

Review

Transforming growth factor- β in breast cancer: too much, too late

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Abstract

The contribution of transforming growth factor (TGF) β to breast cancer has been studied from a myriad perspectives since seminal studies more than two decades ago. Although the action of TGF β as a canonical tumor suppressor in breast is without a doubt, there is compelling evidence that TGF β is frequently subverted in a malignant plexus that drives breast cancer. New knowledge that TGF β regulates the DNA damage response, which underlies cancer therapy, reveals another facet of TGF β biology that impedes cancer control. Too much TGF β , too late in cancer progression is the fundamental motivation for pharmaceutical inhibition.

with the response to TGF β evolving from growth inhibition to tumor progression during advanced malignancy, the majority of breast tumors, including their metastases, are positive for nuclear phosphorylated Smad2, indicating an actively signaling TGF β pathway [6,7].

Loss of TGF β growth inhibition and increased expression of TGF β have been associated with malignant conversion and progression in breast, as well as gastric, endometrial, ovarian, and cervical cancers, glioma and melanoma (reviewed in [4,8]). But specific mutation of TGF β signaling components occurs only occasionally in breast cancers. Rather, TGF β growth response is abrogated by changes in the profile of other active signaling networks or the relative availability of transcriptional co-repressors or co-activators that bind to and modulate the canonical Smad pathway. Estrogens also appear to negatively regulate TGF β signaling in breast cancer [9] and there is evidence that many pathway components may be epigenetically regulated during critical transitions in malignant progression [10].

Transforming growth factor- β in breast cancer progression

The breadth and scope of research to define the complex roles that transforming growth factor (TGF) β plays during mammary development and breast cancer now exceeds a thousand papers. Even by the time the elegant and oft-quoted study by Silberstein and Daniel in 1987 [1] put TGF β on the mammary map as an important regulator of breast development, there was clear evidence that cancer could subvert this powerful growth inhibitory signal [2].

In the past decade or so, animal tumor studies that target over-expression or inactivation of various TGF β signaling components to different epithelial compartments have resulted in a bewildering array of conclusions due to the pleiotropic and highly context-dependent action of TGF β on cancer suppression or progression. It is now generally agreed that during early tumor outgrowth, elevated TGF β is tumor suppressive, whereas at later stages there is a switch towards malignant conversion and progression [3,4], as shown in neu-induced mammary tumors [5]. Inactivation of tumor suppressor genes, the sequential acquisition of oncogenic mutations, and epigenetic changes within the cancer genome divert the canonical growth inhibitory arm of the TGF β signaling pathway towards behaviors that increase motility, invasion and metastasis (reviewed in [4]). Consistent

TGF β genetic predisposition to cancer

Genes encoding components of the TGF β signaling pathway, including *TGFB1* [11], *TGFBRI* [12] and *TGFB2* [13], are functionally polymorphic in humans. *TGFB1* harbors promoter and signal peptide polymorphisms that influence protein secretion and levels of freely circulating TGF β 1 [11,14]. Several groups have demonstrated an association between variant *TGFB1* alleles and breast cancer risk [11,15,16]. The L10P allele increases protein production when expressed in culture and has been associated with high TGF β levels [11]. The Breast Cancer Association Consortium conducted combined case-control analyses for breast cancer risk, and found odds ratios of 1.07 and 1.16 for L10P heterozygotes and homozygotes, respectively [17]. A case-control study of over 3,900 Caucasian women with early onset invasive breast cancer (median age 50 years) and a similar number of matched controls [11] demonstrated association between

EMT = epithelial to mesenchymal transition; FACS = fluorescent-activated cell sorting; TGF = transforming growth factor.

homozygosity for the high producer *TGFB1* L10P allele and an odds ratio of 1.25 for risk of invasive breast cancer. Similar associations have been found between hyperactive *TGFB1* variants and invasive prostate cancer [18], nasopharyngeal cancer [19], malignant melanoma [20], and lung cancer [21]. Conversely, a cohort study of more than 3,000 women aged 65 to 75 years suggested that homozygosity for hyperactive *TGFB1* appeared protective for breast cancer, suggesting that TGF β 1 has a breast tumor suppressing activity [15]. Pasche and colleagues [22] have proposed that hypomorphic variants of the TGF β type I receptor interact with the hyperactive *TGFB1* variant to create 'high' versus 'low' signalers, the latter being associated with elevated breast cancer risk.

