Minireview

TYRO3: A potential therapeutic target in cancer

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Impact statement

Cancer is among the leading causes of death worldwide. In 2016, 8.9 million people are estimated to have died from various forms of 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 developing novel therapy strategies. In this review, we summarize the molecular biology of a unique member of a subfamily of receptor tyrosine kinase, TYRO3 and discuss the new insights in TYRO3-targeted treatment for cancer therapy.

Abstract

TYRO3 belongs to the TAM (TYRO3, AXL, and MER) receptor family, a unique subfamily of the receptor tyrosine kinases. Members of TAM family share the same ligand, growth arrest-specific 6, and protein S. Although the signal transduction pathways of TYRO3 have not been evaluated in detail, overexpression and activation of TYRO3 receptor tyrosine kinase have been reported to promote cell proliferation, survival, tumorigenesis, migration, invasion, epithelial-mesenchymal transition, or chemoresistance in several human cancers. Targeting TYRO3 could break the kinase signaling, stimulate antitumor immunity, reduce tumor 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 this review, we 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.

Keywords: Cancer, drug discovery, TAM, therapeutic potential, TYRO3

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Introduction

Cancer is among the leading causes of death worldwide. In 2016, 8.9 million people are estimated to have died from various forms of 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. 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.[1] 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.[2,3] However, emerging evidence has demonstrated the oncogenic effect of TYRO3 in promoting the survival, chemoresistance, tumorigenesis, and metastasis of cancer cells.[1] This review summaries recent advances about the mechanisms regulated by the TYRO3 to promote oncogenesis. In addition, we will also discuss possible strategies of targeting TYRO3 as an anticancer regimen.

Molecular biology of TYRO3 receptor

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)). TYRO3 receptors bind to their ligands through the Ig-like domains. Gas6 and Pros1 are established nature ligands for TYRO3. After ligand binding, tyrosine residues of TYRO3 are autophosphorylated and downstream signaling 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.[4] These data suggest that even in the absence of its ligand, TYRO3 retains all the properties of the full-length TYRO3 kinase.

There are many aliases for *Tyro3* gene as it was cloned from multiple species by different research groups. In 1991,

