancer cells and early studies demonstrated that these RTKs exhibit the ability to transform NIH3T3 fibroblasts and BaF3 lymphocytes *in vitro* [41]. Some of the most convincing early evidence, however, comes from studies of the avian ortholog of Mer, Eyk [42]. A truncated version of Eyk containing only the tyrosine kinase domain mediates the transforming ability of the virus RLP30, which causes fibrosarcomas, endotheliomas, and visceral lymphomatosis in chickens [43]. Numerous studies have since used a variety of techniques, including immunohistochemistry, Western blotting, microarrays, RT-PCR, and flow cytometry to demonstrate that TAM receptors are ectopically or overexpressed in a wide array of human cancers. Tyro-3 expression has been associated with acute myeloid leukemia (AML) and multiple myeloma. Altered Axl expression has been reported in lung cancer, uterine cancer, breast cancer, ovarian cancer, gastric cancer, colon cancer, prostate cancer, thyroid cancer, liver cancer, renal cell carcinoma, AML, CML, erythroid leukemia, megakaryocytic leukemia, melanoma, osteosarcoma, and glioblastoma. Aberrant expression of Mer has been linked to B-and Tcell acute lymphoblastic leukemias, mantle cell lymphoma, melanoma, rhabdomyosarcoma, pituitary adenoma, gastric cancer, and prostate cancer.

Hanahan and Weinberg have proposed six primary cellular functions as "Hallmarks of Cancer" which normal cells acquire during oncogenesis: self-sufficiency in growth signals, insensitivity to antigrowth signals, limitless replicative potential, tissue invasion and metastasis, sustained angiogenesis, and evasion of apoptosis. In this section, we will discuss evidence which suggests that TAM receptors contribute to at least three of these six fundamental mechanisms of malignancy.

A. Migration and Invasion -TAM receptor signalling pathways have been linked to regulation of the actin cytoskeleton. The resultant changes in cellular morphology are likely to contribute to TAM receptor regulation of normal cellular processes such as platelet spreading and phagocytosis [44]. In glioblastoma cells which express elevated levels of Axl, transfection of a dominant negative Axl (Axl-DN) lacking the kinase domain results in reduced motility, altered morphology characterized by loss of filopodia, and loss of cell-to-cell interactions [45]. Conversely, stimulation of an ectopically expressed EGF/Mer chimera in a murine leukemic cell line induces rapid (8–24 h) changes in cell morphology, including cell flattening, extension of dendrite-like processes, and adherence. Thus, ectopic expression or overexpression of TAM receptors and resultant downstream changes in cellular morphology may contribute to mechanisms of oncogenesis[46].

B. Angiogenesis Formation of new blood vessels is a normal process important during development as well as wound healing. In addition, angiogenesis promotes tumor growth and malignant transformation. Proliferation and migration of vascular smooth muscle cells (VSMCs) are key events required during normal angiogenesis. VSMCs express Gas6 and exogenous application of purified or recombinant Gas6 promotes proliferation and migration of VSMCs. Gas6-induced migration of VSMCs was blocked by inclusion of recombinant Axl–ECD. Furthermore, overexpression of Axl increased migration 2–5-fold whereas expression of a kinase dead mutant reduced migration ~50% relative to parental VSMCs [47]. These results demonstrate that migration of VSMCs correlates with the level of Axl kinase activity. It has also been suggested that Axl plays a role in flow-induced vascular remodeling by regulating VSMC apoptosis[48].

C. Cell Survival and Tumor Growth Several lines of evidence suggest that TAM receptors activate pro-survival signalling pathways in both normal and cancerous cells. In some cases, TAM receptor signalling pathways prevent apoptosis without stimulating proliferation[46]. On the other hand, TAM receptors have also been shown to increase proliferation without

inhibiting apoptosis [49]. A third situation exists, whereby TAM receptors promote both survival and proliferation. Each mechanism provides a means by which TAM receptors may contribute to tumor growth[50].