The disparate conclusions from these studies may be related to the age of the women and tumor grades in different studies. More recently, this apparent genetic dichotomy has been explained in terms of the dual function of TGF β 1 in carcinogenesis evident during neoplastic progression, as demonstrated in mouse models [3]. In a case control study of Asian breast cancer patients stratified according to tumor grade, hyperactive *TGFB1* was associated with decreased risk of early-stage breast cancer but increased risk of advanced breast cancer [23]. Given the complex biology regulated by TGF β , there are probably other processes involved in mediating the TGF β -associated risk of breast cancer. In different mouse strains, for example, homozygosity for a hypomorphic *Tgfb1* variant is genetically linked to skin tumor susceptibility. However, this effect can be completely masked by interacting genetic variants at a distant locus elsewhere in the genome [24]. It is likely that *Tgfb1* genotypes interact with other features in the genetic background [25].

Consequences of too much TGF β

Elevated plasma TGF β 1 in hepatocellular carcinoma and breast, lung and prostate cancer patients correlates with poor outcome (reviewed in [26]). Systemic TGF β 1 levels have been used as a surrogate of tumor load and/or response to therapy [27,28]. Some circulating TGF β 1 may arise from the tumor; however, high plasma TGF β 1 levels can persist after tumor resection, suggesting that there may also be additional sources of the cytokine, such as blood cells, platelet degranulation or liver [29-31]. Compounding this, cancer therapy itself might induce TGF β 1 secretion by a number of routes (reviewed in [32-34]).

Epithelial to mesenchymal transition and the cancer stem cell

The tumor progressing activities of TGF β are multifold, and involve effects on both the tumor cell and the tumor micro-environment [4]. It has been known for some time that TGF β can induce epithelial to mesenchymal transition (EMT) in embryonic or neoplastic epithelial cells. This process is essential for normal embryonic development, and its exploitation during cancer progression has been thought to

contribute to tumor invasion and metastasis [35]. In the mouse skin model of chemical carcinogenesis, overt EMT is a common occurrence, driven by TGF β \rightarrow Smad \rightarrow Snail signaling, and resulting in the formation of highly aggressive, totally fibroblastic spindle carcinoma that have lost all the molecular markers of epithelial cells [3]. Radiation, a carcinogen of human breast, primes non-malignant human mammary epithelial cells to undergo TGF β -mediated EMT [36]. Changes in motility elicited by cytoskeletal re-organization, and enhanced secretion of matrix-remodeling enzymes are classically considered the main driving forces in the contribution of reversible TGF β -driven EMT to invasion and metastasis [37].

A recent paper from Polyak and colleagues [38] suggests an alternative mechanism. Expression profiling of fluorescent-activated cell sorting (FACS) sorted CD44^{HIGH} CD24^{LOW} marked cells, a population enriched for breast epithelial stem cells, showed transcripts associated with cell motility, cell adhesion, cell proliferation, chemotaxis and angiogenesis. The transcriptional similarity between FACS sorted populations enriched for normal and neoplastic stem cells was greater than that between them and the CD44^{LOW} CD24^{HIGH} population. The enrichment in transcripts for TGF β and WNT signaling components was striking in these stem cells [38], suggesting preferential activation of these pathways and their functional involvement in stem cell biology. Indeed, putative stem cells were responsive to TGF β and targeted by TGF β inhibition, whereas the descendant CD44^{LOW} CD24^{HIGH} progenitor cells had lost responsiveness due to methylation of the *TGFBR2* gene. These data suggest that TGF β signaling plays a role in mammary stem cell maintenance [38].

