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Chemotherapeutic Targeting of the Transforming Growth Factor- β Pathway in Breast Cancers

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Abstract

Transforming growth factor (TGF- β) is a multifunctional cytokine that plays essential roles in regulating mammary gland development, morphogenesis, differentiation, and involution. TGF- β also regulates mammary gland homeostasis and prevents its transformation by prohibiting dysregulated cell cycle progression, and by inducing apoptosis; it also creates cell microenvironments that readily inhibit cell migration, invasion, and metastasis. Interestingly, while early-stage mammary tumors remain sensitive to the tumor suppressing activities of TGF- β , late-stage breast cancers become insensitive to the anticancer functions of this cytokine and instead rely upon TGF- β to drive disease and metastatic progression. This switch in TGF- β function is known as the “TGF- β Paradox” and represents the rationale for developing chemotherapies to inactivate the TGF- β pathway and its oncogenic functions in late-stage breast cancers. Here we outline the molecular mechanisms that manifest the “TGF- β Paradox” and discuss the challenges associated with the development and use of anti-TGF- β agents to treat breast cancer patients.

Keywords

Breast Cancer; Chemotherapy; Metastasis; Signaling Transduction; TGF- β

Introduction

Although the widespread use of screening mammography and implementation of adjuvant chemotherapies coalesced to produce a dramatic 24% decrease in the mortality of breast cancers between 1990–2000 [1], this disease nevertheless remains a significant health concern in the United States and accounts for ~30% of all female cancers [2]. Moreover, the acquisition of metastatic phenotypes underlies the deaths of ~90% of breast cancer patients [3, 4]. In fact, metastatic breast cancer is the 2nd leading cause of death in woman in the United States, annually accounting for more than 40,000 fatalities and 227,000 new cases of invasive breast cancer [2]. Generally speaking, metastatic disease is incurable and results in

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a median survival of only 1.5 to 3 years for patients harboring metastatic breast cancers. These statistics are even more daunting due to the fact that breast cancer is not a homogeneous disease, but is instead highly heterogeneous and comprised of at least 5 genetically distinct subtypes that display distinct clinical presentations, prognoses, and outcomes that reflect their unique histopathological features and responses to chemotherapies [5]. Collectively, these challenges highlight the urgent need to decipher the molecular mechanisms that drive breast cancer metastasis, and to develop novel pharmacological agents capable of specifically targeting and alleviating metastatic disease in breast cancer patients.

Transforming growth factor- β (TGF- β) is a pleiotropic cytokine that functions as a potent suppressor of mammary tumorigenesis, doing so by inhibiting mammary epithelial cell (MEC) cell proliferation, and by stimulating their apoptosis, differentiation, and morphogenesis [3, 6]. Interestingly, ~30% of breast cancer patients diagnosed with early-stage disease will ultimately progress to late-stage, metastatic disease, thereby limiting treatment options and contributing to the dismal survival rates of patients with metastatic breast cancers. The underlying cellular and molecular events that mediate this switch in breast cancer prognosis remain incompletely understood; however, accumulating data obtained over the last 25 years clearly implicates inappropriate TGF- β signaling as a driver of metastatic progression in late-stage breast cancers. Indeed, resistance to TGF- β -mediated cytostasis is a hallmark of developing breast cancers [3, 6], which ultimately convert the tumor suppressive signals engendered by this cytokine into oncogenic signals, particularly those coupled to the acquisition of metastatic and chemoresistant phenotypes that arise upon the completion of epithelial-mesenchymal transition (EMT) programs [3, 7]. This pathophysiological conversion of TGF- β function during mammary tumorigenesis is known as the “TGF- β Paradox” and underlies the development of anti-TGF- β agents to alleviate metastatic disease, including that by breast cancers. Here we briefly discuss the TGF- β signaling system and its dichotomous activities in developing and progressing mammary tumors, as well as review the challenges associated with the synthesis and implementation of novel anti-TGF- β agents aimed at alleviating metastasis and disease recurrence in patients with late-stage breast cancers.

The TGF- β signaling system: *A brief overview*

Humans house three distinct genetic loci that produce TGF- β 1 (hChr 19q13), TGF- β 2 (hChr 1q41), and TGF- β 3 (hChr 14q24), all of which are virtually indistinguishable in terms of their mature sequences and biological activities *in vitro* [8]. Within the mammary gland, TGF- β isoforms generally exhibit overlapping expression patterns within the epithelial compartment during branching and alveolar morphogenesis; however, spatiotemporal expression patterns do exist for the TGF- β isoforms in nulliparous and parous mammary glands [6], suggesting that these cytokines modulate unique activities throughout the gland and its supporting stroma. Despite these expression differences, the initiation of transmembrane signaling by individual TGF- β s commences in an identical manner through their binding to two high-affinity receptors, namely the TGF- β type I (T β R-I) and type II (T β R-II), both of which possess intrinsic Ser/Thr protein kinase activity in their cytoplasmic domains. A third TGF- β receptor known as T β R-III (or betaglycan) is also expressed in the

mammary gland and functions as co-receptor that binds and presents TGF- β s to T β R-II [9], which recruits, transphosphorylates, and activates T β R-I, leading to its phosphorylation and activation of the latent transcription factors, Smads 2 and 3. Phosphorylated Smad2/3 form heterocomplexes with the common Smad, Smad4, which collectively accumulate in the nucleus where they interact with a variety of transcriptional activators and repressors to govern the expression of TGF- β -responsive genes [10–12]. The coupling of TGF- β to the activation of Smad2/3 is known as the canonical TGF- β signaling system, which is synonymous with Smad-dependent TGF- β signaling. It is important to note that the functionality of canonical TGF- β signaling is influenced by a variety of auxiliary signaling inputs, including those mediated by a host of TGF- β receptor and Smad2/3 adapter molecules (*e.g.*, Sara, Dab2, Hgs, p130Cas, etc.), by Ser/Thr phosphatases, by the inhibitory Smad, Smad7, and by the E3 ubiquitin ligases, Smurf1/2 [10–12]. Collectively, canonical TGF- β signaling plays a prominent role in mediating the tumor suppressor activities of TGF- β in normal mammary epithelial cells (Figure 1).

In addition to its activation of Smad2/3-dependent signaling, TGF- β also stimulates numerous Smad2/3-independent effector molecules, including (i) β 1 and β 3 integrins and components of focal adhesion complexes; (ii) the PI3K:AKT:mTOR signaling axis; (iii) Rho-family GTPases; (iv) the MAP kinases ERK1/2, p38 MAPK, and JNK; (iv) NF- κ B; and (v) lysyl oxidases ([10, 13]; Figure 1). Collectively, this assortment of signaling molecules and pathways activated by TGF- β are referred as noncanonical TGF- β signaling systems, whose imbalanced activation relative to their canonical counterparts manifest the “TGF- β Paradox” and the acquisition of oncogenic signaling by TGF- β in developing and progressing mammary tumors (Figure 1). Moreover, noncanonical TGF- β signaling systems also enable TGF- β to induce EMT and carcinoma survival programs, to promote the generation and expansion of breast cancer-initiating cells that exhibit stem cell-like characteristics, and to elicit chemoresistance and disease recurrence in late-stage breast cancers [3, 10]. Finally, the magnitude with which TGF- β activates its noncanonical effectors is bolstered by signaling inputs derived from the tumor microenvironment, particularly hypoxia and increased tissue rigidity [14, 15]. Readers desiring additional information related to the pathophysiology of canonical and noncanonical TGF- β signaling systems in mediating the “TGF- β Paradox,” and in driving mammary carcinoma development and metastatic progression are directed to several recent reviews [3, 8, 11]. In the succeeding sections, we review the rationale for targeting the TGF- β pathway in late-stage breast cancers, as well as discuss the major classes of anti-TGF- β agents currently being evaluated in preclinical and clinical settings.