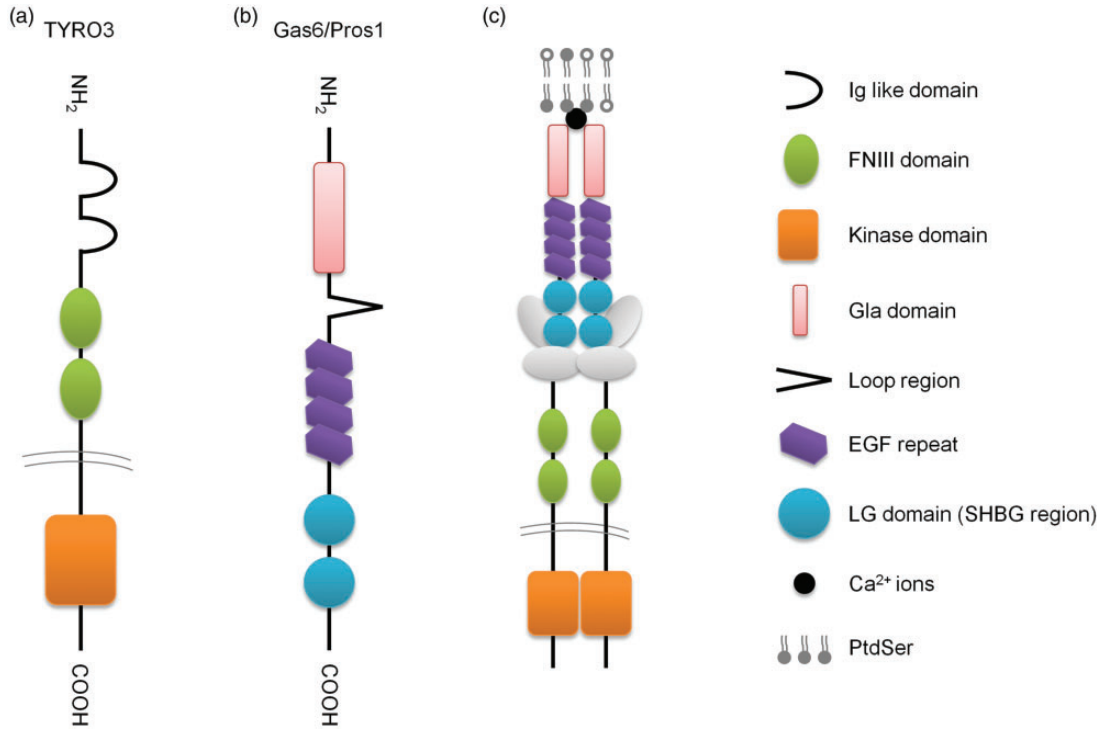


Figure 1. Schematic representation of TYRO3 receptor and ligands protein structure. (a) TYRO3 receptors carry two immunoglobulin (Ig)-like domains in their N-terminus, followed by two fibronectin type III repeats, a transmembrane region and a tyrosine kinase domain in the C-terminal intracellular region. (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.)

Tyro1 to *Tyro13* were found from rat brain.[2] *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.[11] There are three splicing variants for *TYRO3* that contain exons 2A, 2B, and 2C, respectively.[11–13] These exons encode different signaling peptide sequences, indicating that the expression of these alternative splicing variants may affect the subcellular localization and thus the function of TYRO3.

Ligands and structures

The endogenous ligands for TYRO3 receptors are the Gas6 and Pros1. The structure of Gas6 and Pros1 is related to vitamin K. They share approximately 40% sequence identities with an N-terminal γ-carboxyglutamic acid domain, four tandem EGF-like domains, and a C-terminal sex hormone-binding globulin domain (Figure 1(b)).[14,15] Pros1 is known to regulate anticoagulation and complement cascades. It can be purified using TYRO3-phosphorylating activity as an indicator[16] since purified recombinant murine Pros1 binds to and activates both MER and TYRO3 (TYRO3>MER).[17] Currently, there is no evidence that Pros1 activates AXL. Gas6 was originally identified based on its dramatic upregulation after growth arrest with unknown function.[18,19] In 1995, it was reported that Gas6 could bind and activate AXL.[16,20] Shortly thereafter, Gas6 was found to activate all TAM receptors (AXL>TYRO3⋙MER).[21] Since the secretion signal and the γ-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,[22] especially when it is γ-carboxylated.[23,24] 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 signaling pathways including phosphatidylinositol 3-kinase (PI3K)/AKT, ERK, and PLC-γ (Figure 1(c)).[25–27] 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.[28] By co-immunoprecipitation, Tulp1 was found to interact with MER, AXL, and TYRO3, while Tulp2 can be

co-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 homo- and/or hetero-dimerization of TAM receptors, though it is unclear whether one or multiple receptors will be bound.[29] Interestingly, Tulp proteins lack signal peptide and have been identified as intracellular proteins by immunohistochemistry.[30] 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.[31] Indeed, Caberoy and Li[32] 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.

TYRO3 in development

The expression of TYRO3 was found in embryonic developing mouse tissue, including undifferentiated mouse embryonic stem cells. Therefore, TYRO3 was initially named Developmental Tyrosine Kinase.[5] The TAM receptors play overlapping and unique roles in regulating immunity and inflammation. Inactivation of all TAM receptors does not lead to embryonic lethality, indicating the overlapping and compensatory roles of TAM receptors demonstrated in various cell types for embryogenesis.[13,33] However, TYRO3 receptor and their ligands are largely expressed during development in gastrointestinal, nervous, and reproductive systems.[5,13] Inactivation of TYRO3 receptors or their ligands affects postnatal developmental processes. For example, the neural degeneration with seizures and paralysis is observed in *Tyro3* knockout mice.[13] With aging, *Tyro3*-null mice develop cerebellar ataxia, and immune defects are observed with the progressive loss of the TAM family.[29] A previous study indicated that Gas6 supports gonadotropin-releasing hormone (GnRH) neuronal survival through AXL and TYRO3, which are expressed by migratory GnRH neurons. *Axl* and *Tyro3* deficiencies resulted in increased apoptosis and delayed migration with a reduction in GnRH neurons reaching their final destination.[34] This strain manifested later in adulthood in delayed sexual maturation and irregular estrus cycles with a significantly prolonged proestrus phase.[34] Moreover, triple knockouts of *TAM* receptors demonstrated multiple debilitating and degenerative traits, most importantly, impaired hemostasis, immunoregulation, spermatogenesis, and blindness.[13,35]