Taking this observation one step further, Mani and colleagues [39] showed that Snail-driven EMT in human mammary epithelial cells induces stem cell-like properties in terms of expression of stem cell markers, increased mammosphere seeding activity *in vitro* and tumorigenicity *in vivo*. Excessive TGF β levels in the tumor microenvironment may, therefore, not only maintain putative cancer stem cells, but also contribute to their formation if more differentiated progenitors undergo EMT. This latter possibility remains to be tested. However, clinical evidence demonstrates that tumor expression of a 'TGF β cassette' of genes (expressed in CD44^{HIGH} CD24^{LOW} > CD44^{LOW} CD24^{HIGH}) is associated with shorter metastasis-free survival of patients with estrogen receptor-negative breast cancer [38]. These studies suggest that anti-TGF β therapy could hold promise for targeting the cancer stem cell, especially within this TGF β active sub-group of estrogen receptor-negative breast tumors.

Either as part of the stem cell 'phenotype' or independently of it, TGF β can induce several other cell autonomous phenotypic changes that are conducive to tumor progression and metastasis. TGF β signaling is clearly required for efficient

colonization of the lung by transformed cells [40], and expression of a TGF β response expression signature in estrogen receptor-negative primary breast tumors is clinically associated with metastasis specifically to the lung but not to the bone [41]. One molecular mechanism responsible for this organ-specific tropism is TGF β /Smad-driven activation of the gene encoding angiopoietin-like 4 (*ANGPTL4*). Angiopoietin-like 4 is a secreted ligand that disrupts tight endothelial barriers, such as those found in lung but not bone marrow, thus specifically stimulating pulmonary trans-endothelial migration of tumor cells [41]. Importantly, only transient exposure to TGF β is required to induce the TGF β response signature, which includes *ANGPTL4*, and to stimulate the consequent enhanced ability for lung colonization in a mouse metastasis model.

Tumor progression via microenvironment modification

Clearly, TGF β has dramatic effects on epithelial phenotype, growth regulation and cell fate. Importantly, TGF β has comparable control of the microenvironment composition mediated by effects on stromal, immune and vascular cells. Many investigators have argued that disruption of the stroma and tissue architecture can be a primary driver of carcinogenesis [42-46]. Recent experiments published from the labs of Weinberg [47], Moses [48], Sonnenschein [49] and Coussens [50] provide additional evidence that microenvironment composition is a critical determinant of cancer progression, which underscores the flipside of the cancer paradigm, that is, how the tissue becomes a tumor; TGF β has a significant role on this side of the coin.

Tgfb1 null mice crossed onto an immune deficient background (which prevents neonatal death from gross inflammatory disease shortly after birth [51]) show little evidence of spontaneous cancer when housed under germ-free conditions. However, under standard mouse husbandry, these mice develop gastrointestinal cancer, supporting the concept that non-target cells mediate this epithelial tumorigenesis via TGF β [52]. It is perhaps surprising to note that spontaneous cancer is not elevated in *Tgfb1* heterozygote mice up to 2 years, even though TGF β production is severely compromised, even in Balb/C mice that are highly susceptible to breast cancer (MH Barcellos-Hoff and RJ Akhurst, unpublished data).

One of the major stromal targets for TGF β action in tumor progression is the immune system. TGF β acts in the tumor microenvironment to blunt immune-surveillance via multiple mechanisms, including suppression of both cytotoxic T and natural killer (NK) cells (reviewed in [53]). TGF β recruitment of macrophages to the tumor also leads to a pro-inflammatory micro-environment, further exacerbating TGF β production and the vicious cycle of tumor progression. Cell autonomous effects of TGF β on the tumor cell provide protection from elimination by the immune system - for example, by down regulation of the expression of death receptors, major histo-

compatibility complex (MHC) molecules and Rae-1 γ , the NKGD2 ligand required for NK cell activity. Recently, Wakefield and colleagues [54] demonstrated that TGF β stimulates CD8+ T cells that infiltrate the tumor to produce interleukin-17, that in turn acts as a tumor cell survival factor via the interleukin-17 receptor.