TGF- β and the mammary gland: *Rationale underlying the use of anti-TGF- β agents to treat late-stage breast cancers*

Mammary glands are comprised of two major compartments, namely (i) the epithelium and its associated luminal and myoepithelial cells that form the ductal structures, and (ii) the stroma and its composite of adipocytes, fibroblasts, immune, and endothelial cells, as well as their production of extracellular matrix and connective tissue proteins. Importantly, the functions of both mammary gland compartments coalesce to maintain cell and tissue

homeostasis, and to suppress mammary tumorigenesis. TGF- β was originally purified to homogeneity more than 30 years ago based on its ability to stimulate normal rat kidney fibroblasts (NRK-49 cell) to undergo morphological transformation and anchorage-independent growth [16, 17]. Shortly thereafter and in stark contrast to its activities in fibroblasts, TGF- β was determined to function as a potent antiproliferative agent in epithelial cells [18], and subsequently in endothelial [19] and hematopoietic lineages as well [20, 21], thus providing the initial framework for the “TGF- β Paradox” in traditional culture systems. With respect to the mammary gland, TGF- β isoforms are generally expressed in a spatiotemporal manner throughout both mammary gland compartments, particularly during glandular development and branching morphogenesis, as well as during pregnancy, lactation, and involution induced by weaning. Indeed, the first demonstration linking TGF- β 1 to mammary gland biology occurred in 1987 following the implantation of slow-release TGF- β 1 pellets into female mice, which reversibly inhibited glandular growth and morphogenesis [22]. These findings were recapitulated shortly thereafter by the sustained release of either TGF- β 2 or TGF- β 3 in mouse mammary glands [23, 24], suggesting that all three TGF- β s mediate antiproliferative activities within the mammary gland. Interestingly, the cytostatic functions of TGF- β are confined solely to the epithelial compartment and accompanying terminal end buds, being noticeably absent in their surrounding stroma; they are also inactivated in pregnant or estrogen-treated animals, in which TGF- β had little-to-no effect on the proliferation of lobuloalveolar units [25]. Along these lines, transgenic expression of a constitutively-active TGF- β 1 mutant in the mammary glands of mice results in the appearance of severely hypoplastic ductal trees and significantly diminished stem cell compartments [26, 27]. Collectively, these studies implicate TGF- β as a powerful suppressor of MEC growth and proliferation, findings bolstered by the ability of transgenic expression of dominant-negative T β R-II mutants to enhance mammary tumorigenesis in carcinogen-treated mice [28].

It is interesting to note that despite its clear associations with mediating tumor suppression, aberrant expression of either TGF- β or T β R-II are positively correlated with the onset of aggressive breast cancer progression and metastasis, as is the elevated phosphorylation of Smad2 [29, 30]. Thus, these findings suggest that amplified TGF- β signaling may in fact function to enhance the development and progression of late-stage breast cancers. Accordingly, TGF- β is highly expressed at the invading face of primary human breast carcinomas and lymph node metastases [31], while administration of neutralizing TGF- β antibodies inhibits the formation and metastasis of MDA-MB-231 tumors in mice [32]. Likewise, conditional expression of constitutively-active TGF- β 1 in established mammary tumors selectively stimulated their metastatic progression without affecting the proliferative index and growth rate of the corresponding primary tumors [33]. Similarly, inactivation of canonical TGF- β signaling augmented the tumorigenicity of premalignant (MCF10A1AT1k) and nonmetastatic (MCF10CA1h) derivatives of the MCF10A progression series, but attenuated that of their metastatic (MCF10ACA1a) counterparts [34–36]. Along these lines, systemic administration [37] or transgenic expression [38] of a soluble Fc:T β R-II fusion protein, which antagonizes TGF- β signaling by sequestering TGF- β and preventing its activation of T β R-II, inhibited the survival, motility, and metastasis of mammary tumors in mice. Contrary to expectations, lifetime exposure to these anti-TGF- β agents failed to

recapitulate the phenotype and morbidity associated with genetic inactivation of TGF- β 1 in mice [37, 38], thereby provide strong rationale for the development of TGF- β -directed chemotherapies for the treatment of metastatic breast cancers. Finally, conditional deletion of T β R-II in mammary gland fibroblasts elicited abnormal ductal development [39], as well as stimulated the growth and invasion of adjacent breast cancer cells *via* paracrine production of TGF- α , MSP (macrophage-stimulating protein), and HGF (hepatocyte growth factor) [15, 39–41]. Collectively, these findings highlight the dichotomous activities of TGF- β that exist in normal and malignant mammary tumors, as well as establish the pathophysiology of the “TGF- β Paradox” in preclinical models breast cancer development and metastasis regulated by TGF- β . In the succeeding sections, we discuss recent strategies and challenges related to the design and implementation of anti-TGF- β agents to treat metastatic disease.

Development and Implementation of Anti-TGF- β Chemotherapies

As discussed above, mammary tumors exploit aberrant TGF- β expression as a means to escape the confines of the primary tumor and disseminate to distant locales, most notably to the lungs, liver, bone, and brain [42]. In doing so, TGF- β acquires oncogenic functions that enable this cytokine to promote EMT and survival programs in late-stage breast cancers, as well as to engender their development of cancer-initiating, chemoresistant, and immunotolerant phenotypes (*see* [3, 12]). Thus, targeting the TGF- β pathway may offer new inroads to improving the overall survival of patients harboring metastatic breast cancers, a supposition that is being rigorously investigated in a variety of human tumors and fibrotic conditions driven by TGF- β [43]. To date, more than 30 anti-TGF- β agents have been developed and analyzed in preclinical and clinical settings; they also fall into 4 general categories, including large and small molecule inhibitors, antisense oligonucleotides, and various peptidomimetics and peptide aptamers (Table 1; [43–45]). Collectively, these anti-TGF- β agents have demonstrated varying degrees of success that generally track the nuances of the “TGF- β Paradox” and the extent to which TGF- β functions either as a tumor suppressor or oncogene within a given tumor. Additional challenges include the necessity to establish biomarkers capable of identifying patients recommended for anti-TGF- β therapies from those contraindicated for doing so, as well as the development of (*i*) acquired resistance to various anti-TGF- β agents, and (*ii*) secondary malignant lesions or other non-cancer-related complications, such as inflammation, autoimmune, or cardiovascular anomalies [43–45]. Here we discuss the findings associated with the preclinical and clinical administration of anti-TGF- β agents belonging to the 4 aforementioned categories (Table 1).

Large molecule inhibitors

It has long been known that transformed cells significantly upregulate their secretion of TGF- β as compared to their nontransformed counterparts [46], a phenomenon that occurs in breast cancer cells and triggers the activation of autocrine TGF- β signaling operant in mediating tumor promotion [8]. Attempts to alleviate inappropriate TGF- β signaling *via* large molecule inhibitors reflects two major approaches: (*i*) neutralizing anti-TGF- β antibodies, and (*ii*) soluble recombinant Fc fusion proteins that house the extracellular domains of either T β R-II (Fc-T β R-II or SR2F) or T β R-III (Fc-T β R-III). In both scenarios,

these large anti-TGF- β agents function by binding TGF- β and preventing its activation of TGF- β receptors [43–45], thereby eliminating oncogenic TGF- β signaling. As mentioned previously, transgenic expression [38] or systemic administration [37] of Fc-T β R-II significantly reduces the aggressiveness of mammary tumors driven by the transgenic expression of either PyMT or c-Neu. Similarly, administration of Fc-T β R-III was found to inhibit the angiogenesis of human MDA-MB-231 and MDA-MB-435 tumors, thereby reducing their growth and metastasis in nude mice [47]. Despite their apparent preclinical success, Fc-T β R-II and Fc-T β R-III analogues have yet to progress to clinical trials for breast cancer patients.