Disease relevance of TYRO3

TYRO3 appears to have a critical role in the immunity, phagocytosis, hemostasis, and neuronal disease. The first evidence suggests TYRO3 may be involved in platelet aggregation came from studies of *Gas6*-null mice. Knockout of *Gas6* protects mice from thrombosis and causes platelet dysfunction.[36] A follow-up study demonstrated that platelet granule secretion was limited in $Tyro3^{-/-}$ mice. This strain demonstrated reduced thrombus formation and decreased platelet aggregation stability.[37] In neuron, TYRO3 activation due to progranulin reduction results in activation of PKCα via PLCγ, inducing tau phosphorylation, mislocalization of tau to dendritic spines, and spine loss.[38]Genetic variants of *TYRO3* were associated with an increased risk for several immune-related disorders. Functional *TYRO3* variants alter gene expression in the pathogenesis of allergic sensitization and allergic rhinitis. Multiple intronic variants in *TYRO3* were also associated with asthma. The most significant association was at a single-nucleotide polymorphism located within several putative transcription factor binding sites.[39] Moreover, TYRO3 contributes to anxiety phenotypes and can delay hypothalamic neurodegeneration in anx/anx mice. In their study, Kim *et al*.[40] identified a mutation in the signal sequence of the *Tyro3* (R7W-Tyro3) as an enhancing anx phenotype.

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.[8] 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/NFκB signaling, TYRO3 exerts pro-survival effects and promotes cancer cell growth.[42] TYRO3 and AXL protein levels are undetectable in normal thyroid cells but significantly upregulated and activated in thyroid cancer cells.[43] 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.[44]

Activated TYRO3 promotes the survival, invasion, migration, proliferation, and transformation of cancer cells (Figure 2).[45,46] Increasingly, evidence supporting the notion that overexpression of TYRO3 contributes to the resistance to conventional and targeted therapies in thyroid cancer, and blocking its signaling dramatically reduced cell viability and resistance to apoptotic stimuli.[43] TYRO3 was also shown to promote cell proliferation and chemoresistance in breast cancer.[142] Increased resistance to platinum and taxol secondary to TYRO3 overexpression has also been reported in ovarian cancer.[47] Ovarian cancer cells

Table 1. Expression and function of TAM family kinases and ligands in cancer.

TAM kinase or ligand	Ectopic expression or overexpression	Functional roles (proliferation, survival, or tumorigenesis)	Metastatic roles (migration, invasion, or EMT)	Roles in chemoresistance	Prognostic importance	References
TYRO3	Multiple myeloma, lung cancer, breast cancer, melanoma, esophageal cancer, hepatocellular carcinoma, colorectal cancer, prostate cancer, ovarian cancer, endometrial cancer, leiomyosarcoma, thyroid cancer, schwannoma, ALL, AML, CML, and B-CLL	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, breast cancer	41–43,45–69
AXL	Lung cancer, glioblastoma, breast cancer, colorectal cancer, gastric cancer, pancreatic cancer, oesophageal cancer, melanoma, squamous cell skin cancer, prostate cancer, endometrial cancer, ovarian cancer, oral squamous cell carcinoma, thyroid cancer, bladder cancer, renal cancer, schwannoma, mesothelioma, Kaposi's sarcoma, osteosarcoma, AML, CML, and B-CLL,	Prostate cancer, ovarian cancer, breast cancer, thyroid cancer, lung cancer, pancreatic cancer, melanoma, hepatocellular carcinoma, glioblastoma, 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, mesothelioma, oesophageal cancer, glioblastoma, colorectal cancer, cervical cancer, neuroblastoma, and osteosarcoma	Breast cancer, lung cancer, ovarian cancer, oesophageal cancer, AML, and CML	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	43,55,57–59, 70–110
MER	Lung cancer, glioma, melanoma, prostate cancer, schwannoma, mantle 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	57,59,73,79, 83,97,99, 110–118
Gas6	Multiple myeloma, glioblastoma, 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, osteosarcoma, and renal cancer	ALL	Lung cancer, glioblastoma and renal cancer, and AML	43,57–59,78,86, 91,94,96,101, 119–129
Pros1	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	130–141

