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Roles for the Type III TGF- β Receptor in Human Cancer

Catherine E. Gatza¹, Sun Young Oh¹, and Gerard C. Blobe^{1,2,*}

¹Department of Medicine, Duke University Medical Center, Durham, NC, USA

²Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC, USA

Abstract

Transforming growth factor β (TGF- β) superfamily ligands have important roles in regulating cellular homeostasis, embryonic development, differentiation, proliferation, immune surveillance, angiogenesis, motility, and apoptosis in a cell type and context specific manner. TGF- β superfamily signaling pathways also have diverse roles in human cancer, functioning to either suppress or promote cancer progression. The TGF- β superfamily co-receptor, the type III TGF- β receptor (T β RIII, also known as betaglycan) mediates TGF- β superfamily ligand dependent as well as ligand independent signaling to both Smad and non-Smad signaling pathways. Loss of T β RIII expression during cancer progression and direct effects of T β RIII on regulating cell migration, invasion, proliferation, and angiogenesis support a role for T β RIII as a suppressor of cancer progression and/or as a metastasis suppressor. Defining the physiological function and mechanism of T β RIII action and alterations in T β RIII function during cancer progression should enable more effective targeting of T β RIII and T β RIII mediated functions for the diagnosis and treatment of human cancer.

Keywords

T β RIII; TGF- β ; BMP; cancer; receptor

Introduction

Transforming growth factor β (TGF- β) superfamily ligands mediate and regulate cellular homeostasis, including embryonic development, differentiation, proliferation, immune surveillance, angiogenesis, motility, and apoptosis, in both a cell type and context specific manner [1]. TGF- β superfamily signaling pathways also have diverse roles in human disease, with both increases and decreases in these signaling pathways resulting in human disease [2]. In human cancers, TGF- β superfamily ligands and their pathways function as both tumor suppressors and tumor promoters [2-5]. TGF- β superfamily ligands function as tumor suppressors through the ability to inhibit cell proliferation, maintain tissue architecture, inhibit genomic instability, and induce senescence and apoptosis [6-8]. Alterations in many components of the TGF- β superfamily signaling pathways, such as mutation or deletion of receptors and other signaling components, are frequent events in human cancers and lead to a

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*Corresponding Author: Gerard C. Blobe, Duke University Medical Center, Department of Medicine, B354 LSRC, Box 91004, Durham NC, 27708, 919-668-1352, Fax: 919-681-6906, gerard.blobe@duke.edu.

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loss of the tumor suppressor function of these pathways [7,9]. Conversely, many late stage human tumors dysregulate or over-express TGF- β superfamily ligands, which can have tumor promoting effects on both the stromal compartment (suppressing immune surveillance, enhancing angiogenesis) and on the cancer cells themselves (inducing epithelial-to-mesenchymal transition (EMT), enhancing migration and invasion) to promote tumor invasiveness and metastasis [6-7,10-11]. Precise mechanisms for this dual tumor suppressor/tumor promoter role for TGF- β superfamily ligands remains a fundamental problem in the TGF- β superfamily signaling field and major obstacle to targeting these pathways for the treatment of human cancers.

Recently, loss or reduced expression of the TGF- β superfamily co-receptor, the type III TGF- β receptor (T β RIII, also known as betaglycan), has been demonstrated in multiple human cancers (Table 1) [12-17]. T β RIII has also been shown to be an important regulator of cell migration, invasion, cell growth, and angiogenesis in both *in vitro* and *in vivo* cancer models. Taken together, these results support a role for T β RIII as a mediator of TGF- β superfamily function during cancer progression [18]. Here we review what is currently known about T β RIII, both in the context of TGF- β superfamily signaling and in human cancer biology, supporting T β RIII as a guardian of the epithelial phenotype, as a suppressor of cancer progression, and as a metastasis suppressor.

Regulation, Structure, and Function of the Type III TGF- β Receptor

The gene encoding T β RIII, the TGFBR3 gene, is located at chromosome 1p31-32, consists of 16 exons, and has two promoters, a proximal promoter and a distal promoter, which produce two mRNA species [19-20]. In most tissue types, the proximal transcript is the predominant promoter utilized [19-20]. While no mutations in the TGFBR3 gene have been reported in human disease, the chromosomal locus for the TGFBR3 gene, 1p31-32, frequently is lost in human cancer, and loss of heterozygosity of the TGFBR3 gene has been reported in several cancer including breast, prostate, and lung cancer as discussed below [12,14,17,21]. In addition, several studies have identified single nucleotide polymorphisms (SNPs) in the TGFBR3 3' untranslated region (UTR), open reading frame (ORF), and in the 5'UTR [22-23]. However, these SNPs have not been linked to any physiological effects [22-23].

T β RIII is ubiquitously expressed on nearly all cell types and is the mostly highly expressed of the TGF- β superfamily receptors on those cells, with at least 200,000 receptors/cell as opposed to 5,000-10,000 receptors/cell for most TGF- β superfamily type I and type II receptors [19, 24-26]. However, some cell types, including endothelial cells, appear to have little or no T β RIII expression, but instead express the related TGF- β superfamily co-receptor, endoglin [27].

T β RIII expression is regulated at multiple levels. At the transcriptional level, T β RIII expression is negatively regulated by TGF- β 1 through inhibition of the proximal promoter in multiple cell types, including breast, ovarian, and small cell lung cancer cell lines, as well as by bone morphogenetic protein-2 (BMP-2) in osteoblast cell lines [20,28-29]. Expression of T β RIII mRNA has also been reported to decrease with transdifferentiation of hepatic stellate cells (HSC) to myofibroblasts [30] and during induction of EMT in a pancreatic model [16]. In contrast corticosteroids, specifically dexamethasone, aldosterone, and hydrocortisone, transcriptionally up-regulate T β RIII mRNA expression in hepatic stellate cells (HSCs), myofibroblasts, and osteoblasts while both MyoD and retinoic acid increase T β RIII expression in mouse myoblast cells [28-31]. In addition, follicle-stimulating hormone, estrogen, and luteinizing hormone increase T β RIII mRNA expression in ovarian granulosa-luteal cells [32-33]. T β RIII mRNA expression appears to be highly regulated and required during development, demonstrated by the embryonic lethality of the T β RIII null mouse [34-36].

T β RIII expression is increased during C2C12 skeletal muscle differentiation, with an increase in T β RIII expression during the conversion of myoblasts to myotubes [31]. T β RIII expression is also regulated during palatal fusion, with expression temporo-spatially restricted to the medial edge epithelium, suggesting that T β RIII mediates TGF- β 3 effects during palatal fusion [37].

Expression of T β RIII is also regulated at the epigenetic level. Treatment of ovarian and prostate cancer cells that express low levels of T β RIII with methyltransferase inhibitors and/or histone deacetylase inhibitors induces T β RIII expression, indicating that either direct or indirect epigenetic silencing plays a role in the loss of T β RIII expression in these cancer types [14-15]. Interestingly, this methyltransferase and/or histone deacetylase inhibitor mediated induction of T β RIII expression is specific to ovarian cancer cells with low levels of T β RIII, with low to no induction observed in normal ovarian epithelial cells or ovarian cancer cells with basal levels of T β RIII [15]. In addition, the distal TGFBR3 promoter has been shown to be highly methylated, although this has not been shown to correlate with T β RIII expression and effects of epigenetic silencing of this promoter region remain to be examined further [20].