Administration of neutralizing anti-TGF- β monoclonal antibodies has also demonstrated success in alleviating breast cancer metastasis. For instance, the pan anti-TGF- β monoclonal antibody, ID11, which as developed by Genzyme and inactivates all three TGF- β isoforms, significantly decreased the migration and invasion of human MDA-MB-231 breast cancer cells *in vitro* [48]. Moreover, administration of ID11 also inhibited the ability of murine 4T1 tumors to metastasize to the lungs in syngeneic Balb/C mice, an event coupled to (i) enhanced infiltration of tumor-directed NK and T cells into 4T1 tumors [49], and (ii) elevated apoptosis resulting from diminished IL-17 signaling in 4T1 tumors [50]. Similar antitumor activities were observed upon administration of the pan anti-TGF- β monoclonal antibody, 2G7, which inhibited the growth and metastasis of human triple-negative breast cancers in mice [32]. Success in these preclinical studies bolstered efforts for the development and evaluation of humanized neutralizing anti-TGF- β monoclonal antibodies in clinical safety and efficacy analyses. Amongst the monoclonal antibodies against TGF- β that have entered clinical trials are Lerdelimumab (CAT-152; Genzyme) [51], Metelimumab (CAT-192; Genzyme) [52], Fresolimumab (GC-1008; Genzyme) [53], and LY238277 (Ely Lilly). Of these, Lerdelimumab and Metelimumab were both judged to be safe in humans; however, neither anti-TGF- β antibody proved to be effective in improving system sclerosis and corneal scarring, respectively. Likewise, LY2382770 is safe in humans and undergoing evaluation in the treatment of kidney fibrosis. Whether LY2382770, Lerdelimumab, or Metelimumab possess efficacy against breast cancers remains to be determined. The safety of Fresolimumab in humans has been established, which has paved the way for investigations of its efficacy against a variety of human ailments, including system sclerosis, fibrosis, and several cancers (*e.g.*, renal cell carcinoma, melanoma, and mesothelioma). Interestingly, radiation therapy activates latent TGF- β complexes, resulting in the acquisition of EMT, metastatic, and chemoresistant phenotypes in surviving breast cancer cells [54]. As such, the effectiveness of Fresolimumab to increase the radiosensitivity of breast cancer patients is currently being evaluated (clinical trial NCT01401062). Eli Lilly has pursued an alternative approach *via* the development of a humanized anti-T β R-II monoclonal antibody, IMC-TR1, that binds T β R-II and prevents its activation by TGF- β . The effectiveness of IMC-TR1 to alleviate colon and breast cancers in patients is currently being evaluated (clinical trial NCT01646203). Finally, we established the cysteine protease inhibitor Cystatin C as a novel antagonist of TGF- β signaling, doing so by interacting physically with T β R-II and preventing its binding of TGF- β [55, 56]. Importantly, engineering triple-negative breast cancer cells to overexpress human Cystatin C inhibited their formation of tumors and subsequent angiogenesis and metastasis in mice [57]. Moreover, we recently

observed intravenous administration of recombinant human Cystatin C to significantly impede the colonization and metastatic outgrowth of 4T1 breast cancer cells in syngeneic Balb/C mice (R. Kanwal, B.J. Schiemann, and W.P. Schiemann, *unpublished observation*).

Small molecule inhibitors

The use of small molecule ATP analogues that inactivate protein kinases by targeting their ATP-binding sites has revolutionized the treatment of human cancers (*e.g.*, Imatinib against BCR-ABL; [58]). T β R-I and T β R-II are both Ser/Thr protein kinases, whose coupling to the activation of canonical and noncanonical TGF- β signaling systems can be readily inhibited by administration of a plethora of small molecule inhibitors against these receptors [43, 59]. Unlike their large molecule counterparts, small molecule inhibitors of the TGF- β pathway are generally produced in a more efficient and economical fashion, as well as administered in a more straightforward manner; however, these anti-TGF- β agents suffer from their propensity to inhibit off-target protein kinases that can elicit adverse events (see [60, 61]). Amongst the small molecule T β R-I inhibitors is Ki26894, which was developed by Kirin Pharmaceuticals and successfully blocked the invasion and metastasis of human MDA-MB-231 breast cancer cells. Moreover, pretreating these cells with Ki26894 prior to their inoculation into mice also decreased the metastatic ability of MDA-MB-231 cells and prolonged the lifespan of tumor bearing animals [62]. Similarly, LY364947 was observed to reduce the ability of MDA-MB-435 breast (melanoma) cells to metastasize to the bone or lungs of mice [63]. Along these lines, Scios' SD208 has proven effective in decreasing the growth and invasiveness of human and mouse gliomas [64], and in preventing the development of osteolytic bone metastases by human melanoma xenografts in mice [65]. Taking a slightly different approach, Eli Lilly developed a novel dual T β R-I and T β R-II inhibitor called LY2109761, which suppresses the metastasis of pancreatic and colon cancers in mice [66, 67]. Interestingly, LY2109761 proved to be more effective in reducing the growth of early-stage breast cancer bone metastases as compared to their well-established counterparts in mouse xenografts [68]. Additional small molecule inhibitors currently undergoing preclinical evaluation include SB-431542, SD-093, SM16, and IN-1130, all of which demonstrate significant antitumor activity *in vitro* and *in vivo*. For instance, SB-431542 inhibits the invasion of Ras-transformed mammary epithelial cells [69], while SM16 inhibits the phosphorylation of Smad2 in 4T1 tumors and their metastases in mice [70]. Similarly, SD-093 blocks the phosphorylation of Smad2 and Smad3 in pancreatic cancer cells treated with TGF- β [71], while IN-1130 suppresses breast cancer metastasis in MMTV-c-Neu transgenic mice, as well as reduces renal and hepatic fibrosis [72]. Finally, oral administration of LY2157299 was effective in diminishing mammary tumor growth in mice [73] and is currently being evaluated in several clinical trials for solid tumors.

Antisense oligonucleotides

Antisense oligonucleotides (ASOs) complementary to TGF- β mRNA have been developed as a means to prevent its translation, thereby reducing the concentrations of TGF- β within the tumor microenvironment. Challenges associated with the administration of ASOs are significant and include the *(i)* potential to elicit off-target effects, *(ii)* unpredictable RNA binding affinities in biological systems, and *(iii)* efficient and specific delivery to tumors.

Despite these considerable limitations, Antisense Pharma managed to bring AP12009 (Trabedersen) to clinical trials to evaluate its safety and efficacy. Indeed, the progression and immunotolerance exhibited by glioblastomas, melanomas, pancreatic, and some colorectal cancers has been associated to their specific expression of TGF- β 2, which is specifically targeted by AP12009 [74, 75]. To date, Phase I/II trial data demonstrate that AP12009 is safe and well-tolerated, having few severe side effects or adverse reactions; it also provides significant survival benefits in patients with malignant gliomas [75]. Additionally, Antisense Pharma has also developed AP11014 to specifically target the translation of TGF- β 1 mRNA and is undergoing preclinical evaluation for the treatment of non-small cell lung, colorectal, and prostate cancers that overexpress TGF- β 1 (NSCLC) [61]. Finally, NovaRx has developed Lucanix, which involves the transfection of antisense TGF- β 2 cDNA into allogeneic NSCLC for their use as tumor vaccines. Importantly, studies of Lucanix have advanced to Phase III clinical trials, which have shown Lucanix to provide superior tumor vaccine activity and significant survival benefits to NSCLC patients as compared to conventional treatment approaches [76]. Future studies need to address the potential effectiveness of ASOs in the treatment of human breast cancers.