D. TAM Receptors as Prognostic Factors Elevated Axl expression correlated with adherence, motility, and invasiveness of osteosarcoma cell lines selected for their high metastatic ability in an *in vivo* model of lung metastasis. In addition, lung metastasis has been correlated with reduced overall survival of osteosarcoma patients [47]. The previous results therefore suggest that Axl expression may correlate with poor prognosis in osteosarcoma [51]. Similarly, analysis of 58 adenocarcinoma patient samples revealed that Axl expression significantly correlated with metastatic cancer of advanced clinical stage [52]. Axl expression also correlated with invasiveness of lung cancer cell lines in vitro. In 54 patient samples of AML, Axl expression correlated with worse progression-free and overall survival [53]. Interestingly, coexpression of both Mer and Axl correlates with poor prognosis in gastric cancer, suggesting that cooperativity of multiple TAM receptors may play a role in progression and metastasis of some cancers. These data suggest that TAM receptor signalling may play a role in the progression of multiple cancers, including the development of metastasis [54].

Involvement of TYRO3 in cancer

The first study that indicates TYRO3 exerts oncogenic capacity was evidenced by showing Tyro3-transfected Rat-2 fibroblasts could grow in soft agar. Later on, rat fibroblasts overexpressing TYRO3 were shown to be able to form tumors in nude mice and the transcripts of Tyro3 have been associated with human and mouse mammary tumors further support this notion.[4,41] Like other TAM receptor family members, TYRO3 and ligand overexpression have been shown in a wide range of cancers, and correlate with poor prognosis in a variety of tumor types (Table 1). Through AKT/NFjB signalling, TYRO3 exerts pro-survival effects and promotes cancer cell growth. TYRO3 and AXL protein levels are undetectable in normal thyroid cells but significantly upregulated and activated in thyroid cancer cells. [55] TYRO3 also triggers the tyrosyl-phosphorylation of ACTN4, a member of actin binding protein family involved in motility. Knockdown of Tyro3 by siRNA prevents melanoma cell migration and invasion [56].

Table 01: Expression and functions of TAM family kinases and ligands in cancer

TAM Kinase or Ligand	Ectopic expression or over expression	Functional roles (Proliferation,survival or tumorigenesis)	Metastatic roles (Migration, invasion or EMT)	Roles in chemoresistance	Prognostic importance
TYRO3	Multiple myeloma, lung cancer, breast cancer,melanoma esophageal cancer,hepatocellular carcinoma,colorectal cancer, endometrial cancer, leiomyosarcoma, thyroid cancer, schwannoma,	Melanoma,hepatocellular carcinoma, colorectal cancer, breast cancer, ovarian cancer, thyroid cancer	Colorectal cancer, prostate cancer, lung cancer	Melanoma,hepatocellular, carcinoma, colorectal cancer, breast cancer, ovarian cancer, and thyroid cancer	Hepatocellular carcinoma, colorectal cancer, breast cancer.
AXL	Lung cancer,glioblastoma, breast cancer, colorectal cancer, gastric cancer, pancreatic cancer, oesophageal cancer, melanoma, sqamous cell skin cancer, prostate cancer, endometrial cancer, ovarian cancer, oral sqamous cell carcinoma, thyroid cancer, bladder cancer, renal cancer, schwannoma, mesothelioma, Kaposi's	Prostate cancer, ovarian cancer, breast cancer, thyroid cancer, lung cancer, pancreatic cancer, melanoma, hepatocellular carcinoma, giloblastoma, mesothelioma, osteosarcoma, schwannoma, kaposi's sarcoma and oesophageal cancer	Breast cancer, lung cancer, melanoma. Prostate cancer, pancreatic cancer, ovarian cancer, hepatocellular carcinoma, thyroid cancer, bladder cancer, kaposi's sarcoma and oesophageal cancer glioblastoma, colorectal cancer,	Breast cancer, lung cancer ovarian cancer, oesophageal cancer	Lung cancer, glioblastoma, osteosarcoma, oral squamous cell carcinoma, breast cancer, head and neck cancer, colorectal cancer, pancreatic cancer, oesophageal cancer, ovarian cancer, gastric cancer, bladder cancer and AML