Levels of T β RIII expression are also regulated at the protein level. T β RIII interacts with the PDZ-protein GAIP-interacting protein, C terminus (GIPC), which stabilizes T β RIII at the cell surface and enhances TGF- β signaling [38]. Conversely, interaction with β -arrestin2 mediates co-internalization of T β RIII, T β RII, and T β RI and down-regulation of receptor levels, causing a decrease in TGF- β signaling [39-40]. In addition, the induction of EMT in a pancreatic cell line causes a reduction in cell surface T β RIII levels due to increased ectodomain shedding [16]. T β RIII protein levels also decrease in response to exposure to chronic hypoxia in neonatal and adult rat lung tissues, where TGF- β has an important role in mediating lung development, suggesting that loss of T β RIII at the protein level is a mechanism to regulate TGF- β signaling [41]. The regulation of T β RIII receptor levels by receptor trafficking will be discussed in further detail below.

Regulation of T β RIII expression occurs at multiple levels, via regulation of mRNA expression, epigenetic silencing, protein levels, and receptor trafficking. This multi-tiered regulation supports the importance of regulating T β RIII expression levels to maintain its physiological functions.

T β RIII Structure

T β RIII is an 851 amino acid transmembrane proteoglycan, which contains a large 766 amino acid extracellular domain, a hydrophobic transmembrane domain, and a short 42 amino acid cytoplasmic domain, which lacks intrinsic enzymatic activity [19,42-44] (Figure 1). T β RIII is expressed on the cell surface as a non-covalently linked homodimer [43,45].

The extracellular domain of T β RIII contains an N-terminal orphan domain with no known homology, a zona pelucida domain (ZPD), which may be involved in receptor oligomerization, and two independent TGF- β ligand binding domains, a distal binding domain near the N-terminus of the extracellular domain and a proximal binding domain near the C-terminus of the extracellular domain, which are separated by a non-structured linker region [19,43, 46-50]. T β RIII binds to TGF- β 1, TGF- β 2, and TGF- β 3, BMP-2, BMP-4, and BMP-7, GDF-5, and inhibin A via the ligand binding domains, in addition to bFGF2 via the glycosaminoglycan (GAG) side chain modifications [27,47,51-56]. T β RIII contains distinct inhibin and TGF- β binding domains, although these domains overlap, as the inhibin binding site is solely within the ZP domain in the membrane proximal region of T β RIII [57]. The extracellular T β RIII domain contains two sites of heparin and chondroitin sulfate GAG side chain modifications at serine 535 and serine 546 [42,47]. The T β RIII core protein has a predicted molecular weight

of 100 kDa, however fully processed T β RIII migrates at an apparent molecular weight of 180 to 300 kDa due to the glycosaminoglycan post-translational modifications [19,47]. Mutation of the GAG sites to alanine completely abrogates GAG modification of T β RIII, but does not alter T β RIII stability or the ability to bind ligand [24,45,47,58].

However, post-translational modification of T β RIII does appear to be important for T β RIII function. Oncogenic Ras has been shown to modify T β RIII post-translational modifications, most likely GAG modifications, leading to an increased proliferative response to TGF- β and down-regulation of p21 in colon cancer cells [59]. In addition, T β RIII has also been shown to undergo incomplete post-translational glycosylation modification in goblet cell lines, correlating with their insensitivity to TGF- β 1 [60]. Expression of T β RIII in LLC-PK1 renal epithelial cells, which lack endogenous T β RIII, results in alterations in T β RIII GAG chain modifications which prevent the formation of T β RI/T β RII complexes, inhibiting TGF- β signaling, TGF- β induced growth arrest, and altering TGF- β induced collagen deposition [61]. The T β RIII GAG modifications may also contribute to the ability of T β RIII to regulate cell migration, as a T β RIII mutant lacking the GAG chains fails to fully induce alterations in the actin cytoskeleton or inhibit migration in ovarian cells [62].

T β RIII also undergoes ectodomain shedding, with proteolytic cleavage at a site in the extracellular domain proximal to the transmembrane domain releasing the soluble extracellular domain, known as soluble T β RIII (sT β RIII). sT β RIII can be detected in the extracellular matrix and serum [43,63]. sT β RIII levels have been demonstrated to correlate with the cell surface expression of T β RIII, suggesting constitutive shedding, however little is known about the regulation of sT β RIII production and the specific site of T β RIII cleavage has not been established [43,63]. Although the mechanism of sT β RIII cleavage is not well understood, T β RIII shedding can be modulated by pervanadate, a tyrosine phosphatase inhibitor, and may be mediated in part by the membrane type matrix metalloproteases (MT-MMP) MT1-MMP and/or MT3-MMP, and plasmin, a serine proteinase which has been shown to cleave the extracellular domain of T β RIII [64-65]. However, in contrast to other shed transmembrane proteins, T β RIII shedding is not enhanced by the protein kinase C activator PMA [66]. The restoration of T β RIII expression in multiple cancer types can inhibit tumor progression *in vivo* in part through sT β RIII production, which binds to and neutralizes TGF- β , antagonizing the tumor promoting effects of TGF- β signaling in late stage tumors [12,14-15,17,67-68]. The tumor suppressor function of sT β RIII will be discussed further below.

T β RIII has a short cytoplasmic domain that lacks intrinsic enzymatic activity and contains a class I PDZ binding motif [38,43,69]. Although the cytoplasmic domain of T β RIII is not essential for mediating ligand presentation, it has been shown to play a role in regulating TGF- β mediated signaling and growth inhibition [38,47,69-71]. The cytoplasmic domain of the T β RIII receptor binds to the autophosphorylated cytoplasmic domain of the type II receptor (T β RII), which promotes the formation of an active T β RII-T β RI signaling complex followed by dissociation of T β RIII from this complex [69]. In addition, the T β RIII cytoplasmic domain is phosphorylated by T β RII at threonine (Thr) 841 which facilitates the interaction of T β RIII with the scaffolding protein β -arrestin2 [39,69]. The phosphorylation of T β RIII at Thr 841 by T β RII is required for the interaction with β -arrestin2, as mutation of the T β RIII 841 threonine to alanine (T β RIII-T841A) completely abrogates the interaction of T β RIII and β -arrestin2 [39]. The interaction of the T β RIII cytoplasmic domain with β -arrestin2 results in the co-internalization of the β -arrestin2/T β RII/T β RIII complex into endocytic vesicles and subsequent down-regulation of TGF- β signaling [39]. Recently, the interaction between T β RIII and β -arrestin2 has been shown to regulate BMP signaling as well as TGF- β signaling [72]. T β RIII complexes with the BMP type I receptor, ALK6, in a β -arrestin2 dependent manner to mediate the internalization of ALK6 and stimulation of specific BMP signaling events downstream of ALK6 [72]. In addition, the ability of T β RIII to negatively regulate

NF κ -B signaling in breast cancer is dependent on its interaction with β -arrestin2 [73]. The T β RIII/ β -arrestin2 interaction also mediates the ability of T β RIII to regulate cell migration, as T β RIII activates Cdc42, alters the actin cytoskeleton and suppress migration in both normal and cancerous ovarian epithelial cells in a β -arrestin2 dependent manner [62].

The cytoplasmic domain of T β RIII also interacts with GIPC (GAIP-interacting protein C terminus), a PDZ domain-containing protein, via the T β RIII class I PDZ binding domain in the cytoplasmic domain [38]. Interaction with GIPC leads to stabilization of T β RIII cell surface expression and enhancement of TGF- β signaling [38]. The interaction between T β RIII and GIPC is required for the stabilization of T β RIII, as T β RIII-DEL, a mutant which lacks the PDZ binding domain and does not bind to GIPC, is unable to stabilize T β RIII levels or enhance TGF- β signaling [38]. Furthermore, the interaction between T β RIII and GIPC has been shown to play an important role in T β RIII mediated inhibition of TGF- β signaling, cell migration, and invasion during breast cancer progression as discussed further below [71].