Peptidomimetics and peptide aptamer

Recently, several peptidomimetics and peptide aptamers have been developed as a means to disrupt canonical TGF- β signaling. For instance, peptide aptamers are small peptides comprised of targeting and scaffolding domains that function to disrupt the formation of signaling complexes in diseased cells. With respect to targeting the TGF- β pathway, the Trx-SARA and Trx-xFoxH1b aptamers both bind to Smads 2 and 3 and prevent their association with Smad4 and FoxH1, respectively, thereby inhibiting canonical TGF- β signaling and its induction of EMT [77, 78]. Along these lines, Digna Biotech developed a novel 14-mer peptidomimetic, P144, based on the TGF- β -binding sequence housed in T β R-III. Thus far, P144 has demonstrated efficacy in preventing fibrosis, as well as in enhancing antitumor immunotherapies [79, 80]; this peptidomimetic has also completed phase I/II clinical evaluation for the treatment of scleroderma and skin fibrosis, but awaits evaluation in human breast cancers. Lastly, an innovative Leu-Ser-Lys-Leu peptide has been developed that interacts with LAP and prevents its release of active TGF- β in response to thrombospondin, thereby reducing inappropriate TGF- β signaling during fibrosis and diabetic nephropathy [81]. As above, the potential clinical utility of peptidomimetics and peptide aptamers to alleviate metastatic breast cancers remains to be thoroughly investigated.

Future Perspective

The oncogenic activities of TGF- β in breast cancers transpire through its ability to induce EMT programs, to elicit tumor immunotolerance, to promote survival signaling, and to enhance the selection and expansion of cancer stem cells (for review see [3, 11, 12]). These aberrant TGF- β activities, coupled with its dramatically upregulated expression in mammary tumors and their accompanying microenvironments has led to development of targeted anti-TGF- β therapies, many of which have been highly effective in preclinical analyses, as well as in some clinical settings. Despite these successes, challenges remain associated with the routine use of these agents in human patients. Indeed, while large molecule inhibitors

possess unparalleled target specificity, they nevertheless suffer from difficulties related to their size and ability to readily penetrate tumor tissues. In contrast, small molecule inhibitors readily penetrate tumor sites, but these agents suffer from diminished target specificity and a propensity to elicit adverse reactions. Thus, developing compounds that strike a balance between these classes of anti-TGF- β agents may offer new avenues in alleviating the oncogenic activities of TGF- β in breast cancer cells.

Most cancers eventually develop resistance to chemotherapies, including agents that originally provided significant antitumor activities. For example, Erlotinib (EGFR inhibitor), Imatinib (ABL inhibitor), and Sorafenib (VEGFR/PDGFR inhibitor) initially elicit disease remission in clinical settings, which eventually succumb to aggressive disease progression mediated by cellular reprogramming and/or EMT reactions [82]. Whether these events reflect the development of acquired resistance or the selective evolution and outgrowth of rare tumor variants remains to be determined. Interestingly, acute administration of the dual T β R-I/T β R-II inhibitor, LY2109761, inhibits the induction of EMT programs by TGF- β , while long-term administration of this same molecule elicits drug resistance coupled to the generation of an EMT phenotype [83]. Collectively, these findings suggest that monotherapy approaches to anti-TGF- β agents will almost certainly fail, thereby requiring the coupling of these agents with other treatment modalities, particularly DNA damaging agents and/or radiation. Indeed, combining doxorubicin and small molecule T β R-I inhibitors proved to be significantly more effective in alleviating the metastasis of MDA-MB-231 tumors in mice than either compound alone [84]. Future studies also need to address the role that breast cancer heterogeneity plays in determining carcinoma response to anti-TGF- β agents. Part of this heterogeneity reflects the expression status of estrogen receptor- α (ER- α), whose activation by hormone typically opposes the actions of TGF- β in mammary epithelial cells. Mechanistically, hormone-bound ER α interacts physically with Smad2/3, leading to the recruitment of Smurf1 and its ubiquitination and eventual degradation of Smad2/3 [85]. Additionally, the inhibitor of TGF- β signaling SnoN binds to and enhances the transcriptional activity of ER- α [86] and its suppression of TGF- β signaling [87]. In stark contrast, administration of anti-estrogens to ER- α -positive cells stimulates the production of TGF- β and expression of T β R-II [88–90], as well as facilitates the inactivation of ER- α *via* its interaction with Smad4 [91]. Collectively, these TGF- β events coalesce in augmenting the antiproliferative activities of anti-estrogens. Along these lines, TGF- β stimulation of the EMT transcription factor Snail represses the ER- α expression, leading to enhanced expression of TGF- β effectors and increased tumorigenicity of mammary tumors [92]. Indeed, ER- α expression plays a pivotal role in predicting patient disease recurrence and overall patient survival in TGF- β -driven breast cancers. For instance, gene signatures corresponding to a loss of TGF- β function in estrogen receptor-positive mammary tumors predict for disease recurrence and poor patient prognosis [30, 93]. Interestingly, these same clinical outcomes in estrogen receptor-negative mammary tumors reflects a gene signature indicative of their stimulation by TGF- β , thereby highlighting the (i) dichotomous functions of TGF- β in genetically distinct breast cancer subtypes, (ii) necessity to develop predictive biomarkers for directing and optimizing the use of anti-TGF- β agents in breast cancer patients, and (iii) distinct possibility that anti-TGF- β agents may prove to be more effective in ER- α -negative tumors as compared to their ER- α -positive counterparts. In summary, the

future remains bright for the implementation of anti-TGF- β agents to treat a variety of human cancers, including those of the breast; however, garnering a greater understanding of molecular mechanisms and events operant in manifesting the “TGF- β Paradox” and its stimulation of oncogenic TGF- β functions will undoubtedly assist in the development of novel combinatorial therapies to alleviate breast cancer metastasis and disease recurrence.

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References

1. Berry DA, Cronin KA, Plevritis SK, et al. Effect of screening and adjuvant therapy on mortality from breast cancer. *N Engl J Med*. 2005; 353:1784–1792. [PubMed: 16251534]
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin*. 2013; 63:11–30. [PubMed: 23335087]
3. Taylor MA, Parvani JG, Schiemann WP. The pathophysiology of epithelial-mesenchymal transition induced by transforming growth factor- β in normal and malignant mammary epithelial cells. *J Mammary Gland Biol Neoplasia*. 2010; 15:169–190. [PubMed: 20467795]
4. Nguyen DX, Massague J. Genetic determinants of cancer metastasis. *Nat Rev Genet*. 2007; 8:341–352. [PubMed: 17440531]
5. Perou CM, Borresen-Dale AL. Systems biology and genomics of breast cancer. *Cold Spring Harb Perspect Biol*. 2011; 3 pii: a003293.
6. Moses H, Barcellos-Hoff MH. TGF- β biology in mammary development and breast cancer. *Cold Spring Harb Perspect Biol*. 2011; 3:a003277. [PubMed: 20810549]
7. Wendt MK, Allington TM, Schiemann WP. Mechanisms of the epithelial-mesenchymal transition by TGF- β . *Future Oncol*. 2009; 5:1145–1168. [PubMed: 19852727]
8. Drabsch Y, Ten Dijke P. TGF- β signaling in breast cancer cell invasion and bone metastasis. *J Mammary Gland Biol Neoplasia*. 2011; 16:97–108. [PubMed: 21494783]
9. Gatza CE, Oh SY, Blobe GC. Roles for the type III TGF- β receptor in human cancer. *Cell Signal*. 2010; 22:1163–1174. [PubMed: 20153821]
10. Parvani JG, Taylor MA, Schiemann WP. Noncanonical TGF- β signaling during mammary tumorigenesis. *J Mammary Gland Biol Neoplasia*. 2011; 16:127–146. [PubMed: 21448580]
11. Heldin CH, Landstrom M, Moustakas A. Mechanism of TGF- β signaling to growth arrest, apoptosis, and epithelial-mesenchymal transition. *Curr Opin Cell Biol*. 2009; 21:166–176. [PubMed: 19237272]
12. Massague J. TGF- β in Cancer. *Cell*. 2008; 134:215–230. [PubMed: 18662538]
13. Moustakas A, Heldin CH. Non-Smad TGF- β signals. *J Cell Sci*. 2005; 118:3573–3584. [PubMed: 16105881]
14. Taylor MA, Lee YH, Schiemann WP. Role of TGF- β and the tumor microenvironment during mammary tumorigenesis. *Gene Expr*. 2011; 15:117–132. [PubMed: 22268294]
15. Bieri B, Moses HL. Tumour microenvironment: TGF- β : the molecular Jekyll and Hyde of cancer. *Nat Rev Cancer*. 2006; 6:506–520. [PubMed: 16794634]
16. Roberts AB, Anzano MA, Lamb LC, Smith JM, Sporn MB. New class of transforming growth factors potentiated by epidermal growth factor: isolation from non-neoplastic tissues. *Proc Natl Acad Sci USA*. 1981; 78:5339–5343. [PubMed: 6975480]
17. Moses HL, Branum EL, Proper JA, Robinson RA. Transforming growth factor production by chemically transformed cells. *Cancer Res*. 1981; 41:2842–2848. [PubMed: 6265069]
18. Carr BI, Hayashi I, Branum EL, Moses HL. Inhibition of DNA synthesis in rat hepatocytes by platelet-derived type beta transforming growth factor. *Cancer Res*. 1986; 46:2330–2334. [PubMed: 3008986]