	sarcoma, ostepsarcoma		cervical cancer, neruoblastoma and osteosarcoma		
MER	Lung cancer, glioma, melanoma, Prostate cancer, schwannoma, mantel cell lymphoma, AML and ALL	Glioma, lung cancer, melanoma. AML and ALL	Glioblastoma and melanoma	Glioma, lung cancer, pancreatic cancer, breast cancer and ALL	Gastric cancer
Gas 6	Multiple myeloma, gliobalstoma, breast cancer, gastric cancer, endometrial cancer, ovarian cancer, thyroid cancer, renal cancer, schwannoma, AML,ALL and CML	Lymphoma, breast cancer, prostate cancer, colorectal; cancer, pancreatic cancer, thyroid cancer, schwannoma, gastric cancer, osteosarcoma and renal cancer	Breast cancer, prostate cancer, pancreatic cancer, hepatocellular carcinoma. Gastric cancer, ostesarcoma and renal cancer	ALL	Lung cancer, Glioblastoma, and renal cancer and AML
Pros 1	Thyroid cancer, colorectal cancer, pancreatic cancer, brain tumours, lung cancer, prostate cancer, ovarian cancer, glioblastoma, osteosarcoma and AML	Thyroid cancer and glioblastoma	Prostate cancer and Thyroid cancer	Prostate cancer	Prostate cancer and Glioblastoma

Activated TYRO3 promotes the survival, invasion, migration, proliferation, and transformation of cancer cells[57-58]. Increasingly, evidence supporting the notion that overexpression of TYRO3 contributes to the resistance to conventional and targeted therapies in thyroid cancer, and blocking its signalling dramatically reduced cell viability and resistance to apoptotic stimuli [55]. TYRO3 was also shown to promote cell proliferation and chemoresistance in breast cancer [56]. Increased resistance to platinum and taxol secondary to TYRO3 overexpression has also been reported in ovarian cancer. Ovarian cancer cells overcome treatment resistance via upregulation of TYRO3 and AXL expression, AKT phosphorylation, and Bcl-xl expression. A growing body of evidence demonstrated that epithelial-mesenchymal transition may be a major mechanism in drug resistance. TYRO3 promotes phagocytosis and inhibits inflammation, allowing resistance to antitumor treatments to further cancer progression [59]. Interestingly, recent study demonstrated that treatment with the human TYRO3 antibody abolished TYRO3-induced EMT process in colon cancer. Taken together, these studies suggested that inhibition of TYRO3 and its signalling pathways could have therapeutic benefits in cancer development [60].

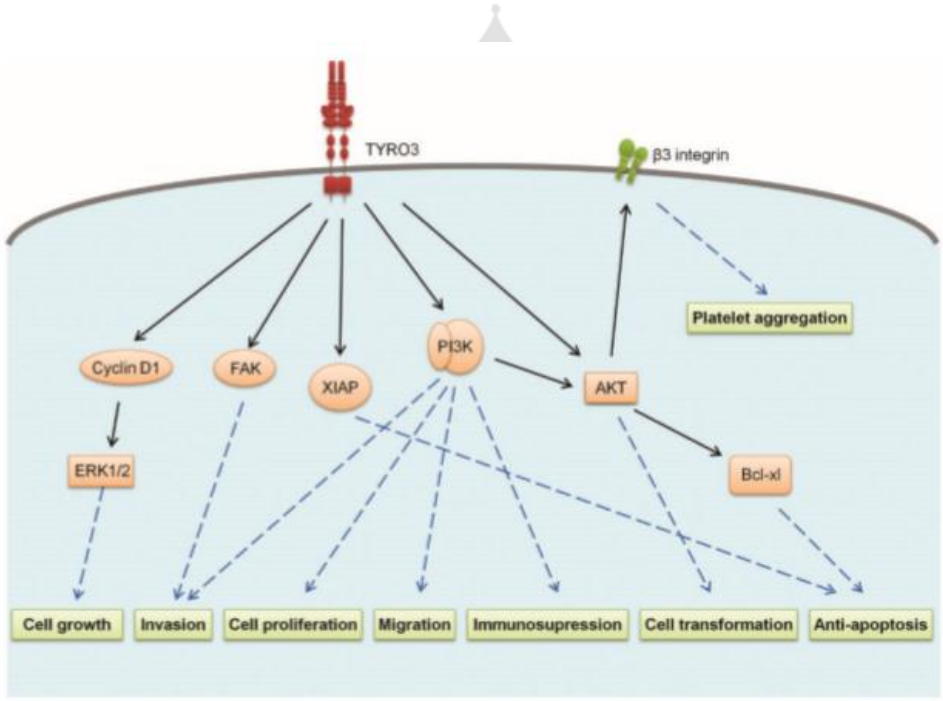

Figure 2. Schematic representation of TYRO3 signaling pathway. TYRO3 has been reported to mediate numerous cellular activities, including platelet aggregation, cell growth, invasion, cell proliferation, migration, immunosupression, cell transformation, and anti-apoptosis. Molecules in orange have been shown to associate with TYROS through either a direct or indirect interaction. (A color version of this figure is available in the online journal.)