Role of T β RIII in TGF- β Superfamily Signaling

T β RIII has a complex and context dependent role in regulating TGF- β superfamily signaling (Figure 2, 3) [1,74-75]. As the most abundantly expressed TGF- β superfamily receptor, the most well characterized role for T β RIII is as a TGF- β superfamily co-receptor [27,54]. In this co-receptor role, T β RIII directly binds ligands in the TGF- β superfamily, including TGF- β 1, 2, and 3, inhibin, BMP-2, 4, and 7 and GDF-5. In most cases, the ability of T β RIII to present ligand increases their binding to their respective cognate type I and type II TGF- β superfamily receptors to increase their signaling through the Smad proteins (i.e. Smad-dependent signaling) [27,47,51-52,54,56]. In the case of inhibin binding, T β RIII is able to inhibit both activin and BMP signaling, by promoting the binding of inhibin to its cognate receptors, the activin type II and BMP type II receptors, as inhibin opposes the action of activin and BMP (Figure 3) [53,74]. The ability of T β RIII to present ligand is particularly important for some TGF- β superfamily ligands, for example, TGF- β 2 and inhibin, as these ligands cannot bind their cognate receptors, T β RII and BMPRII respectively, on their own [47,53-54,76]. The importance of T β RIII in regulating TGF- β signaling has been demonstrated through both gain and loss of function studies. For example, endothelial cells can be rendered sensitive to TGF- β 2 by expressing T β RIII and pituitary corticotrophe tumor cell and ovarian cells can be rendered sensitive to inhibin by expression of T β RIII [53,77]. In a reciprocal manner, knockdown of T β RIII expression has been demonstrated to decrease responsiveness to TGF- β , with a reduction in Smad2 phosphorylation and impaired activation of a TGF- β responsive promoter [78]. Cell surface T β RIII may also regulate TGF- β signaling in a context dependent manner, as it has been demonstrated to inhibit TGF- β signaling by preventing the formation of T β RI/T β RII complexes in a GAG modification-dependent manner in LLC-PK1 renal epithelial cells [61]. Mechanisms for the context dependent regulation of TGF- β signaling by T β RIII remain to be fully defined.

Just as membrane tethered T β RIII is able to alter TGF- β superfamily ligand binding in a cell autonomous manner, T β RIII is able to regulate TGF- β superfamily ligand binding and signaling in a cell non-autonomous manner by undergoing ectodomain shedding to produce sT β RIII. Indeed as sT β RIII has been detected in serum and milk, in addition to autocrine and paracrine effects, sT β RIII has the potential to have systemic effects on signaling [63,79]. For ligands in which cell surface T β RIII enhances signaling, sT β RIII has been demonstrated to bind and sequester these ligands to inhibit signaling. Specifically, sT β RIII has been demonstrated to bind TGF- β and BMP and to inhibit TGF- β signaling [47,80]. However, at low concentrations of TGF- β , sT β RIII has also been shown to enhance TGF- β ligand binding to T β RII, suggesting complex roles for sT β RIII in regulating TGF- β superfamily signaling

[46]. The specific role of sT β RIII in regulating BMP and inhibin/activin signaling remains to be defined.

T β RIII has also been shown to have effects on non-Smad signaling pathways. T β RIII negatively regulates NF κ -B signaling in both normal mammary epithelial cells and breast cancer cells in a β -arrestin2 dependent manner [73]. Accordingly, knockdown of T β RIII expression in normal mammary epithelial cells results in an increase in NF κ -B activity, which mediates a reduction in E-cadherin expression, suggesting a role for T β RIII in maintaining an epithelial phenotype through the regulation of E-cadherin expression [73,81]. Knockdown of T β RIII expression in NMuMG cells, a normal mammary epithelial cell line, also results in the formation of invasive high grade carcinomas an *in vivo* orthotopic xenograft [81], suggesting that in normal epithelial cells T β RIII plays a role in regulating cell growth, motility, and invasion through the negative regulation of NF κ -B signaling.

T β RIII mediates both ligand dependent and independent p38 pathway signaling, which has been shown to play an important role in regulating growth inhibition and apoptosis [70, 82-84]. Expression of T β RIII in L6 myoblasts causes a ligand independent increase in p38 phosphorylation and enhances TGF- β mediated growth inhibition in a Smad 3/T β RI and p38 pathway dependent manner [70,83]. In a metastatic renal cancer cell line (UMRC3) T β RIII expression increases phosphorylation of p38 independent of functional T β RII, inducing apoptosis in clear renal cell carcinoma *in vitro* and *in vivo* [82]. The effects of T β RIII on p38 phosphorylation are at least partially dependent on the T β RIII cytoplasmic domain [70,82].

T β RIII has also been shown to mediate effects on cellular migration through β -arrestin2 dependent activation of Cdc42 in a TGF- β signaling independent manner [62]. Knockdown of T β RIII expression in normal ovarian surface epithelial cells alters the actin cytoskeleton, with an increase in stress fibers and lamellapodia, and reduces directional and random cell migration in the absence of ligand stimulation [62]. Reciprocally, increasing the expression of T β RIII inhibits migration in normal epithelial and cancer cells via constitutive activation of Cdc42, independently of T β RII, ALK5, and Smad2 [62]. These effects on migration are mediated in part through the GAG side chains and the cytoplasmic domain, as T β RIII mutants lacking the GAG side chains (T β RIII Δ GAG) or lacking the cytoplasmic domain (T β RIII Δ cyto) do not suppress migration to the extent of full length T β RIII [62].

Regulation of T β RIII Mediated Signaling and Receptor Levels by Receptor Trafficking

TGF- β signaling can be regulated by controlling receptor cell surface expression, receptor endocytosis, and subsequent trafficking events [85]. Many T β RIII-dependent signaling events are regulated through altering these properties for T β RIII, as well as the ability of T β RIII to regulate these events for interacting receptors. Interaction of T β RIII with the PDZ-protein GIPC stabilizes T β RIII at the cell surface to enhance TGF- β signaling [38]. Conversely, internalization of T β RIII, mediated in part by interaction with β -arrestin2, has been shown to result in the co-internalization of T β RII and T β RI and down regulation of TGF- β signaling (Figure 2) [39-40]. T β RIII internalization is both ligand and GAG modification independent and requires the cytoplasmic domain, as deletion of the cytoplasmic domain (T β RIII Δ cyto) results in a slower rate of internalization [40]. Specifically, interaction with β -arrestin2, which is mediated by phosphorylation of threonine 841 by T β RII, increases T β RIII internalization and decreases cell surface levels [39-40]. T β RIII endocytosis can occur by both clathrin-mediated and clathrin-independent (lipid raft) pathways [40]. However, inhibition of the clathrin-mediated endocytosis pathway does not affect down-regulation of T β RIII, while inhibition of the clathrin-independent pathway blocks down-regulation of T β RIII, suggesting that the clathrin-independent endocytosis pathway is the preferred pathway mediating T β RIII

trafficking and regulation [40]. In addition, inhibition of the clathrin-independent, but not the clathrin-dependent endocytosis pathway down-regulates Smad dependent and Smad independent (p38) TGF- β mediated signaling [40]. These data demonstrate that T β RIII endocytosis occurs via the clathrin-independent pathway, down-regulating T β RIII receptor levels and subsequent TGF- β signaling in a β -arrestin2 dependent manner [39-40].