19. Frater-Schroder M, Muller G, Birchmeier W, Bohlen P. Transforming growth factor- β inhibits endothelial cell proliferation. *Biochem Biophys Res Commun*. 1986; 137:295–302. [PubMed: 3459460]
20. Kehrl JH, Wakefield LM, Roberts AB, et al. Production of transforming growth factor β by human T lymphocytes and its potential role in the regulation of T cell growth. *J Exp Med*. 1986; 163:1037–1050. [PubMed: 2871125]
21. Kehrl JH, Roberts AB, Wakefield LM, Jakowlew S, Sporn MB, Fauci AS. Transforming growth factor β is an important immunomodulatory protein for human B lymphocytes. *J Immunol*. 1986; 137:3855–3860. [PubMed: 2878044]
- 22**. Silberstein GB, Daniel CW. Reversible inhibition of mammary gland growth by transforming growth factor- β . *Science*. 1987; 237(4812):291–293. First study to establish TGF- β as a potent regulator of mammary gland development and proliferation. [PubMed: 3474783]
23. Robinson SD, Roberts AB, Daniel CW. TGF- β suppresses casein synthesis in mouse mammary explants and may play a role in controlling milk levels during pregnancy. *J Cell Biol*. 1993; 120:245–251. [PubMed: 8416990]
24. Robinson SD, Silberstein GB, Roberts AB, Flanders KC, Daniel CW. Regulated expression and growth inhibitory effects of transforming growth factor- β isoforms in mouse mammary gland development. *Development*. 1991; 113:867–878. [PubMed: 1821856]
25. Daniel CW, Silberstein GB, Van Horn K, Strickland P, Robinson S. TGF- β 1-induced inhibition of mouse mammary gland ductal growth: Developmental specificity and characterization. *Dev Biol*. 1989; 135:20–30. [PubMed: 2767334]
26. Kordon EC, Mcknight RA, Jhappan C, Hennighausen L, Merlino G, Smith GH. Ectopic TGF- β 1 expression in the secretory mammary epithelium induces early senescence of the epithelial stem cell population. *Dev Biol*. 1995; 168:47–61. [PubMed: 7883078]
27. Jhappan C, Geiser AG, Kordon EC, et al. Targeting expression of a transforming growth factor β 1 transgene to the pregnant mammary gland inhibits alveolar development and lactation. *EMBO J*. 1993; 12:1835–1845. [PubMed: 8491177]
28. Bottinger EP, Jakubczak JL, Haines DC, Bagnall K, Wakefield LM. Transgenic mice overexpressing a dominant-negative mutant type II transforming growth factor β receptor show enhanced tumorigenesis in the mammary gland and lung in response to the carcinogen 7,12-dimethylbenz-[a]-anthracene. *Cancer Res*. 1997; 57:5564–5570. [PubMed: 9407968]
29. Figueroa JD, Flanders KC, Garcia-Closas M, et al. Expression of TGF- β signaling factors in invasive breast cancers: relationships with age at diagnosis and tumor characteristics. *Breast Cancer Res Treat*. 2010; 121:727–735. [PubMed: 19937272]
30. Brier B, Chung CH, Parker JS, et al. Abrogation of TGF- β signaling enhances chemokine production and correlates with prognosis in human breast cancer. *J Clin Invest*. 2009; 119:1571–1582. [PubMed: 19451693]
31. Blobel GC, Schiemann WP, Lodish HF. Role of transforming growth factor β in human disease. *N Engl J Med*. 2000; 342:1350–1358. [PubMed: 10793168]
32. Arteaga CL, Hurd SD, Winnier AR, Johnson MD, Fendly BM, Forbes JT. Anti-transforming growth factor (TGF)- β antibodies inhibit breast cancer cell tumorigenicity and increase mouse spleen natural killer cell activity Implications for a possible role of tumor cell/host TGF- β interactions in human breast cancer progression. *J Clin Invest*. 1993; 92:2569–2576. [PubMed: 7504687]
33. Muraoka-Cook RS, Kurokawa H, Koh Y, et al. Conditional overexpression of active transforming growth factor β 1 in vivo accelerates metastases of transgenic mammary tumors. *Cancer Res*. 2004; 64:9002–9011. [PubMed: 15604265]
34. Tian F, Byfield SD, Parks WT, et al. Smad-binding defective mutant of transforming growth factor β type I receptor enhances tumorigenesis but suppresses metastasis of breast cancer cell lines. *Cancer Res*. 2004; 64:4523–4530. [PubMed: 15231662]
35. Tian F, Dacosta Byfield S, Parks WT, et al. Reduction in Smad2/3 signaling enhances tumorigenesis but suppresses metastasis of breast cancer cell lines. *Cancer Res*. 2003; 63:8284–8292. [PubMed: 14678987]