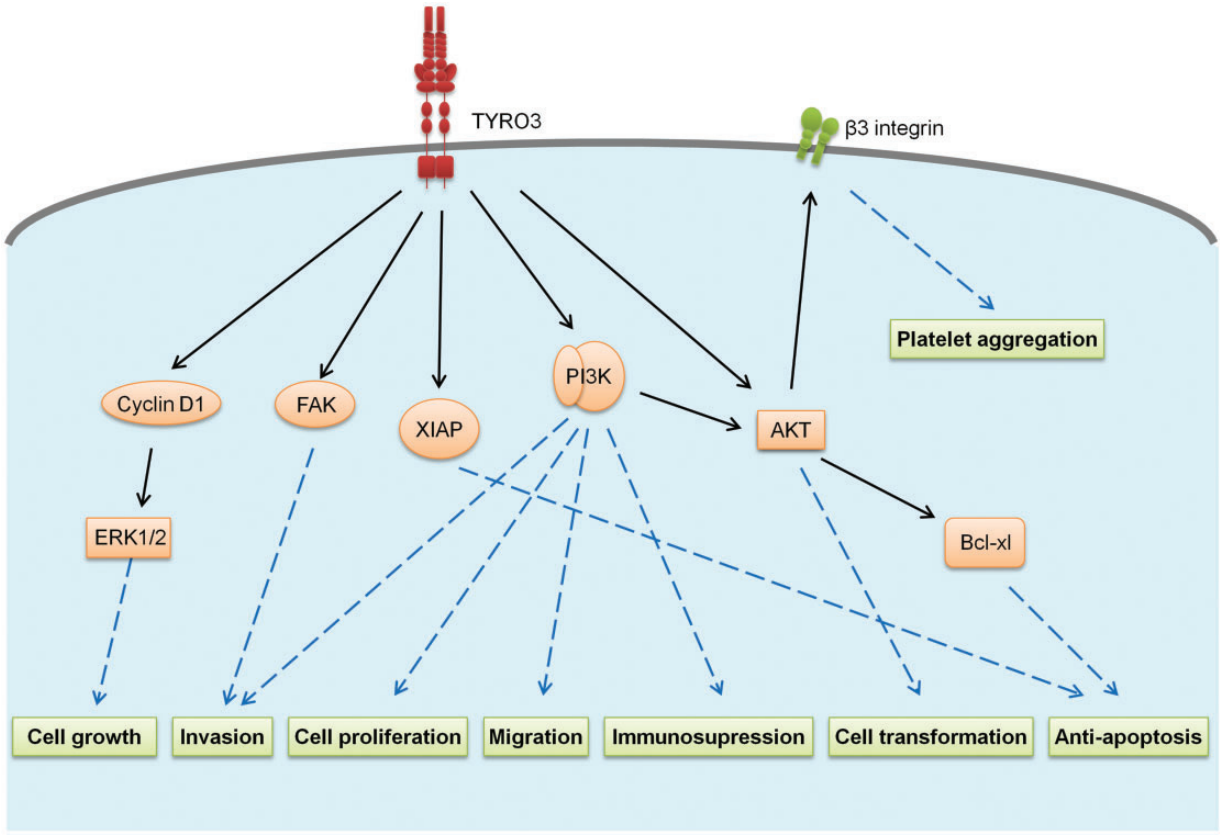


Figure 2. Schematic representation of TYRO3 signaling pathway. TYRO3 has been reported to mediate numerous cellular activities, including platelet aggregation, cell growth, invasion, cell proliferation, migration, immunosupression, cell transformation, and anti-apoptosis. Molecules in orange have been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

overcome treatment resistance via upregulation of TYRO3 and AXL expression, AKT phosphorylation, and Bcl-xl expression.[47] A growing body of evidence demonstrated that epithelial-mesenchymal transition may be a major mechanism in drug resistance.[143,144] TYRO3 promotes phagocytosis and inhibits inflammation, allowing resistance to antitumor treatments to further cancer progression.[145] Interestingly, recent study demonstrated that treatment with the human TYRO3 antibody abolished TYRO3-induced EMT process in colon cancer.[48] Taken together, these studies suggested that inhibition of TYRO3 and its signaling pathways could have therapeutic benefits in cancer development.