Internalization of T β RIII has also been shown to regulate BMP signaling (Figure 3). T β RIII can interact with both type I BMP receptors, ALK3 and ALK6 [72]. T β RIII forms a complex with ALK6 in a β -arrestin2 dependent manner, and this β -arrestin2/T β RIII/ALK6 complex is internalized, where they co-localize in endocytic vesicles [72]. This T β RIII mediated internalization of ALK6 leads to maximal ALK6 mediated BMP signaling, as the ability of ALK6 to induce transcriptional activation is dependent on clathrin-mediated endocytosis of the receptor [72,86]. Both the extracellular and cytoplasmic domains of T β RIII are required to interact with ALK6 [72]. In contrast to ALK6, T β RIII interacts with ALK3 via the extracellular domain in a β -arrestin2 independent manner and stabilizes ALK3 at the cell surface, stimulating distinct ALK-3 mediated transcription [72]. These studies demonstrate a role for T β RIII in mediating distinct TGF- β and BMP receptor trafficking events resulting in distinct and downstream signaling events. The precise mechanisms by which alteration of receptor trafficking results in distinct signaling events remains to be defined.

Phenotype of T β RIII Null Mice

To examine the role of T β RIII *in vivo*, several groups have created T β RIII knockout mice. In wild type murine embryos T β RIII mRNA is expressed widely throughout gestation, but is localized to the heart and liver during midgestation [87]. Deletion of T β RIII by the disruption of exon 2 of T β RIII resulted in embryonic lethality for the majority of the null mice, with only 0.3% of T β RIII $-/-$ mice viable [87]. The T β RIII null mice that survived perinatally had a reduction in fertility, however were otherwise pathologically normal. Beginning at day 13.5, T β RIII null embryos began to exhibit enhanced apoptosis in the liver and defects in the development of the myocardial wall of the heart ventricles, with both phenotypes thought to contribute to embryonic lethality [87]. In addition, MEFs (murine embryonic fibroblasts) derived from null T β RIII mice exhibited a reduced sensitivity to TGF- β 2, with alterations in growth inhibition and Smad2 nuclear localization. This correlates with previous data in demonstrating that blocking the binding of TGF- β 2 to T β RIII or utilizing T β RIII neutralizing antibodies inhibits TGF- β 2 induced mesenchymal transformation in chick heart development [34-35]. This suggests that alterations in T β RIII mediated regulation of TGF β 2 signaling may contribute to the liver and cardiac phenotypes observed in the T β RIII null mice.

A second T β RIII knockout mouse supports the observed defects in heart development. Compton et. al. deleted exon 3 of T β RIII, leading to a loss of T β RIII protein expression [36]. Deletion of T β RIII results in embryonic lethality at D14.5 due to defects in coronary vasculogenesis, including reduced size and irregular shape of coronary vessels [36]. T β RIII null embryos also exhibited abnormal epicardium, with increased space between the epicardium and myocardium, abundant subepicardial mesenchyme, and persistent blood islands. The effects of T β RIII on vasculogenesis and angiogenesis appear to be confined to coronary vessels, as the vasculature outside of the coronary circulation appears normal and smooth muscle recruitment is normal in both coronary and extracardiac vessels [36].

These mouse models demonstrate a requirement for T β RIII in murine somatic development, specifically in murine hepatic and cardiac development, which are essential for embryonic viability. In addition, they support essential and non-redundant roles for T β RIII in embryonic development.

TβRIII: A Suppressor of Cancer Progression

Breast Cancer

Breast cancer is the most frequently diagnosed cancer and the second leading cause of cancer death among women [88]. TGF-β signaling plays an important role in mammary gland development through the inhibition of epithelial cell proliferation and the regulation of mammary ductal and alveolar development [89]. The over-expression of TGF-β in mammary tissue potently inhibits mammary gland development, with reduced ductal outgrowth and branching observed in virgin glands of MMTV-TGF-β mice in comparison to wild type mice [89]. In early stage breast tumors TGF-β functions as a tumor suppressor, while in late-stage tumors TGF-β signaling is frequently up-regulated and has a tumor promoting effect, enhancing tumor progression and metastasis [6]. Interestingly, despite the prominent role TGF-β plays in regulating mammary carcinogenesis, mutations or alterations in components of the TGF-β pathway are infrequent in human breast cancers [6]. However, it has been recently demonstrated that the loss of TβRIII expression, due to a loss of heterozygosity (LOH) or through transcriptional down-regulation, is a frequent early event in the development of human breast cancer, beginning during ductal carcinoma *in situ* (DCIS) [12,20]. Importantly, the restoration of TβRIII expression in murine mammary cancer cells inhibits tumor invasion, angiogenesis, and metastasis *in vivo*, in part through sTβRIII production, which binds to and neutralizes TGF-β, antagonizing the tumor promoting effects of TGF-β signaling in late stage mammary tumors [12,90]. Several studies have demonstrated that treatment with sTβRIII alone inhibits breast cancer tumor growth, angiogenesis, and reduces metastasis in nude mice *in vivo* xenograft studies of breast cancer cells [67-68,90-91]. A recent study has also shown a requirement for the TβRIII cytoplasmic domain, specifically interaction with the scaffolding protein GIPC, in mediating the inhibition of TGF-β signaling, cell migration, invasion, and *in vivo* breast cancer metastasis [71]. These data support that the loss of TβRIII is required for breast cancer progression and metastasis.

In reciprocal studies, knockdown of TβRIII expression in non-tumorigenic NMuMG murine mammary epithelial cells has been demonstrated to down regulate TGF-β signaling resulting in increased growth, motility, and invasion [81]. In contrast, another study demonstrated that knockdown of TβRIII in human and murine breast cancer cells resulted in decreased proliferation, migration, and invasion, and increased apoptosis as well as impairing *in vivo* tumorigenicity and inhibiting lung metastasis [78]. Mechanistically, loss of TβRIII reduced NF-κB activity and restoration of NF-κB activity restored tumor cell invasiveness and resistance to apoptosis [78]. Although this data is in contrast to previous reports of a role for TβRIII in inhibition of tumor progression, all data supports a role for TβRIII in the regulation of migration and invasion. The effects of TβRIII on tumor cell invasion and migration may be dependent on the activation or inhibition of other oncogenes or tumor suppressors. Taken together, these studies suggest that the loss of TβRIII expression during breast cancer progression alters the regulation of TGF-β signaling and may contribute to the loss of TGF-β mediated inhibition of proliferation and the increase in migration and invasion observed in late stage breast tumors.

Renal cancer

Renal cell carcinoma (RCC) represents approximately 3% of all cancers worldwide [92]. Localized renal cell carcinoma lesions can be surgically cured. However, metastatic disease is incurable and systemic chemotherapy is usually ineffective. Loss of TβRIII expression has also been described during renal cell carcinoma progression. When comparing TβRIII expression between normal renal epithelium, non-metastatic renal cell carcinoma, and primary and disseminated lesions in metastatic renal cell carcinoma, loss of TβRIII was an early event in renal cell carcinoma carcinogenesis, with loss observed in non-metastatic renal cell

carcinoma specimens, and subsequent loss of T β RII associated with the acquisition of a metastatic phenotype [13]. In contrast, T β RI levels were not altered across cancer stages [13]. When correlated with TNM score, T β RIII mRNA expression was suppressed in the earliest stage (stage I) and in all tumor stages (I-V) [13]. Restoration of T β RIII expression in clear cell RCC resulted in a marked induction of apoptosis both *in vitro* and *in vivo* models [13,82]. The ability of T β RIII to induce apoptosis was ligand, T β RI, and T β RII independent. The effects of T β RIII were dependent on the cytoplasmic domain, as the expression of the T β RIII cytoplasmic domain, but not the extracellular domain, could mimic the induction of apoptosis by full-length T β RIII in cell culture and inhibited tumor growth in athymic nude mice [82]. This T β RIII associated apoptosis was not dependent on signaling through the canonical TGF- β /Smad pathway but was mediated through the p38 pathway [82]. These studies demonstrate that T β RIII plays an important role in renal cell carcinoma tumorigenesis.