These observations suggest that microenvironmental effects of TGF β , together with its roles in EMT and metastasis, stimulate cancer progression and override any effects of TGF β as a tumor suppressor in epithelia. These studies underscore the consensus opinion that TGF β 1 levels in cancer mediate a neoplastic plexus, driving cancer cells towards more aggressive behaviors and supporting their survival, while simultaneously limiting suppression by the host and perhaps augmenting normal tissue complications. The concept, put forward by Wakefield and colleagues [54], is that since excessive TGF β action is mostly localized within the tumor, TGF β inhibition could be therapeutically advantageous.

TGF β , a malicious bystander during cancer therapy

TGF β inhibition in either mouse or human mammary epithelial cells increases the cytotoxic response to ionizing radiation and several chemotherapeutic drugs [55-60]. Both radiation and chemotherapy induce TGF β activity [61]. More importantly, Teicher and colleagues [62] showed that tumors secreting high levels of TGF β are more resistant to chemotherapy. Cis-platinum treatment of MDA-MB-231 breast cancer cells increased both TGF β mRNA levels and the secretion of active TGF β , which the authors suggest enhances growth arrest that facilitates repair of damage, thus rendering these cells resistant to cis-platinum killing [63]. Furthermore, treatment of MDA-MB-231 cells with anti-TGF β antibodies greatly enhanced cis-platinum-induced DNA fragmentation, augmented cell cycle progression and restored cellular sensitivity to cis-platinum [55]. Treatment of animals bearing cis-platinum-resistant tumors with TGF β neutralizing antibody or with the TGF β inhibitor decorin restored drug sensitivity of the tumor [56,57]. These authors suggested that inhibiting TGF β -mediated cell cycle control would augment therapeutic efficacy.

Recent data suggest an even more proximal role for TGF β in radiotherapy (reviewed in [64]). Breast cancer radiotherapy targets the tumor with the goal of inducing DNA damage resulting in cancer cell death, which increases long term patient survival [65]. Radiation-induced DNA damage elicits a signal transduction pathway that begins with sensor/activator proteins that lead to the activation of transducers that further convey the signal to multiple downstream effectors [66]. Recent studies have focused on ATM, a serine/threonine protein kinase required for the rapid response to radiation-induced DNA double strand breaks [67], as a means to amplify the therapeutic efficacy of radiation. Remarkably, the DNA damage response and subsequent cell fate decisions

are severely compromised if TGF β is inhibited prior to irradiation in mouse epithelial tissues [59], human mammary epithelial cells [60,68] and lung cancer cells [60,68].

TGF β depletion or signal inhibition does not affect ATM protein abundance, but actually blocks ATM kinase activity [60]. Both ATM autophosphorylation and phosphorylation of critical substrates, such as p53, Chk2 and Rad17, are abrogated, which in turn prevents cells from undergoing apoptosis or cell cycle arrest following DNA damage. As a consequence, epithelial cells are sensitized to radiation toxicity as assessed by clonogenic assays, just as if ATM is inhibited. Whether this potentially important therapeutic consequence will extend the use of TGF β inhibitors in breast cancer treatment is unknown. Although a lung cancer cell line was rendered more resistant to radiation by use of small hairpin RNA inhibition of TGF β receptors [68], preliminary studies using small molecule inhibition of TGF β type I receptor kinase resulted in significant radiosensitization in four of five breast cancer cell lines (MH Barcellos-Hoff and A Pal, unpublished data). If TGF β control of ATM is confirmed in tumors, then high tumor levels of TGF β might actually amplify DNA damage signaling and repair, preventing tumor cell death, thereby limiting response to radiotherapy as Teicher and colleagues have shown for the response to chemotherapy [58].