Figure No. 2:: Schematic representation of TYRO3 Signalling pathway.

TYRO3 signalling pathways

The TYRO3 receptor is the least studied TAM receptors with largely unknown signalling pathways. However, some sporadic studies did report the involvement of different signalling molecules involved in transmitting signals of TYRO3. One study indicated a possible interaction between TYRO3 and a phosphorylated Src family kinase in COS cells[61]. Previous studies identified multiple proteins that may interact with TYRO3, including Ran binding protein in microtubule organizing center, protein phosphatase 1, and the p85 b-subunit of PI3K[62]. Another study showed that Pros1/TYRO3 activates the PI3K/AKT pathway, which protects neurons from excitotoxic injury and apoptosis in mouse cortical neurons [63]. Epidermal growth factor receptor (EGFR)/TYRO3 chimeric receptor also showed that the cytoplasmic domain of TYRO3 associated with PI3K and led to transformation of NIH3T3 cells. The mitogen-activated protein kinase (MAPK) signalling pathway has also been linked to TYRO3 activation. It has been shown that Gas6 increased the phosphorylation of AKT and ERK1/2. TYRO 3 acts on mature osteoclasts through activation of ERK1/2 MAPK, possibly contributing to the bone loss by estrogen deficiency [64]. TYRO3 also regulates the proliferation of MCF-7 breast cancer cells through control of cyclin D1 expression and phosphorylation of ERK1/2 or STAT3. These studies reveal that TYRO3 may utilize distinct signalling pathways to transmit its message in different cell types. Further study is warranted to systemically characterize the second messengers directly downstream of TYRO3 [65].

Therapeutic potential of targeting TYRO3

Because the TAM family has been implicated in the pathogenesis of several cancers, the therapeutic potential of targeting the TAM family has been validated. To date, many pan-TAM inhibitors have proven to be both efficacious and less toxic than standard chemotherapies. These inhibitors may prevent activation of the TAM family and other RTKs leading to promotion of cell death, thereby preventing cancer metastasis. It is found that the selectivity is primarily determined by the size and configuration of kinase's ATP binding site. The location and shape of active-site residues of MER and AXL are highly consistent, suggesting that small-molecule inhibitors generally have a low MER over-AXL selectivity and a high MER-over-TYRO3 selectivity. Due to the centrality of TYRO3 in immune disease and cancer development, there is interest in targeting TYRO3. A recent study showed that TYRO3 was overexpressed in nearly all melanoma cell lines. Knockdown of TYRO3 by short hairpin RNA led to significantly inhibited cell proliferation [66]. Administration of anti-TYRO3 antibody

increased cell death signal and drug sensitivity *in vitro* and *in vivo* [67]. Furthermore, miR-7 was identified as a potent tumor suppressor of human hepatocellular carcinoma. It targets TYRO3 and regulates proliferation, migration, and invasion through the PI3K/protein kinase B pathway [68]. These findings indicated that TYRO3 is a druggable target in cancer. However, because no specific TYRO3-targeted drugs are available, the role of specific inhibitors against TYRO3 as a therapeutic target has not previously been evaluated in detail. Below is a summary of some representative TAM inhibitors that also work on TYRO3.

Metformin

Since the primary effect of metformin is glucose metabolism modification, it has been widely used to treat type 2 diabetes [69]. Accumulating evidence suggests it also possesses anticancer effects on cell proliferation in various cancers and tumor growth in xenograft model [70]. The efficacy of metformin for the treatment of endometrial, cervical, breast, and ovarian cancer has been suggested in preclinical studies and clinical trials [71]. The anticancer mechanisms of metformin have been assessed by its ability to inhibit pro-survival and anti-apoptotic signals mediated by mammalian target of rapamycin complex 1, EGFR, and MAPK [72]. Metformin may target TYRO3 to prevent cell proliferation and reduce chemo-resistance [73]. Collectively, these studies indicated a potent therapeutic strategy to facilitate the anticancer activity of metformin and overcome chemoresistance in cancer cells.