Prostate Cancer

TGF- β signaling plays an important role in the regulation of normal prostate epithelium through the mediation of differentiation, growth arrest, and apoptosis [93]. During prostate cancer, prostate cancer cells become resistant to TGF- β mediated effects, due to in part to a loss of T β RI or T β RII in 30% of tumors [94]. Several studies have shown that the loss of T β RIII is the most common alteration in the TGF- β pathway during prostate cancer progression, with a significant reduction or complete loss of T β RIII in the majority of human prostate cancers [14,95]. The loss of T β RIII expression has been shown to occur at the mRNA and protein level, and is due to LOH at the genomic locus and either direct or indirect epigenetic regulation of T β RIII expression [14]. Loss of T β RIII correlates with disease stage, including metastatic disease and prostate-specific antigen (PSA) recurrence, suggesting a role for T β RIII in disease progression [14]. In addition, T β RIII expression was shown to decrease further in metastases in comparison to primary prostate tumors.

The knockdown of T β RIII in immortalized prostate epithelial cells causes increased focus formation, although these cells did not form tumors in NOD/SCID mice [95]. In addition, the knockdown of T β RIII led to increased expression of the prostate stem cell marker, CD133, suggesting an expansion of cells with stem cell characteristics [95]. Microarray analysis of changes in gene expression between immortalized prostate epithelial cells expressing T β RIII and cells with knockdown of T β RIII revealed alterations in 101 genes, including down-regulation of genes commonly altered in prostate cancer: bone morphogenic protein 4 (BMP4), latent transforming growth factor beta binding protein 1 (LTBP1), vimentin, coagulation factor C homolog (COCH), SERPINF1, peripheral myelin protein 22 (PMP22), and dihydropyrimidinase-like 3 (DPYSL3) [95]. Interestingly, SERPINF1 is a potent inhibitor of angiogenesis and vimentin expression has been shown to decrease in the transition from benign prostate to local prostate cancer in a number of studies [95].

The restoration of T β RIII expression in prostate cancer cells inhibits migration and invasion, both basally and in response to TGF- β treatment, however this has no effect on proliferation [14]. Restoration of T β RIII in an *in vivo* prostate cancer xenograft model decreased tumor growth, supporting a tumor suppressor role of T β RIII in prostate cancer [14]. In addition, treatment of a prostate cancer xenograft model with sT β RIII significantly inhibited tumor growth, angiogenesis, decreased expression of MMP-9, and enhanced apoptosis [96]. This suggests that loss of T β RIII with disease progression may enable prostate cancer cells to become more motile and invasive. These studies support a role for T β RIII as a suppressor of prostate cancer progression.

Non-small cell lung Cancer (NSCLC)

Lung cancer accounts for the highest number of cancer related deaths in both men and women [88]. The TGF- β signaling pathway plays an important role in lung development and many lung cancers become resistant to the homeostatic effects of TGF- β [97]. This occurs in part through loss of T β RII, Smad2, and Smad4, although these mutations are not common in non-small cell lung cancer (NSCLC), which accounts for greater than 80% of all lung cancers [98-99]. However, T β RIII expression is decreased in NSCLC at both the mRNA level and protein level, due to LOH at the TGFBR3 genomic locus [17]. In addition, decreasing levels of T β RIII expression correlates with increasing tumor grade and disease progression [17]. One study also defined a correlation between decreased T β RIII expression and a decrease in squamous cell carcinoma patients' 5-year survival [100].

Expression of T β RIII in NSCLC cells, which exhibit normal to low levels of T β RIII, does not affect proliferation or TGF- β dependent Smad phosphorylation [17]. However, T β RIII expression inhibits lung cancer cell motility and invasion in NSCLC cells and knockdown of T β RIII expression increased invasion [17]. The ability of T β RIII to mediate migration and invasion is due, in part, to production of sT β RIII as treatment with conditioned media from cells over-expressing T β RIII was able to inhibit invasion in NSCLC cells. In addition, expression of T β RIII inhibits anchorage-independent growth of NSCLC cells in a soft agar assay [17]. Over-expression of T β RIII in an NSCLS *in vivo* xenograft assay reduced tumor incidence, tumor growth, and invasiveness of tumors [17]. These studies demonstrate a role for T β RIII in suppression of cancer progression in NSCLC, with T β RIII inhibiting migration, invasion, anchorage independent growth, and *in vivo* tumorigenicity of lung cancer cells.

Pancreatic Cancer

The expression of multiple components of the TGF- β signaling pathway, including T β RI, T β RII, and Smad4, are altered at both the mRNA and protein levels during pancreatic cancer [101]. T β RIII expression has recently been shown to decrease at the mRNA and protein level in pancreatic adenocarcinomas in comparison to matched normal samples [16]. In addition, the genomic locus for T β RIII on chromosome 1p is deleted in 49% of human pancreatic cancers [102]. The loss of T β RIII increases as tumor grade increases, suggesting that T β RIII plays an important role in mediating the progression of pancreatic cancer [16].

Epithelial to mesenchymal transition (EMT) plays an important role in the metastatic process. During the process of EMT, adherent epithelial cells become motile and invasive, and subsequently lose expression of epithelial E-cadherin cytokeratin and gain expression of vimentin, N-cadherin, and transcription factors Snail and Slug. The TGF- β pathway regulates EMT during development and cancer progression, in multiple systems including pancreatic cancer [11,103]. TGF- β , BMP2, 4, and 7 have been shown to induce both time and dose dependent EMT in a pancreatic cancer cell line [16,104]. T β RIII expression decreases during TGF- β and BMP4 induced EMT in PANC-1 cells in a time and dose dependent manner, although expression of T β RI and T β RII is not altered [16]. There is an increase in levels of sT β RIII in pancreatic cancer cells undergoing EMT, suggesting that T β RIII cell surface expression decreases rapidly during EMT due to increased T β RIII shedding [16]. Inhibiting the loss of T β RIII during both TGF- β and BMP4 induced EMT by adenoviral over-expression did not alter ligand induced EMT. However inhibiting loss of T β RIII expression does significantly inhibit the EMT-associated increases in motility and invasiveness in pancreatic cancer cells [16,104]. Conversely, knockdown of T β RIII expression in pancreatic cancer cells increased motility and invasion in response to TGF- β treatment. A T β RIII mutant lacking the cytoplasmic domain (T β RIII- Δ cyto) inhibited basal motility and invasion to the same extent as full length T β RIII, suggesting that the cytoplasmic domain does not play a role in mediating the effects of T β RIII on motility and invasion in pancreatic cells [16]. In addition, maintaining

TβRIII expression during EMT inhibits both TGF-β mediated induction of Smad2 phosphorylation and BMP mediated Smad1 phosphorylation. Loss of Smad1 in pancreatic cancer cells inhibits BMP mediated induction of MMPs and cell invasiveness, suggesting that TβRIII may mediate BMP effects on invasiveness through its effects on Smad1 [104]. The ability of TβRIII to inhibit TGF-β mediated motility and invasion occurs at least in part through production of sTβRIII, as conditioned media from cells over-expressing TβRIII inhibits basal and TGF-β mediated increases in invasion [16,104]. These data demonstrate that while the loss of TβRIII is not required for EMT induction, loss of TβRIII is required for the increase in motility and invasiveness associated with EMT and pancreatic cancer progression.

Ovarian cancer

Ovarian cancer is the most lethal of all gynecological cancers and the fifth leading cause of death among women in United States [88]. Due to the lack of early symptoms or effective screening tests, most ovarian cancer patients are diagnosed with metastatic disease.

