

Featured Review Article

Vicious cycle of TGF- β signaling in tumor progression and metastasis

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Abstract: TGF- β is an important biological mediator. It regulates a wide range of functions including embryonic development, wound healing, organ development, immuno-modulation, and cancer progression. Interestingly, TGF- β is known to inhibit cell growth in benign cells but promote progression in cancer cells, a phenomenon known as TGF- β paradox. TGF- β stimulation in cancer cells leads to a differential Erk activation, which serves as the basis of TGF- β paradox between benign and cancer cells. The critical events of TGF- β mediated Erk activation are suppressed TBRs and elevated TGF- β in tumor cells but not in benign cells. These events form the basis of the “vicious cycle of TGF- β signaling”. The term “vicious cycle”, implies that, with each advancing cycle of TGF- β signaling, the tumor will accumulate more TGF- β and will be more “aggressive” than that of the previous cycle. Understanding this vicious cycle of TGF- β signaling in tumor progression and metastasis will help us to predict indolent from aggressive cancers and will help us to develop novel anti-cancer strategies.

Keywords: Cancer, TGF- β signaling, TGF- β receptors, Erk, Smad, TGF- β auto-induction, negative feedback, positive feedback, vicious cycle

Introduction

With rare exceptions, the natural history of all types of tumors is known to progress from localized indolent stages to aggressive metastatic stages [1, 2]. Recent advancements in biomarker research have made significant progresses to help prediction of cancer progression and disease outcome [3-7]. However, the molecular mechanism behind tumor progression remains elusive. In this review, we propose that a vicious cycle of TGF- β signaling is a universal mechanism that leads to tumor progression. The following paragraphs will define the role of TGF- β signaling in cancer progression and metastasis.

Biology of TGF- β signaling

There are three known mammalian isoforms of TGF- β (TGF- β 1, - β 2, and - β 3) with significant structural and functional similarity [8]. The bio-

logical effect of TGF- β is mediated through type I and type II receptors (TBRI and TBRII) [9]. The canonical downstream events involve the activation of Smad pathways [10]. TGF- β first binds to TBRII, which recruits and activates TBRI [9, 11]. The latter then activates Smad2/3. The activated Smad2/3 combines with Smad4 and migrates to the nucleus to regulate transcription [12]. In addition to the Smad pathway, TGF- β also signals through a number of non-canonical pathways, including m-TOR, RhoA, Ras, MAPK, PI3K/AKT, PP2A/p70s6K, and JNK [13]. The relative importance and interplay of these pathways of TGF- β signaling is still under investigation [14, 15]. In this review, we will limit our discussion to TGF- β mediated Smad and Erk activation.

TGF- β paradox

TGF- β is known to inhibit cell cycle in benign cells but promote progression and metastasis

Differential activation of Erk by TGF- β

TGF- β (low dose) Benign Cells \rightarrow Erk activation
TGF- β (High dose) Benign Cells \rightarrow Erk inactivation
TGF- β (low dose) Cancer Cells \rightarrow Erk activation
TGF- β (high dose) Cancer Cells \rightarrow Erk activation

Figure 1. Differential activation of Erk by TGF- β . Treatment of benign cells with a low dose of TGF- β (0.1 ng/ml) will lead to Erk activation. But, treatment of benign cells with a high dose of TGF- β (10.0 ng/ml) will lead to Erk inactivation (26). However, in malignant cells, especially the advanced cancer cells, the treatment of the same cells with a high dose of TGF- β (10 g/ml) will result in Erk activation. The use of different dosages of TGF- β in these studies is critical as they bring out the interesting phenomenon of differential responses to TGF- β stimulation. It should be pointed out that cancer cells in the early stage of carcinogenesis retain some of the features of benign cells in that they can be inhibited by TGF- β . However, in advanced cancer cells, treatment with any dose of TGF- β would result in Erk activation and cell proliferation.

in cancer cells [16, 17], a phenomenon known as TGF- β paradox [18]. Although there are numerous articles with different approaches tackling this topic, to date, a logical explanation leading to TGF- β paradox remains elusive and is accepted as a scientific mystery [17-20]. Recently, we reported that a differential activation of Erk in cancer cells is the underline molecular mechanism for TGF- β paradox [21]. In this review, we will further elaborate the role of a vicious cycle in TGF- β signaling as the mechanism of tumor progression and metastasis.