Compounds 21 and 24

High-throughput screening identified a novel series of spiroindoline-based inhibitors as the first TYRO3-selective tyrosine kinase inhibitors [74]. Among these, compounds 21 and 24, 2,4-diaminopyrimidine-5-carboxamide inhibitors, are potent inhibitors of TYRO3 kinase (Sky IC50 ¼0.0007 mM and 0.015 mM, respectively).156 The compound 21, which replaces the entire amide sidechain with a 3-methylisoxazole from an 2, 4-diaminopyrimidine-5-carboxamide inhibitor, exhibited excellent selectivity in 46/48 kinases with some activity in MAP4K4 and Mer. Compound 24 which replaces amide sidechain by a simple bromine atom has moderate functional P-selectin inhibition, good human liver microsomes, and rat liver microsomes metabolism stability. However, the low aqueous solubility and PAMPA permeability were not predictive of good oral bioavailability [75].

LDC1267

LDC1267 is an inhibitor of the TAM kinase family in cells at low nanomolar levels with IC50 ofRXDX-106 RXDX-106 is an oral immunomodulatory agent that can restore and enhance overall immune function. It inhibits the activity of TAM- and c-Met-induced pro-tumorigenesis by a decrease in downstream MAPK and PI3K signaling and cell viability. RXDX-106 could reverse immunosuppression of innate immune cells, inhibit tumors harboring activating TAM gene fusions, and affect the TAM-expressing tumor microenvironment resulting in a global anti-cancer environment. Phase 1 study is expected to commence in early 2018 [76].

6g

The affinity of 6g for AXL, MER, Met, and TYRO3 is 39, 42, 65, and 200 nM, respectively. The absence of cytotoxicity against several tumor cell lines in culture makes this inhibitor a good candidate for the growth inhibition of tumor cells that would overexpress a gene belonging to the TAM subfamily [77].

Sitravatinib (MGCD516)

MGCD516 is a novel small molecule inhibitor targeting TAM family (IC50 < 1 nM) and multiple RTKs involved in driving sarcoma cell growth. MGCD516 treatment induced potent anti-proliferative effects in vitro and suppressed tumor growth *in vivo*[78]. As an immuno-oncology agent, MGCD516 may target the tumor microenvironment, resulting in innate and adaptive immune cell changes that augment immune checkpoint blockade [79].MGCD516 is being evaluated in combination with checkpoint blockade (nivolumab) for refractory non-small cell lung cancer in phase 2 clinical study [80].

UNC compound 5

More recently, UNC2541-derived compound 5, a TYRO3- selective pyrrolopyrimidine-based inhibitor was mentioned. They reported this inhibitor with more selective against TYRO3 over MER (3-fold) and AXL (31-fold). However, the pharmacokinetic properties of Compound 5 are unclear. Thus, more work is needed in the development of more potent TYRO3 inhibitors [81].

Current and future development

Despite numerous efforts, many traditional therapies are ineffective due to the pathological and etiological complexity of cancer. As with most drugs, chemotherapy drugs do have side effects. Therefore, it is important to develop effective and safe strategies for cancer prevention and treatment. The important role of TYRO3 in cancer development has been elucidated. Targeting TYRO3 represents a novel therapeutic approach by suppressing tumor cell survival, proliferation, invasion, chemoresistance, and de-repression of the immune activities. Therefore, therapeutic TYRO3 inhibition may sensitize tumor cells to killing by chemotherapy, radiation, or other targeted agents. Specific TYRO3- targeted drug may enhance immunotherapeutic efficacy in combination with immune checkpoint inhibitors. If these combination therapies are effective against metastatic disease, then TYRO3-targeted drug could be used in early stages as an adjuvant to provide cancer patients with new options for durable responses. However, development of autoimmunity is a consideration for TYRO3 inhibition treatment. Although many potent drugs have been developed as mention in this review, including different compounds, multi-target TYRO3 inhibitors, and antibody, the ability of these drugs to defeat cancers by TYRO3 and reduce drug resistance is unclear. A better understanding of TYRO3 could lead to more effective anti-cancer strategies.

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