TYRO3 signaling pathways

The TYRO3 receptor is the least studied TAM receptors with largely unknown signaling pathways. However, some sporadic studies did report the involvement of different signaling molecules involved in transmitting signals of TYRO3 (Figure 2). One study indicated a possible interaction between TYRO3 and a phosphorylated Src family kinase in COS cells.[146] Previous studies identified multiple proteins that may interact with TYRO3, including Ran binding protein in microtubule organizing center, protein phosphatase 1, and the p85 β-subunit of PI3K.[147,148] Another study showed that Pros1/TYRO3 activates the PI3K/AKT pathway, which protects neurons from excitotoxic injury and apoptosis in mouse cortical neurons.[149,150] 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.[147] The mitogen-activated protein kinase (MAPK) signaling pathway has also been linked to TYRO3 activation. It has been shown that Gas6 increased the phosphorylation of AKT and ERK1/2.[151] TYRO 3 acts on mature osteoclasts through activation of ERK1/2 MAPK, possibly contributing to the bone loss by estrogen deficiency.[152] TYRO3 also regulates the proliferation of MCF-7 breast cancer cells through control of cyclin D1 expression and phosphorylation of ERK1/2 or STAT3.[142,153] These studies reveal that TYRO3 may utilize distinct signaling pathways to transmit its message in different cell types. Further study is warranted to systemically characterize the second messengers directly downstream of TYRO3.

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.[49,50] Administration of anti-TYRO3 antibody increased cell death signal and drug sensitivity *in vitro* and *in vivo*.[48,49] 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.[45] 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 (Table 2).

Metformin

Since the primary effect of metformin is glucose metabolism modification, it has been widely used to treat type 2 diabetes.[173] Accumulating evidence suggests it also possesses anticancer effects on cell proliferation in various cancers and tumor growth in xenograft model.[174–177] The efficacy of metformin for the treatment of endometrial, cervical, breast, and ovarian cancer has been suggested in preclinical studies and clinical trials.[154] 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.[178–181] Metformin may target TYRO3 to prevent cell proliferation and reduce chemoresistance.[155] 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.[156,182] Among these, compounds 21 and 24, 2,4-diaminopyrimidine-5-carboxamide inhibitors, are potent inhibitors of TYRO3 kinase (Sky IC_{50} =0.0007 μM and 0.015 μM, 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.

LDC1267

LDC1267 is an inhibitor of the TAM kinase family in cells at low nanomolar levels with IC50 of <5 nM, 8 nM, and 29 nM for MER, TYRO3, and AXL, respectively.[157] Treatment of wild-type natural killer cells with LDC1267 conferred therapeutic potential, efficiently enhancing anti-metastatic NK cell activity *in vivo*. Administration of LDC1267 markedly reduced murine mammary cancer development and metastasis of melanoma.[157]

BMS-777607

BMS-777607 is a small molecule inhibitor for c-Met, AXL, Ron, and TYRO3 with IC50 of 3.9 nM, 1.1 nM, 1.8 nM, and 4.3 nM, 40-fold more selective for Met-related targets versus Lck, VEGFR-2, and TrkA/B, and more than 500-fold greater selectivity versus all other receptor and non-receptor kinases.[158] Previous studies demonstrated the effect of BMS-777607 in antitumor treatment may be mediated by blocking hepatocyte growth factor-stimulated phosphorylation of Met and downstream pathways.[159,183,184] BMS-777607 inhibits hepatocyte growth factor-stimulated cell scatter, motility, and invasion *in vitro*.[159,185] Surprisingly, BMS777607 was also reported to have inhibitory effects on TYRO3 in cell-free conditions.[169] However, the efficacy of BMS-777607 against TYRO3 as a therapeutic target has not been evaluated. Due to its potency and promising pharmacokinetic and preclinical safety profiles, BMS-777607 has been advanced into phase 1/2 clinical trials.