36. Tang B, Vu M, Booker T, et al. TGF- β switches from tumor suppressor to prometastatic factor in a model of breast cancer progression. *J Clin Invest*. 2003; 112:1116–1124. [PubMed: 14523048]
- 37***. Muraoka RS, Dumont N, Ritter CA, et al. Blockade of TGF- β inhibits mammary tumor cell viability, migration, and metastases. *J Clin Invest*. 2002; 109:1551–1559. Together with Reference 38, these studies provide the rationale for developing anti-TGF- β agents, whose activities do not recapitulate the lethality associated with genetic inactivation of TGF- β alleles in mice. [PubMed: 12070302]
- 38***. Yang YA, Dukhanina O, Tang B, et al. Lifetime exposure to a soluble TGF- β antagonist protects mice against metastasis without adverse side effects. *J Clin Invest*. 2002; 109:1607–1615. See [37]. [PubMed: 12070308]
39. Cheng N, Bhowmick NA, Chytil A, et al. Loss of TGF- β type II receptor in fibroblasts promotes mammary carcinoma growth and invasion through upregulation of TGF- α -, MSP- and HGF-mediated signaling networks. *Oncogene*. 2005; 24:5053–5068. [PubMed: 15856015]
40. Bhowmick NA, Moses HL. Tumor-stroma interactions. *Curr Opin Genet Dev*. 2005; 15:97–101. [PubMed: 15661539]
- 41***. Bhowmick NA, Chytil A, Plieth D, et al. TGF- β signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science*. 2004; 303:848–851. Important study demonstrates the essential role of paracrine TGF- β signaling networks to govern tissue homeostasis and prevent tumorigenesis. [PubMed: 14764882]
42. Chiang AC, Massague J. Molecular basis of metastasis. *N Engl J Med*. 2008; 359:2814–2823. [PubMed: 19109576]
43. Akhurst RJ, Hata A. Targeting the TGF- β signalling pathway in disease. *Nat Rev Drug Discov*. 2012; 11:790–811. [PubMed: 23000686]
44. Connolly EC, Freimuth J, Akhurst RJ. Complexities of TGF- β targeted cancer therapy. *Int J Biol Sci*. 2012; 8:964–978. [PubMed: 22811618]
45. Nagaraj NS, Datta PK. Targeting the transforming growth factor- β signaling pathway in human cancer. *Expert Opin Investig Drugs*. 2010; 19:77–91.
46. Anzano MA, Roberts AB, De Larco JE, et al. Increased secretion of type β transforming growth factor accompanies viral transformation of cells. *Mol Biol Cell*. 1985; 5:242–247.
47. Bandyopadhyay A, Zhu Y, Malik SN, et al. Extracellular domain of TGF- β type III receptor inhibits angiogenesis and tumor growth in human cancer cells. *Oncogene*. 2002; 21:3541–3551. [PubMed: 12032856]
48. Ganapathy V, Ge R, Grazioli A, et al. Targeting the transforming growth factor- β pathway inhibits human basal-like breast cancer metastasis. *Mol Cancer*. 2010; 9:122. [PubMed: 20504320]
49. Nam JS, Terabe M, Kang MJ, et al. Transforming growth factor β subverts the immune system into directly promoting tumor growth through interleukin-17. *Cancer Res*. 2008; 68:3915–3923. [PubMed: 18483277]
50. Nam JS, Terabe M, Mamura M, et al. An anti-transforming growth factor β antibody suppresses metastasis via cooperative effects on multiple cell compartments. *Cancer Res*. 2008; 68:3835–3843. [PubMed: 18483268]
51. Mead AL, Wong TT, Cordeiro MF, Anderson IK, Khaw PT. Evaluation of anti-TGF- β 2 antibody as a new postoperative anti-scarring agent in glaucoma surgery. *Invest Ophthalmol Vis Sci*. 2003; 44:3394–3401. [PubMed: 12882787]
52. Denton CP, Merkel PA, Furst DE, et al. Recombinant human anti-transforming growth factor β 1 antibody therapy in systemic sclerosis: a multicenter, randomized, placebo-controlled phase I/II trial of CAT-192. *Arthritis Rheum*. 2007; 56:323–333. [PubMed: 17195236]
53. Trachtman H, Fervenza FC, Gipson DS, et al. A phase 1, single-dose study of fresolimumab, an anti-TGF- β antibody, in treatment-resistant primary focal segmental glomerulosclerosis. *Kidney Int*. 2011; 79:1236–1243. [PubMed: 21368745]
54. Barcellos-Hoff MH, Akhurst RJ. Transforming growth factor- β in breast cancer: too much, too late. *Breast Cancer Res*. 2009; 11:202. [PubMed: 19291273]
55. Sokol JP, Schiemann WP. Cystatin C antagonizes transforming growth factor β signaling in normal and cancer cells. *Mol Cancer Res*. 2004; 2:183–195. [PubMed: 15037657]

56. Sokol JP, Neil JR, Schiemann BJ, Schiemann WP. The use of cystatin C to inhibit epithelial-mesenchymal transition and morphological transformation stimulated by transforming growth factor- β . *Breast Cancer Res.* 2005; 7:R844–853. [PubMed: 16168131]
57. Tian M, Schiemann WP. Preclinical efficacy of cystatin C to target the oncogenic activity of transforming growth factor β in breast cancer. *Transl Oncol.* 2009; 2:174–183. [PubMed: 19701502]
58. Hunter T. Treatment for chronic myelogenous leukemia: the long road to imatinib. *J Clin Invest.* 2007; 117:2036–2043. [PubMed: 17671637]
59. Yingling JM, Blanchard KL, Sawyer JS. Development of TGF- β signalling inhibitors for cancer therapy. *Nat Rev Drug Discov.* 2004; 3:1011–1022. [PubMed: 15573100]
60. Akhurst RJ. Large- and small-molecule inhibitors of transforming growth factor- β signaling. *Curr Opin Investig Drugs.* 2006; 7:513–521.
61. Saunier EF, Akhurst RJ. TGF- β inhibition for cancer therapy. *Curr Cancer Drug Targets.* 2006; 6:565–578. [PubMed: 17100564]
62. Ehata S, Hanyu A, Fujime M, et al. Ki26894, a novel transforming growth factor- β type I receptor kinase inhibitor, inhibits in vitro invasion and in vivo bone metastasis of a human breast cancer cell line. *Cancer Sci.* 2007; 98:127–133. [PubMed: 17129361]
63. Bandyopadhyay A, Agyin JK, Wang L, et al. Inhibition of pulmonary and skeletal metastasis by a transforming growth factor- β type I receptor kinase inhibitor. *Cancer Res.* 2006; 66:6714–6721. [PubMed: 16818646]
64. Uhl M, Aulwurm S, Wischhusen J, et al. SD-208, a novel transforming growth factor β receptor I kinase inhibitor, inhibits growth and invasiveness and enhances immunogenicity of murine and human glioma cells in vitro and in vivo. *Cancer Res.* 2004; 64:7954–7961. [PubMed: 15520202]
65. Mohammad KS, Javelaud D, Fournier PG, et al. TGF- β -RI kinase inhibitor SD-208 reduces the development and progression of melanoma bone metastases. *Cancer Res.* 2011; 71:175–184. [PubMed: 21084275]
66. Melisi D, Ishiyama S, Sclabas GM, et al. LY2109761, a novel transforming growth factor β receptor type I and type II dual inhibitor, as a therapeutic approach to suppressing pancreatic cancer metastasis. *Mol Cancer Ther.* 2008; 7:829–840. [PubMed: 18413796]
67. Zhang B, Halder SK, Zhang S, Datta PK. Targeting transforming growth factor- β signaling in liver metastasis of colon cancer. *Cancer Lett.* 2009; 277:114–120. [PubMed: 19147275]
68. Korpai M, Yan J, Lu X, Xu S, Lerit DA, Kang Y. Imaging transforming growth factor- β signaling dynamics and therapeutic response in breast cancer bone metastasis. *Nat Med.* 2009; 15:960–966. [PubMed: 19597504]
69. Wiercinska E, Naber HP, Pardali E, Van Der Pluijm G, Van Dam H, Ten Dijke P. The TGF- β /Smad pathway induces breast cancer cell invasion through the up-regulation of matrix metalloproteinase 2 and 9 in a spheroid invasion model system. *Breast Cancer Res Treat.* 2011; 128:657–666. [PubMed: 20821046]
70. Rausch MP, Hahn T, Ramanathapuram L, et al. An orally active small molecule TGF- β receptor I antagonist inhibits the growth of metastatic murine breast cancer. *Anticancer Res.* 2009; 29:2099–2109. [PubMed: 19528470]
71. Subramanian G, Schwarz RE, Higgins L, et al. Targeting endogenous transforming growth factor β receptor signaling in SMAD4-deficient human pancreatic carcinoma cells inhibits their invasive phenotype1. *Cancer Res.* 2004; 64:5200–5211. [PubMed: 15289325]
72. Kim YW, Kim YK, Lee JY, et al. Pharmacokinetics and tissue distribution of 3-((5-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-imidazol-2-yl)methyl)benzamide; a novel ALK5 inhibitor and a potential anti-fibrosis drug. *Xenobiotica.* 2008; 38:325–339. [PubMed: 18274960]
73. Bueno L, De Alwis DP, Pitou C, et al. Semi-mechanistic modelling of the tumour growth inhibitory effects of LY2157299, a new type I receptor TGF- β kinase antagonist, in mice. *Eur J Cancer.* 2008; 44:142–150. [PubMed: 18039567]
74. Schlingensiepen KH, Fischer-Blass B, Schmaus S, Ludwig S. Antisense therapeutics for tumor treatment: the TGF- β 2 inhibitor AP 12009 in clinical development against malignant tumors. *Recent Results Cancer Res.* 2008; 177:137–150. [PubMed: 18084956]