TβRIII expression is significantly decreased or lost in human epithelial origin ovarian tumors compared to normal tissue, with loss of TβRIII expression correlating with tumor grade and ovarian cancer progression [15]. Treatment with methyltransferase and histone deacetylase inhibitors induced TβRIII expression in ovarian cancer cell lines, indicating that either direct or indirect epigenetic silencing plays a role in the loss of TβRIII expression in ovarian cancer cells [15]. When TβRIII expression is restored in Ovca429, an ovarian epithelial cell line which lacks TβRIII yet retains high TβRI and TβRII levels, cell invasion and migration were significantly reduced [15]. In addition, re-introduction of TβRIII into Ovca429 cells enhanced the anti-migratory effect of inhibin, which could be due in part to the decrease of MMP2 and MMP9 levels [15]. This anti-migratory effect of TβRIII was independent of TβRI or TβRII and was mediated through the interaction of TβRIII with βarrestin2, resulting in activation of Cdc42, which alters the actin cytoskeleton and reduces directional persistence to inhibit random migration of both cancer and normal ovarian epithelial cells [62].

One possible anti-tumorigenic effect of TβRIII in ovarian cancer is through its enhancement of inhibin binding to ActRII (Activin specific type II receptor serine kinase), which results in enhancement of activin antagonism [53]. Activin and inhibin are structurally related TGF-β superfamily members and are mutually antagonistic regulators of reproductive function. In the ovary, activin and inhibin subunits are expressed mainly in granulosa cells but are also detected in normal epithelial cells [105]. Malignant ovarian epithelial tumors have decreased inhibin production relative to activin production in comparison with normal ovarian surface epithelial cells and nonmalignant ovarian surface epithelial cells [105]. Activin binds to ActRII and recruits ALK4, which then activates the Smad pathway, while inhibin binds to ActRII but does not recruit ALK4, thereby inhibiting the pathway. TβRIII binds inhibin with high affinity, enhancing inhibin binding to ActRII and blocking TβRII/I downstream signal transduction.

The effect of TβRIII has also been investigated in ovarian sex cord stromal tumors, specifically granulosa cell tumor (GCT), which account for 7% of total ovarian cancer incidences [106]. In this study, 53% (9 of 17) of human GCT samples exhibit significantly lower TβRIII mRNA expression compared to normal premenopausal ovary, suggesting that loss of TβRIII is a feature of clinical disease and may contribute to GCT progression [106]. Restoration of TβRIII expression in COV434 cells derived from metastatic GCT and in KGN cells derived from stage III GCT, increased TGF-β responsiveness and adherence to the extracellular matrix component and decreased migration. However, knockdown of inhibin A abrogated TβRIII mediated inhibition of wound healing and invasion. In addition, when inhibin A and TGF-β were neutralized, this abolished the TβRIII mediated increase in adhesion to substrate [106]. TβRIII did not have effect on COV434 and KGN cell proliferation or apoptosis [106]. These studies

support a role for T β RIII in ovarian cancer progression through the inhibition of migration, invasion, and adhesion.

Endometrial carcinoma

In endometrial adenocarcinomas, expression of both T β RIII and inhibin α are reduced at the mRNA and protein level [107]. In normal endometrial tissue, T β RIII is localized to vascular endothelial cells lining vessels in the endometrium and myometrium and also in endometrial epithelial cells. However, T β RIII expression is weak in well-differentiated endometrial carcinoma and lacking in poorly differentiated endometrial carcinoma. [107]. Interestingly, tumor endothelial cells exhibit high T β RIII levels, suggesting that a selective vascular response to TGF- β and inhibin may be possible [107]. This suggests that in addition to the decreased expression of inhibin α , the loss of T β RIII may disrupt the inhibin/activin pathway and TGF- β signaling in endometrial cancer.

Role of T β RIII in Mediating Invasion and Migration

Metastasis is the most common cause of death in cancer patients. The classical view of the metastatic cascade, starting from a primary, epithelial, neoplastic lesion include: EMT and breach of the basement membrane barrier, dissociation of tumor cells from the bulk tumor, invasion of the neighboring tissue, intravasation into pre-existing and newly formed blood and lymph vessels, transport through vessels, extravasation from vessels and establishment of disseminated cells at a secondary anatomic site and outgrowth of micrometastases and macrometastases/secondary tumors [108]. During these processes, cancer cells undergo changes in cell motility parameters, stiffness, and shape as seen during EMT, which allow these cells to enter lymphatic and blood vessels and disseminate. In addition, the cancer cells need to degrade the basement membrane to enter the blood or lymphatic vessels and parenchyma of the target organ where they form secondary tumors. This process can be mediated by secretion or regulation of matrix degrading enzymes including matrix metalloproteinases, proteinases of the plasminogen activator system, and heparanase and by alterations in cell morphology and cytoskeleton remodeling which facilitate the invasion process [109]. Cell-cell adhesion is important for maintaining an epithelial phenotype, and loss of cell-cell adhesion is associated with EMT, increasing migration, and invasion [109]. The TGF- β superfamily, including TGF- β and BMP, regulates EMT during development and cancer progression in multiple systems [11].

T β RIII has been shown to regulate both MMP secretion and cell invasion in multiple cancer systems. Expression of T β RIII in multiple human cancer types that lose T β RIII expression with disease progression has been shown to inhibit cell migration, invasion, and metastasis both *in vitro* and *in vivo* [12,14-17,106]. In ovarian cancer, T β RIII decreases the level of MMP2 and 9 at both the mRNA and protein level [15]. In pancreatic cancer, reintroduction of T β RIII expression during EMT inhibits BMP mediated Smad1 phosphorylation, which abrogates BMP mediated induction of MMPs and cell invasiveness [104]. Systemic treatment with sT β RIII reduced MMP-9 expression in a prostate cancer xenograft model [96]. Loss of T β RIII expression in human ovarian cancers results in an increase of N-Cadherin and Slug mRNA and also alterations in the cytoskeleton including filopodia and lamellipodia [62]. In pancreatic cancer, loss of T β RIII is associated with TGF- β induced EMT, and this is required for EMT associated increases in migration and invasion possibly via alterations in cell-cell junctions [16].

T β RIII has an emerging role in the regulation of migration in both normal and cancerous cells in both a ligand dependent and independent manner. Re-introduction of T β RIII expression in multiple cancer systems that lose T β RIII expression with disease progression, including breast, ovarian, lung, prostate, and pancreatic cancer, has been shown to inhibit ligand induced

migration [12,14-17,71]. Recent studies have suggested essential, non-redundant roles for T β RIII in regulating migration signals independently from T β RI/RII in renal cell tumors and in ovarian epithelial and cancer cells [13,62]. The short T β RIII cytoplasmic domain has been classically considered to lack signaling function. However recent studies suggest that this domain may play an important role in regulation of cell migration. In normal ovarian surface epithelial and ovarian cancer cells, the interaction of the T β RIII cytoplasmic domain and β -arrestin2 regulates the activation of Cdc42 to alter migration [62]. The T β RIII mediated effects on migration are also regulated in part through the GAG side chains, as T β RIII mutants lacking the GAG side chains (T β RIII Δ GAG) or lacking the cytoplasmic domain (T β RIII Δ cyto) do not suppress migration to the extent of full length T β RIII [62]. However, the dependence on the cytoplasmic domain for regulation of cell migration may be cell type dependent as T β RIII Δ cyto was just effective as T β RIII in decreasing migration and invasion in breast and pancreatic cancer cells, in part, via effects of sT β RIII [12,16].

Soluble T β RIII, generated by ectodomain shedding, has also been demonstrated to regulate cell adhesion and migration. sT β RIII is able to recapitulate the inhibitory effect of membrane bound T β RIII on invasion in breast, pancreatic, and non-small cell lung cancer models [12, 16-17]. sT β RIII may function either by sequestering TGF- β superfamily ligands and suppressing signaling or by binding to extracellular matrix proteins and blocking their ability to interact with T β RIII on the cell surface.