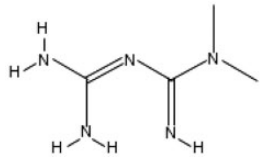
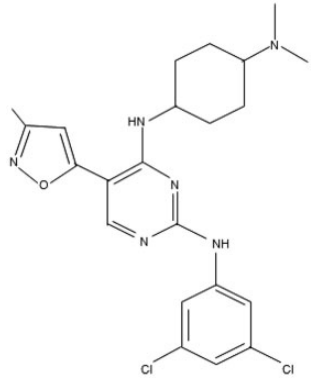
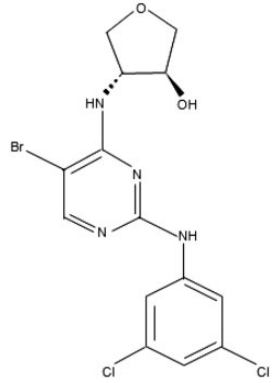
UNC2250

UNC2250 is a potent and selective MER inhibitor with IC50 of 1.7 nM, about 160- and 60-fold selectivity over the closely related kinases AXL/TYRO3.[165] MER inhibition by UNC2250 decreased activation of downstream AKT and p38, inhibited proliferation and invasion in mantle cell lymphoma (MCL), and sensitized MCL cells to treatment with vincristine *in vitro* and doxorubicin *in vitro* and *in vivo*.[166]

UNC2881

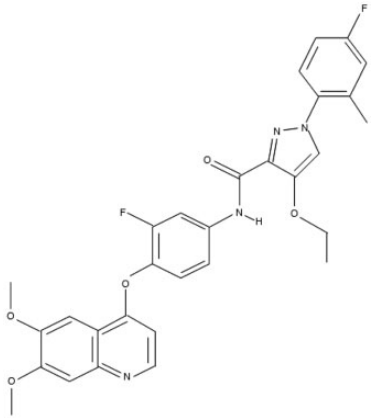
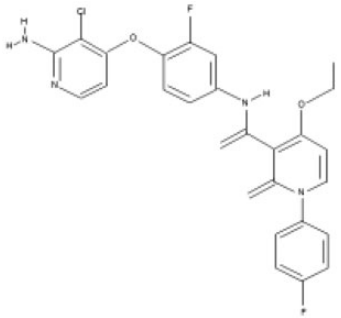
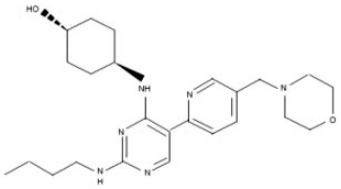
UNC2881 is a specific MER tyrosine kinase inhibitor with IC50 of 4.3 nM, about 83- and 58-fold selectivity over AXL and TYRO3, respectively. UNC2881 inhibits steady-state MER kinase phosphorylation with an IC50 value of 22 nM *in vitro*. Treatment with UNC2881 is also sufficient to block EGF-mediated stimulation of EGFR/MER chimeric receptor. In addition, UNC2881 may have utility for prevention and/or treatment of pathologic thrombosis by inhibiting platelet aggregation.[167]

Table 2. TYRO3 as a therapeutic target.

Name / Structure	Target profile					
		Activity				
	Target(s)	TYRO3	AXL	MER	Development stage/ outcome	References
Metformin 	TYRO3, AXL	–	–	–	Approved (Diabetes) Approved for ovarian cancer cells, endometrial, breast, and ovarian cancer	154,155
Compound 21 	TYRO3	IC_{50}=0.7 nM	–	–	Preclinical	156
Compound 24 	TYRO3	IC_{50}=15 nM	–	–	Preclinical	156

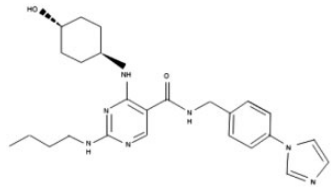
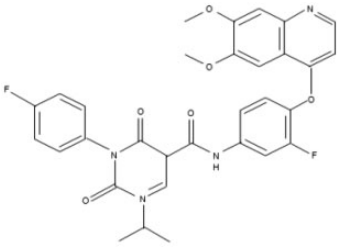
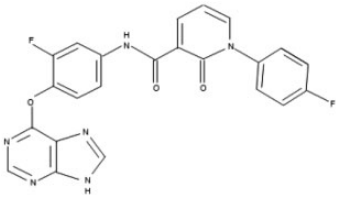
(continued)