75. Hau P, Jachimczak P, Schlingensiepen R, et al. Inhibition of TGF- β 2 with AP 12009 in recurrent malignant gliomas: from preclinical to phase I/II studies. *Oligonucleotides*. 2007; 17:201–212. [PubMed: 17638524]
76. Nemunaitis J, Dillman RO, Schwarzenberger PO, et al. Phase II study of belagenpumatucel-L, a transforming growth factor β 2 antisense gene-modified allogeneic tumor cell vaccine in non-small-cell lung cancer. *J Clin Oncol*. 2006; 24:4721–4730. [PubMed: 16966690]
77. Cui Q, Lim SK, Zhao B, Hoffmann FM. Selective inhibition of TGF- β responsive genes by Smad-interacting peptide aptamers from FoxH1, Lef1 and CBP. *Oncogene*. 2005; 24:3864–3874. [PubMed: 15750622]
78. Zhao BM, Hoffmann FM. Inhibition of transforming growth factor- β 1-induced signaling and epithelial-to-mesenchymal transition by the Smad-binding peptide aptamer Trx-SARA. *Mol Biol Cell*. 2006; 17:3819–3831. [PubMed: 16775010]
79. Santiago B, Gutierrez-Canas I, Dotor J, et al. Topical application of a peptide inhibitor of transforming growth factor- β 1 ameliorates bleomycin-induced skin fibrosis. *J Invest Dermatol*. 2005; 125:450–455. [PubMed: 16117784]
80. Hermida N, Lopez B, Gonzalez A, et al. A synthetic peptide from transforming growth factor- β 1 type III receptor prevents myocardial fibrosis in spontaneously hypertensive rats. *Cardiovasc Res*. 2009; 81:601–609. [PubMed: 19019833]
81. Lu A, Miao M, Schoeb TR, Agarwal A, Murphy-Ullrich JE. Blockade of TSP1-dependent TGF- β activity reduces renal injury and proteinuria in a murine model of diabetic nephropathy. *Am J Pathol*. 2011; 178:2573–2586. [PubMed: 21641382]
82. Engelman JA, Settleman J. Acquired resistance to tyrosine kinase inhibitors during cancer therapy. *Curr Opin Genet Dev*. 2008; 18:73–79. [PubMed: 18325754]
83. Connolly EC, Saunier EF, Quigley D, et al. Outgrowth of drug-resistant carcinomas expressing markers of tumor aggression after long-term T β RI/II kinase inhibition with LY2109761. *Cancer Res*. 2011; 71:2339–2349. [PubMed: 21282335]
84. Bandyopadhyay A, Wang L, Agyin J, et al. Doxorubicin in combination with a small TGF- β inhibitor: a potential novel therapy for metastatic breast cancer in mouse models. *PLoS One*. 2010; 5:e10365. [PubMed: 20442777]
85. Ito I, Hanyu A, Wayama M, et al. Estrogen inhibits transforming growth factor β signaling by promoting Smad2/3 degradation. *J Biol Chem*. 2010; 285:14747–14755. [PubMed: 20207742]
86. Band AM, Laiho M. SnoN oncoprotein enhances estrogen receptor- α transcriptional activity. *Cell Signal*. 2012; 24:922–930. [PubMed: 2227247]
87. Band AM, Laiho M. Crosstalk of TGF- β and estrogen receptor signaling in breast cancer. *J Mammary Gland Biol Neoplasia*. 2011; 16:109–115. [PubMed: 21390570]
88. Buck MB, Pfizenmaier K, Knabbe C. Antiestrogens induce growth inhibition by sequential activation of p38 mitogen-activated protein kinase and transforming growth factor- β pathways in human breast cancer cells. *Mol Endocrinol*. 2004; 18:1643–1657. [PubMed: 15056732]
- 89*. Knabbe C, Lippman ME, Wakefield LM, et al. Evidence that transforming growth factor- β is a hormonally regulated negative growth factor in human breast cancer cells. *Cell*. 1987; 48:417–428. First study to show the interplay between TGF- β and ER- α signaling in breast cancer cells. [PubMed: 2879636]
90. Chen H, Tritton TR, Kenny N, Absher M, Chiu JF. Tamoxifen induces TGF- β 1 activity and apoptosis of human MCF-7 breast cancer cells in vitro. *J Cell Biochem*. 1996; 61:9–17. [PubMed: 8726350]
91. Wu L, Wu Y, Gathings B, et al. Smad4 as a transcription corepressor for estrogen receptor α . *J Biol Chem*. 2003; 278:15192–15200. [PubMed: 12576474]
92. Dhasarathy A, Kajita M, Wade PA. The transcription factor snail mediates epithelial to mesenchymal transitions by repression of estrogen receptor- α . *Mol Endocrinol*. 2007; 21:2907–2918. [PubMed: 17761946]
93. Yang L, Huang J, Ren X, et al. Abrogation of TGF- β signaling in mammary carcinomas recruits Gr-1+CD11b+ myeloid cells that promote metastasis. *Cancer Cell*. 2008; 13:23–35. [PubMed: 18167337]

94. Nam JS, Suchar AM, Kang MJ, et al. Bone sialoprotein mediates the tumor cell-targeted prometastatic activity of transforming growth factor β in a mouse model of breast cancer. *Cancer Res.* 2006; 66:6327–6335. [PubMed: 16778210]
95. Muraoka-Cook RS, Dumont N, Arteaga CL. Dual role of transforming growth factor β in mammary tumorigenesis and metastatic progression. *Clin Cancer Res.* 2005; 11:937s–943s. [PubMed: 15701890]
96. Lahn M, Kloeker S, Berry BS. TGF- β inhibitors for the treatment of cancer. *Expert Opin Investig Drugs.* 2005; 14:629–643.
97. Zhong Z, Carroll KD, Policarpo D, et al. Anti-transforming growth factor β receptor II antibody has therapeutic efficacy against primary tumor growth and metastasis through multieffects on cancer, stroma, and immune cells. *Clin Cancer Res.* 2010; 16:1191–1205. [PubMed: 20145179]
98. Sawyer JS, Anderson BD, Beight DW, et al. Synthesis and activity of new aryl- and heteroaryl-substituted pyrazole inhibitors of the transforming growth factor- β type I receptor kinase domain. *J Med Chem.* 2003; 46:3953–3956. [PubMed: 12954047]
99. Sawyer TK. Novel oncogenic protein kinase inhibitors for cancer therapy. *Curr Med Chem Anticancer Agents.* 2004; 4:449–455. [PubMed: 15379702]
100. Hjelmeland MD, Hjelmeland AB, Sathornsumetee S, et al. SB-431542, a small molecule transforming growth factor- β -receptor antagonist, inhibits human glioma cell line proliferation and motility. *Mol Cancer Ther.* 2004; 3:737–745. [PubMed: 15210860]
101. Suzuki E, Kim S, Cheung HK, et al. A novel small-molecule inhibitor of transforming growth factor β type I receptor kinase (SM16) inhibits murine mesothelioma tumor growth in vivo and prevents tumor recurrence after surgical resection. *Cancer Res.* 2007; 67:2351–2359. [PubMed: 17332368]
102. Moon JA, Kim HT, Cho IS, Sheen YY, Kim DK. IN-1130, a novel transforming growth factor- β type I receptor kinase (ALK5) inhibitor, suppresses renal fibrosis in obstructive nephropathy. *Kidney Int.* 2006; 70:1234–1243. [PubMed: 16929250]
103. Dumont N, Arteaga CL. Targeting the TGF- β signaling network in human neoplasia. *Cancer Cell.* 2003; 3:531–536. [PubMed: 12842082]
104. Tojo M, Hamashima Y, Hanyu A, et al. The ALK-5 inhibitor A-83-01 inhibits Smad signaling and epithelial-to-mesenchymal transition by transforming growth factor- β . *Cancer Sci.* 2005; 96:791–800. [PubMed: 16271073]
105. Tran TT, Uhl M, Ma JY, et al. Inhibiting TGF- β signaling restores immune surveillance in the SMA-560 glioma model. *Neuro Oncol.* 2007; 9:259–270. [PubMed: 17522330]
106. Nemunaitis J, Jahan T, Ross H, et al. Phase 1/2 trial of autologous tumor mixed with an allogeneic GVAX vaccine in advanced-stage non-small-cell lung cancer. *Cancer Gene Ther.* 2006; 13:555–562. [PubMed: 16410826]