Arteaga and colleagues [69] demonstrate that radiation-induced systemic TGF β can also promote metastatic disease in breast cancer. In these studies, irradiated MMTV/PyVmT transgenic mice showed increased circulating levels of TGF β 1, circulating tumor cells, and lung metastases, which was abrogated by administration of a pan-neutralizing TGF β antibody to the irradiated host. Hence, TGF β inhibitors could block this tumor survival pathway and increase radiosensitivity, as well as preventing metastasis [69].

Radiotherapy-induced TGF β activity is also implicated in late tissue toxicities that limit the use of radiotherapy for cancer treatment (reviewed in [32,33]). Normal tissues are spared from radio-toxicity in large part by physical targeting of tumors with conformal and targeted radiotherapy. Nonetheless, in some individuals, fibrosis can develop several years after therapy, which can affect quality of life or, in the case of lung tissue, be life-threatening. Unlike tumor control mediated by cell killing, fibrosis results from aberrant cytokine cascades principally initiated by TGF β . Recent studies by Anscher and colleagues [33] have shown that even a single dose of anti-TGF β antibody blocked radiation-induced lung injury, inflammatory response, and expression and activation of TGF β from 6 weeks to 6 months after irradiation. Interestingly, EMT can contribute to fibrotic processes [70], and radiation appears to sensitize cells to TGF β -mediated EMT [36].

These studies demonstrating that TGF β activation is an undesirable side effect of radiotherapy provide further impetus for therapeutic inhibition. Along with the idea that

TGF β promotes breast cancer cell survival and metastasis at multiple levels, these data support the use of TGF β inhibition during radiotherapy and chemotherapy. If effective, increased tumor response and decreased late tissue effects would result in a vastly improved therapeutic index for radiation treatment in breast cancer.

Future directions

The dysregulation of TGF β in breast cancer, which in turn deregulates cellular and multicellular interactions to promote cancer, underlies one rationale for pharmaceutical TGF β inhibition for breast cancer treatment. Immediate gain could be achieved by using TGF β inhibitors to improve the response to chemo- and radiotherapy. Attenuation of undesirable effects, such as fibrosis, is yet another benefit of TGF β inhibition, based on directly blocking processes that initiate pathology, or indirectly due to the anticipated reduction in radiation dose or scheduling necessary because of improved tumor response.

Concerns about limiting the activity of a growth factor whose action is essential to normal development and that plays crucial roles in wound healing and inflammation are valid but have yet to be confirmed in experimental cancer models. Perhaps, as suggested by several studies, the high levels of both protein and activity in the context of cancer elicit very different effects to those found in normal tissues where TGF β activation is highly controlled. As proposed by Wakefield and colleagues [54], the 'locally distributed' activity may be the key to rational targeting. TGF β inhibitors that reduce, rather than eliminate, TGF β effects, used in combination with either targeted delivery to the tumor or a targeted therapy like radiation, may spare normal tissue at the expense of tumors (reviewed in [34]).

TGF β -specific inhibitors based on blockade of synthesis, ligand/receptor binding or receptor kinase signaling are in clinical trials (reviewed in [53]). Pre-clinical models using TGF β inhibitors have not yet elicited overt toxicity, and have shown efficacy by suppressing tumor metastasis, enhancing tumor responses to radio- and chemotherapy, and reducing normal tissue late effects. Given its complex biology, the biological target in breast cancer may be stromal, immune, vascular, or cancer stem cells, or all of these. Further research can refine the therapeutic rationale by focusing on drug scheduling and delivery, identifying patients who will benefit most from such therapy, and combining therapeutic modalities such that cancer is eliminated without normal tissue toxicity or long term health effects.

Competing interests

The authors declare that they have no competing interests.

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