T β RIII has been shown to have an essential role in mediating the migration and invasion of both normal epithelial and epithelial-derived cancer cells. The ability of T β RIII to suppress cancer progression is due in part to inhibition of migration and invasion in cancer cells. Further understanding the role of T β RIII in regulating these mechanisms that contribute to metastasis will increase our ability to target T β RIII and the TGF- β superfamily signaling pathways in human cancer.

Targeting T β RIII in Human Cancers

Cancer progression, including breast, prostate, and glioma progression, is associated with the increased expression of TGF- β and other TGF- β superfamily ligands, which may function through effects on the cancer cells and the stroma to promote cancer progression [6]. Therefore antagonizing TGF- β superfamily ligands is a promising strategy to counteract malignant properties of tumors. T β RIII has been shown to have anti-tumor and anti-metastatic effects through the inhibition of migration, invasion, cell growth, apoptosis, and metastasis *in vitro* and *in vivo*. The ability of T β RIII to inhibit tumorigenesis may occur in part through the ability of sT β RIII to sequester TGF- β superfamily ligands and antagonize TGF- β superfamily signaling, suggesting that sT β RIII or agents derived from sT β RIII could be used as therapeutic agents [47,80].

Several studies have been performed with sT β RIII demonstrating its inhibitory effects on tumor progression. Treatment of mink lung epithelial cells with conditioned medium from MDA-MB-231 cells stably expressing sT β RIII significantly suppressed cell growth *in vitro* due to the ability of sT β RIII to sequester extracellular TGF- β isoforms and antagonize their function [91]. Treatment of the breast cancer cell line MDA-MB-231 with recombinant sT β RIII or stable expression of sT β RIII in MDA-MB-231 cells antagonized TGF- β signaling, inhibiting anchorage dependent and independent cell growth, and induced apoptosis *in vitro* and *in vivo* [91,110]. MDA-MB-231-sT β RIII cells showed reduced tumor incidence and growth as well as a reduction in lung metastasis compared to MDA-MB-231-Neo cells *in vivo* [91,110]. MDA-MB-231-sT β RIII cells also exhibited increased PTEN levels and a concurrent decrease in phosphorylated Akt levels in comparison to the Neo cells [110]. Further, ectopic expression of sT β RIII in human colon (HCT116) and breast carcinoma (MDA-MB-435) *in vivo* xenograft

models inhibited tumor growth and the metastatic potential of tumors, as well as inhibiting angiogenesis in primary tumors [68]. In addition, recombinant sTβRIII was shown to have potential therapeutic utility in breast, colon, and prostate cancer, with systemic treatment of sTβRIII reducing tumor growth, metastasis, and angiogenesis *in vivo* [91,96]. Treatment of human endothelial cells with a recombinant sTβRIII significantly inhibited capillary formation, suggesting that the anti-tumorigenic effect of sTβRIII is partially via suppression of TGF-β induced angiogenesis [67-68]. Continuous or bolus administration of sTβRIII inhibited prostate xenograft growth associated with reduced tumor blood volume and microvessel density and an enhanced intra-tumoral apoptosis [96]. Furthermore, sTβRIII treatment inhibited TGF-β induced MMP-9 activity in this system, suggesting inhibition of invasive properties. sTβRIII has also been shown to reduce renal damage in the *db/db* mouse model of type 2 diabetes [111]. In this model of nephropathy, systemic treatment with sTβRIII prevented up-regulation of TGF-β1, 2, and 3, as well as decreasing fibronectin and collagen IV levels in the kidney, reducing renal damage progression [111]. Treatment of mice with sTβRIII did not cause any deleterious side effects, such as weight loss or behavior changes, suggesting that sTβRIII is well tolerated.

sTβRIII has also been shown to have promise in combination with other agents. For example, treatment of malignant gliomas with sTβRIII alone was not effective, however when combined with soluble TβRII (sTβRII), sTβRIII increased the anti-tumor effect of sTβRII, reducing Smad2 phosphorylation and abrogating TGF-β effects [112]. In addition, combinatorial treatment with sTβRII and sTβRIII in a glioma model blocked the immunosuppressive effect of TGF-β on NK cells, suggesting that sTβRII and sTβRIII may restore an effective anti-glioma immune response by neutralizing TGF-β [112]. This study demonstrated an *in vivo* survival benefit of treatment with sTβRII and sTβRIII. Although there have been attempts to use other soluble TGF-β receptors as TGF-β inhibitors, their antagonistic potency against different TGF-β isoforms varies and TGF-β isoforms are differentially expressed depending on cell type and context [113-118]. sTβRIII may be more effective than sTβRII, as it can bind and sequester TGF-β2, while sTβRII cannot [80,119-120]. In addition, TβRIII can bind BMPs and inhibin, suggesting that sTβRIII can bind these ligands as well [52-53].

Recently, a recombinant fusion protein containing the endoglin domain of TβRIII (BGE) and the extracellular domain of TβRII has been constructed for use as a TGF-β ligand antagonist [121]. This fusion protein (BGERII) bound with a higher affinity to TGF-β1 and TGF-β3 than a commercially available sTβRII and with a higher affinity to TGF-β2 than a commercially available sTβRIII [121]. Whereas BGE or TβRII alone showed no antagonistic activity towards TGF-β2, BGERII inhibited the signaling of both TGF-β1 and TGF-β2 in cell-based assays more effectively than equimolar concentrations of either sTβRII or sTβRIII alone, including reducing TGF-β induced phosphorylation of Smad2 and Smad3 and inhibiting transcription from a TGF-β responsive promoter. This study suggests that sTβRIII may be useful for engineering additional reagents which target TGF-β signaling.

It is promising that systemic administration of sTβRIII has been shown to have an anti-tumorigenic effect in breast, prostate, colon, and glioma *in vivo* cancer models without deleterious side effects. The ability of sTβRIII treatment to reduce tumor growth, inhibit angiogenesis, induce apoptosis, and inhibit metastasis in multiple cancer systems suggests that sTβRIII may be a viable target to inhibit cancer progression.

In addition, TβRIII expression may also have prognostic value as a biomarker, as loss of TβRIII expression increases with clinical stage and correlates with metastatic disease in multiple tumor types, including ovarian, breast, prostate, and NSCLC [12,14-15,17,71]. Levels of TβRIII or sTβRIII expression could be assessed by immunohistochemistry or RT-PCR of

tumor tissue or ELISA analysis of blood serum for sTβRIII and could be utilized to determine disease stage or direct treatment.

TβRIII also has functions beyond the production of sTβRIII, including regulation of migration and invasion, therefore restoring the expression of cell surface TβRIII in tumors could have therapeutic benefits. In addition, expression of membrane bound TβRIII has been shown to correlate with production of sTβRIII [43,63]. As expression of TβRIII is down-regulated via epigenetic silencing in multiple human tumor types, treatment with histone deacetylase inhibitors or DNA methylation inhibitors could be utilized to restore TβRIII expression [14]. Restoration of TβRIII expression inhibits tumor migration, invasion, metastasis, and angiogenesis in multiple *in vivo* models of tumorigenesis thereby supporting reintroduction of TβRIII expression as a viable therapy [12,14,17,71].

Future Directions

TβRIII mediates and regulates cell migration, invasion, angiogenesis, apoptosis, and metastasis, all mechanisms that contribute to cancer progression. The ability of TβRIII to suppress cancer progression is due in part to the inhibition of migration and invasion of cancer cells. Further understanding the role of TβRIII in regulating these mechanisms that contribute to metastasis will increase our ability to target TβRIII and the TGF-β superfamily signaling pathways in human cancer.