Executive Summary

The TGF- β Signaling System

- Human express three distinct isoforms of TGF- β , namely TGF- β 1, TGF- β 2, and TGF- β 3.
- TGF- β signaling commences by its binding to three high affinity receptors, namely T β R-I, T β R-II, and T β R-III, which collectively promote the phosphorylation and activation of the latent transcription factors, Smad2 and Smad3.
- Phosphorylated Smad2/3 form heterocomplexes with Smad4, which translocate into the nucleus en masse to govern the expression of TGF- β -responsive genes.
- Biological functions mediated by Smad2/3 are known as canonical TGF- β signaling.
- Biological functions independent of Smad2/3 are known as noncanonical TGF- β signaling.
- Inappropriate activation of noncanonical TGF- β signaling induces EMT and promotes the selection and expansion of cancer stem and progenitor cells; it also manifests the “TGF- β Paradox.”

TGF- β and the Mammary Gland

- TGF- β plays a prominent role in all stages of mammary gland development, differentiation, and homeostasis.
- TGF- β suppresses mammary tumorigenesis by inhibiting MEC proliferation, or by stimulating MEC apoptosis or differentiation.
- Intriguingly, mammary tumorigenesis converts the functions of TGF- β from that of a tumor suppressor to a promoter of breast cancer invasion and metastasis.
- Oncogenic TGF- β signaling also promotes the acquisition of EMT, cancer stem cell-like, and chemoresistant phenotypes.
- Aberrant expression of either TGF- β or T β R-II is strongly associated with the onset of aggressive disease states and metastasis.

Development and Implementation of Anti-TGF- β Chemotherapies

- More than 30 anti-TGF- β agents have been developed and are under evaluation in preclinical and clinical settings.
- Anti-TGF- β agents fall into 4 general categories (*i*) large molecule inhibitors, (*ii*) small molecule inhibitors, (*iii*) antisense oligonucleotides, and (*iv*) peptidomimetics and peptide aptamers.
- Large molecule inhibitors of TGF- β action are (*i*) neutralizing anti-TGF- β monoclonal antibodies, and (*ii*) soluble recombinant Fc fusion proteins that

contain extracellular domains of either T β R-II (Fc-T β R-II) or (Fc-T β R-III). As a group, these reagents bind TGF- β and prevent its activation of T β R-II.

- Administration of large molecule inhibitors alleviates breast cancer metastasis in preclinical studies.
- Fc-T β R-II and Fc-T β R-III analogues have yet to progress to clinical trial for breast cancer patients.
- Neutralizing anti-TGF- β monoclonal antibodies are under clinical safety and efficacy analysis. Fresolimumab is currently being investigated clinically as a means to enhance the radiosensitivity of breast cancers.
- Cystatin C, a novel antagonist of TGF- β signaling, inhibits tumor formation, angiogenesis, and metastasis in mice.
- Small molecule inhibitors target the ATP binding sites of T β R-I and T β R-II and have exhibited effectiveness to decrease tumor growth and metastasis, and to prolong survival in preclinical studies.
- Among the small molecule inhibitors, only LY2157299 is currently being evaluated in clinical trials.
- Antisense oligonucleotides targeting TGF- β 1 mRNA are being evaluated in preclinical studies, while those targeting TGF- β 2 mRNA are being evaluated in clinical trials for glioblastomas and pancreatic cancers; however, the effectiveness of this class of anti-TGF- β agents against breast cancers awaits further testing.
- The peptidomimetic P114 is under clinical study, but awaits evaluation in human breast cancers.

Figure 1. Schematic of Canonical and Noncanonical TGF-β Signaling Systems and Their Targeting by Anti-TGF-β Agents

TGF-β regulates normal and malignant MEC behavior by binding and activating two transmembrane Ser/Thr protein kinase receptors, namely TβR-I and TβR-II, whose binding to TGF-β is modulated by TβR-III. Activation of these ligand:receptor ternary complexes requires TβR-II to transphosphorylate TβR-I, which subsequently phosphorylates and activates Smad2/3. The phosphorylated forms of Smad2/3 form heterocomplexes with Smad4, which collectively accumulate in the nucleus to mediate canonical TGF-β signaling. Likewise, noncanonical TGF-β signaling transpires *via* the activation of an ever expanding list of downstream effectors, including integrins, FAK and Pyk2, MAP kinases, Src, PI3K/AKT/mTOR, NF-κB, and Cox-2. Also shown are various anti-TGF-β agents and their molecular targets. See text for additional details.

Table 1Anti-TGF- β agents undergoing preclinical and clinical evaluation.

Agent	Manufacturer	TGF- β Target	Clinical Status	Reference
<i>Large Molecule Inhibitors</i>				
1D11	Genzyme	Pan TGF- β 1-3	Preclinical	[94]
2G7	Genentech	Pan TGF- β 1-3	Preclinical	[95]
SR2F	NCI/NIH	Pan TGF- β 1-3	Preclinical	[96]
Lerdelimumab (CAT-152)	Genzyme/CAT	TGF- β 2	Phase III	[51]
Metelimumab (CAT-192)	Genzyme/CAT	TGF- β 1	Phase I/II	[52]
Fresolimumab (GC-1008)	Genzyme/CAT	Pan TGF- β 1-3	Phase I/II	[53]
LY2382770	Eli Lilly	TGF- β 1	Phase II	--
IMC-TR1	Eli Lilly	T β R-II	Preclinical	[97]
Cystatin C	--	T β R-II & Cathepsin B	Preclinical	[55–57]
<i>Small Molecule Inhibitors</i>				
Ki26894	Kirin Pharma	T β R-I	Preclinical	[62]
LY364947	Eli Lilly	T β R-I & T β R-II	Preclinical	[98]
SD-208	Scios	T β R-I	Preclinical	[64]
LY2109761	Eli Lilly	T β R-I & T β R-II	Preclinical	[99]
SB-431542	GlaxoSmithKline	T β R-I	Preclinical	[100]
SD-093	Scios	T β R-I	Preclinical	[71]
SM16	--	T β R-I	Preclinical	[101]
IN-1130	In2Gen Co	T β R-I	Preclinical	[102]
LY2157299	Eli Lilly	T β R-I	Phase II	[59]
LY550410	Eli Lilly	T β R-I	Preclinical	[59]
LY580276	Eli Lilly	T β R-I	Preclinical	[99]
SB-505124	GlaxoSmithKline	T β R-I	Preclinical	[61]
NPC-30345	Scios	T β R-I	Preclinical	[103]
A-83-01	Kyoto Pharma	T β R-I	Preclinical	[104]
SX-007	Scios	T β R-I	Preclinical	[105]
<i>Antisense Oligonucleotides</i>				
AP11014	Antisense Pharma	TGF- β 1 mRNA	Preclinical	[61]
AP12009	Antisense Pharma	TGF- β 2 mRNA	Phase III	[74]
Lucanix (tumor vaccine)	NovaRx	TGF- β 2 mRNA	Phase III	[106]
<i>Peptidomimetics & Peptide Aptamers</i>				
Trx-SARA	--	Smad2/3	Preclinical	[78]
Trx-xFoxH1b	--	Smad2/3	Preclinical	[77]
P114	Digna Biotech	TGF- β	Phase II	[79]
LSKL	--	Latent TGF- β	Preclinical	[81]

CAT: Cambridge Antibody Technology