Table 2. Continued

Name / Structure	Target profile				Development stage/ outcome	References
	Target(s)	Activity TYRO3	AXL	MER		
LDC1267 	TYRO3,AXL, MER	IC_{50}=8 nM	IC_{50}=29 nM	IC_{50}<5 nM	Preclinical Approved for murine mammary cancer and melanoma metastases dependent on NK cells	157
BMS-777607 	c-Met, TYRO3,AXL, Ron,	IC_{50}=4.3 nM	IC_{50}=1.1 nM	–	Phase 1/2. Approved for advanced or metastatic solid tumors	158–164
UNC2250 	TYRO3,AXL, MER	IC_{50}~100 nM	IC_{50}~270 nM	IC_{50}=1.7 nM	Phase 1 Approved for mantle cell lymphoma	165,166

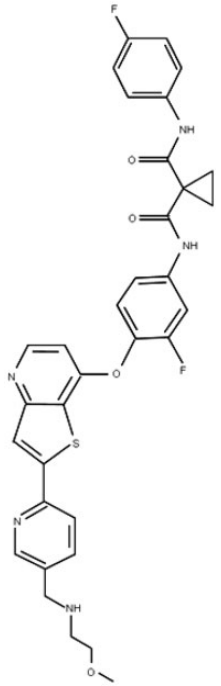
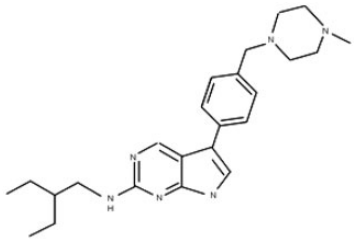
(continued)

Table 2. Continued

Name / Structure	Target profile				Development stage/ outcome	References
		Activity				
	Target(s)	TYRO3	AXL	MER		
UNC2881 	TYRO3,AXL, MER	IC_{50}~250 nM	IC_{50}~350 nM	IC_{50}=4.3 nM	Preclinical Approved for pathologic thrombosis	[167]
RXDX-106 	TYRO3,AXL, MER, c-Met	IC_{50}=19 nM	IC_{50}=7 nM	IC_{50}=29 nM	Phase 1 Approved for immuosuppression of innate immune cells and gastric cancer cell lines	[168]
6g 	TYRO3,AXL, MER, Met	Kd=200 nM	Kd=39 nM	Kd=42 nM	Preclinical	[169]

(continued)

Table 2. Continued

Name / Structure	Target profile				Development stage/ outcome	References
	Target(s)	Activity TYRO3	AXL	MER		
Sitravatinib (MGCD516) 	TYRO3,AXL, MER	IC_{50}<1 nM	IC_{50}<1 nM	IC_{50}<1 nM	Phase 1/2	170,171
UNC Compound 5 	TYRO3	IC_{50}=6.7 nM	IC_{50}=206 nM	IC_{50}=19 nM	Preclinical	172
shRNA	TYRO3	–	–	–	Preclinical Approved for leiomyosarcoma and melanoma	49,50
Antibodies	TYRO3	–	–	–	Preclinical Approved for colon cancer and melanoma cell lines	48,49

Kd=dissociation constant.

RXDX-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 protumorigenesis by a decrease in downstream MAPK and PI3K signaling and cell viability. RXDX-106 could reverse immuosuppression 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.[168] Phase 1 study is expected to commence in early 2018.

6g

The affinity of 6g for AXL, MER, Met, and TYRO3 is 39, 42, 65, and 200 nM, respectively.[169] 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.

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*.[170] As an immuno-oncology agent, MGCD516 may target the tumor microenvironment, resulting in innate and adaptive immune cell changes that augment immune checkpoint blockade.[171]MGCD516 is being evaluated in combination with checkpoint blockade (nivolumab) for refractory non-small cell lung cancer in phase 2 clinical study.[171]

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).[172] However, the pharmacokinetic properties of Compound 5 are unclear. Thus, more work is needed in the development of more potent TYRO3 inhibitors.

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 anticancer strategies.

Authors' contributions: PL Hsu and J Jou wrote the article, SJ Tsai conceived and coordinated the project and revised the article.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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