The effects of TβRIII are due at least in part to the production of sTβRIII, as treatment with sTβRIII reduces migration, invasion, and metastasis in *in vivo* models and is able to recapitulate the inhibitory effect of membrane bound TβRIII on invasion in breast, pancreatic, and non-small cell lung cancer models [12,16-17]. A number of questions regarding sTβRIII remain to be answered. While TβRIII functions by sequestering TGF-β superfamily ligands and suppressing signaling, the specific roles and the ratio of membrane bound TβRIII and sTβRIII still remain to be fully elucidated. Currently, the specific site of TβRIII cleavage remains unknown. Identification of the cleavage site would allow mutation to inhibit TβRIII cleavage, further enhancing our understanding of the contribution of soluble and membrane bound TβRIII to the inhibition of cancer progression. Finally, the mechanisms regulating ectodomain shedding and production of sTβRIII remain to be discovered. A further understanding of the production of sTβRIII could lead to mechanisms to enhance ectodomain shedding as a therapeutic target.

TβRIII expression is significantly decreased or lost in multiple types of human cancers. Loss of TβRIII expression occurs via epigenetic silencing in multiple human tumor types, suggesting that treatment with histone deacetylase inhibitors or DNA methylation inhibitors could be utilized to restore TβRIII expression [14]. Further understanding the cell type specific regulation of TβRIII mRNA expression may identify possible treatments to enhance or restore TβRIII expression in different human tumors.

Many TβRIII dependent signaling events are regulated through alteration of receptor cell surface expression, receptor endocytosis, and subsequent trafficking events, as well as the ability of TβRIII to regulate these events for interacting receptors [85]. The interaction of TβRIII with GIPC has been shown to stabilize TβRIII at the cell surface resulting in enhanced TGF-β signaling, while interaction with β-arrestin2 leads to co-internalization of TβRIII, TβRII and TβRI and down regulation of TGF-β signaling respectively [39-40]. TβRIII plays a role in mediating distinct TGF-β and BMP receptor trafficking events resulting in distinct and downstream signaling events. The interaction of TβRIII with other scaffolding proteins and subsequent receptor regulation still remains to be discovered. In addition, the precise mechanisms by which alteration of receptor trafficking results in distinct signaling events remains to be defined.

Conclusion

TGF- β signaling plays a dichotomous role in human cancers, functioning as both a tumor suppressor and a tumor promoter. T β RIII plays an important role in mediating ligand dependent TGF- β superfamily signaling as well having as ligand independent functions, including the regulation of migration. The loss of T β RIII expression in early disease stages of multiple human cancer types suggests that T β RIII may inhibit tumor progression [12-17]. Indeed, T β RIII has direct roles in inhibiting cell migration, invasion, angiogenesis, apoptosis, and metastasis in *in vivo* models suggesting an emerging role for T β RIII as an inhibitor of tumor progression and/or metastasis. Supporting this theory, reintroduction of T β RIII expression or treatment with sT β RIII inhibits metastasis in multiple *in vivo* tumor models [12,14,17,67,82,91,96]. These studies demonstrate the important and non-redundant roles of T β RIII beyond that of a co-receptor, including regulation of migration, invasion, and metastasis. The anti-tumor and anti-metastatic effects of T β RIII and promising results with sT β RIII treatment suggest that T β RIII may be an important target for treatment of human cancers. Further work remains to more fully understand the role and mechanism of T β RIII in human cancer and to explore its possible role as a therapeutic target.

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Abbreviations

TGF- β	Transforming growth factor β
T β RIII	Type III TGF- β receptor
ST β RIII	Soluble type III TGF- β receptor
BMP	Bone morphogenetic protein
EMT	Epithelial-to-mesenchymal transition
GIPC	GAIP-interacting protein, C terminus
GAG	Glycosaminoglycan
MMP	Matrix metalloproteases
RCC	Renal cell carcinoma
NSCLC	Non-small cell lung cancer

Structure of TβRIII

■ N-Linked glycan ⬠ Glycosaminoglycan ○ O-Linked glycan

ECD: Extracellular domain, TMD: Transmembrane domain, CD: Cytoplasmic domain

Figure 1.

TβRIII Structure. TβRIII consists of a large extracellular domain (ECD), which has multiple sites of glycan modification (proteoglycan and glycosylation), a hydrophobic transmembrane domain, and a short cytoplasmic domain, which interacts with β-arrestin2 via phosphorylation of amino acid site 841, and with GIPC via the PDZ domain.

Figure 2.

TβRIII mediates both ligand dependent and independent effects. A. TβRIII can mediate cell migration in a β-arrestin2 dependent manner through the activation of Cdc42 in a ligand independent manner. TβRIII activates p38 in both a ligand independent and dependent manner. TβRIII undergoes ectodomain shedding producing soluble TβRIII, which can bind to and sequester ligand, inhibiting TGF-β signaling. B. TβRIII presents ligand to TβRII, which phosphorylates TβRIII, and recruits and phosphorylates TβRI, causing phosphorylation of the R-Smads. Phosphorylation of the R-Smads allows interaction with co-Smad4, mediating nuclear translocation of the Smad complex and regulation of transcriptional activity. Interaction of TβRIII with β-arrestin2 results in the internalization of the TβRIII/TβRII/β-arrestin2 complex and subsequent down-regulation of TGF-β signaling. TβRIII also negatively regulates NF B signaling in a β-arrestin2 dependent manner. Interaction of TβRIII with GIPC stabilizes TβRIII at the cell membrane and enhances TGF-β signaling, as well as mediating effects on migration and invasion.

Figure 3.

TβRIII Regulates BMP Mediated Signaling. A. TβRIII binds to inhibin and promotes the binding of inhibin to the type II receptor. The binding of inhibin to the type II receptor prevents binding of BMP (or activin, not shown) and fails to recruit the type I receptor, antagonizing both BMP (and activin) signaling. B. TβRIII interacts with ALK3 via the extracellular domain in a β-arrestin2 independent manner and stabilizes ALK3 at the cell surface stimulating ALK3 mediated transcription. C. TβRIII interacts with ALK6 via the extracellular and cytoplasmic domains in a β-arrestin2 dependent manner. This β-arrestin2- TβRIII-ALK6 complex is internalized and localized to endocytic vesicles, leading to maximal ALK6 mediated BMP signaling.

T β RIII in Human Cancers

Table 1

Cancer	T β RIII Expression (Mechanism)	Effects on Signaling	Proliferation	Migration	Invasion	Angiogenesis	<i>In Vivo</i> /Other T β RIII Effects	References
Breast	Decreased (LOH)	Decreased	No effect	Decreased	Decreased	Decreased	Xenograft: Reduced tumor invasiveness, angiogenesis, metastasis; sT β RIII reduces tumor growth, angiogenesis, metastasis	[12,67,68,90,91]
Lung (NSCLC)	Decreased (LOH)	No effect	No effect	Decreased	Decreased	Decreased	Xenograft: Reduced tumor incidence, growth, & invasiveness	[20]
Prostate	Decreased (LOH; epigenetic regulation)	Not examined	No effect	Decreased	Decreased	Decreased	Xenograft: Reduced tumor incidence, & growth; sT β RIII reduced tumor growth, angiogenesis, & MMP induction, increased apoptosis	[14,95,96]
Pancreatic	Decreased	Decreased	Not examined	Decreased	Decreased	Not examined	Decreased MMP Induction	[16,104]
Ovarian	Decreased (Epigenetic regulation)	Not examined	Not examined	Decreased	Decreased	Not examined	Decreased MMP Induction	[15,62,106]
Renal	Decreased	Decreased (p38)	Not examined	Not examined	Not examined	Decreased	Xenograft: Reduced tumor growth, increased apoptosis	[13,82]