TGF- β mediates a differential activation of Erk between benign and cancer cells (Figure 1)

It is well known that TGF- β is able to activate Erk in cancer cells [22-24] and inactivate Erk in non-cancer cells [25]. However, a direct link of TGF- β mediated differential activation of Erk between cancer and non-cancer cells in the same cell system has not been reported until our recent report [26]. In that study, we treated benign cells with a low concentration of TGF- β (0.1 ng/ml) which led to Erk activation; while the treatment of the same cells with a high concentration of TGF- β (10 ng/ml) resulted in Erk inactivation. Activated Erk is a key regulator for cell proliferation. Consistent with this finding, we have observed cell proliferation in benign

cells with a low dose of TGF- β but growth arrest with a high dose in benign stromal cells [27] as well as in benign epithelial cells [23]. The use of different dosages of TGF- β in these studies is critical as they bring out the interesting phenomenon of differential responses to TGF- β stimulation. It should be pointed out that cancer cells in the early stage of carcinogenesis retain some of the features of benign cells in that they can be inhibited by TGF- β [28, 29]. However, in advanced cancer cells, treatment with TGF- β would result in Erk activation and cell proliferation [22, 23, 27, 30].

In contrast to the traditional concept of TGF- β paradox [18], TGF- β treatment in benign cells does not always result in growth arrest. Under normal physiological conditions, cellular activities are carefully monitored by TGF- β . Differential Erk activation seems to play a central role in this regulation. When TGF- β level in the local environment is low, cells will activate Erk and induce TGF- β expression [26]. On the other hand, when the local concentration of TGF- β is more than sufficient, cells have a mechanism to shut off Erk activation, thus, prevent further expression of TGF- β .

It is important to note that Erk activation or inactivation by TGF- β in benign cells is not a case of all-or-none phenomenon. In order to demonstrate the gradual changes in Erk or Smad activation in benign cells, multiple doses of TGF- β at different cell density must be employed as described by Clarke and associates [31]. Indeed, they demonstrated a linear increment of Smad activation within a wide range of available TGF- β per cell in mink lung epithelial cells [31]. In an attempt to validate the same linear relationship exists between TGF- β dosage and Erk inactivation, we repeated the same experiment performed by Clarke and associates [31] by using a different set of benign epithelial cells (RWPE1 and BPH1). Indeed, a linear Erk inactivation was demon-

TGF- β paradox between benign & malignant cells:

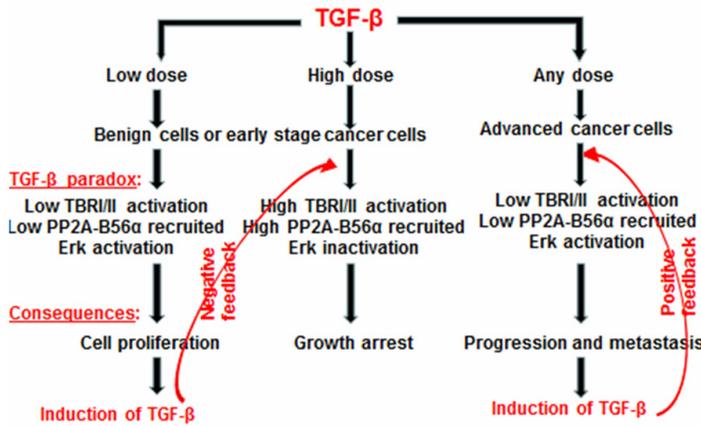


Figure 2. TGF- β paradox between benign and malignant cells. The effects of TGF- β on benign cells or the early stage of carcinogenesis are two-fold. At low doses of TGF- β , TBRs are activated at a low level, which will recruit a low level of PP2A-B56 α and will activate a high level of Erk. At high doses, a high level of TBRs is activated, which will lead to high recruitment of PP2A-B56 α and low level of Erk activation. In advanced cancer cells, due to a severely down-regulated TBR, TGF- β , at any dose, will recruit low levels of PP2A-B56 α and will activate high levels of Erk. The net consequence will be tumor progression and tumor invasion. An important implication in TGF- β paradox is that, in benign cells or early stage cancer cells, TGF- β offers a mechanism for cellular homeostasis; while in advanced cancer cells it promotes tumor progression.

benign cells or early stage cancer cells, TGF- β offers a mechanism for homeostasis; while in advanced cancer cells it promotes tumor progression.

Activated Erk is a master regulator for tumor progression and metastasis

The activated Erk is a master regulator for tumor progression [22] (Figure 3), it is responsible for a host of oncogenic signaling events including NF- κ B activation [23], promotion of angiogenesis [32], evasion of immune surveillance [33, 34], stimulation of cancer cell proliferation, inhibition of cancer cell apoptosis [17], and facilitation of epithelial-to-mesenchymal transition (EMT) and metastasis [23].

p-Erk is a master regulator of tumor progression

TGF- β → Cancer Cells → Erk activation

Figure 3. Activated Erk is a master regulator of tumor progression. Activated Erk is a key regulator for cell proliferation. Its downstream effects are the activation of NF- κ B which lead to upregulation DNA methyltransferases and TBRs down-regulation, loss of E-cadherin which results in β -catenin to interact with Wnt signaling, vimentin expression which results in epithelial-to-mesenchymal transition, and TGF- β auto-induction.

1. NF- κ B activation
2. Wnt activation
3. Loss of E-cadherin
4. Vimentin expression
5. TGF- β auto-induction
6. TBR down regulation

TGF- β mediated Erk activation leads to up-regulation of DNA methyltransferases (DNMTs) and down-regulation of TBRs

It is known that TGF- β mediated Erk activation in cancer cells will lead to up-regulates DNA methyltransferases (DNMTs) [35]. Targets of DNMTs promoter methylation in many tumor suppressor genes are TBRs [35, 36]. A search of the literature has revealed that down regulation of TBRs is an

strated [21]. This phenomenon is only applied to benign cells or early stage cancer cells, as in advanced cancer cells, there will be no such linear relationship in Smad activation and Erk inactivation upon TGF- β stimulation. In advanced cancer cells, Erk is constantly in an activated state [23, 26] and Smad activation is suppressed, regardless the level of TGF- β employed. This finding has an important implication in TGF- β paradox (Figure 2), that is, in

early event of carcinogenesis for all types of cancer [37]. The biological, consequence of a down-regulated TBR will be an attenuate Smad2/3 activation and an elevated Erk1/2 activation in advanced cancer cells. The availability of TBRs dictates the relative levels of activated Erk1/2 and inactivated Smad2/3, thus determines the fate of the TGF- β paradox [31, 38, 39]. It follows that any condition that results in down regulation of functional TBRs,

Vicious cycle of TGF- β signaling in tumor progression



Figure 4. Vicious cycle of TGF- β signaling in tumor progression and metastasis. The characteristic feature of TGF- β action in cancer cells is the auto-induction of TGF- β by cancer cells, resulting in an elevated TGF- β in the microenvironment through a positive feedback loop (see **Figure 3**). The term “vicious cycle” is coined in this study. It implies that, with each advancing cycle of TGF- β signaling, the tumor will accumulate more TGF- β and will be more “aggressive” than that of the previous cycle.

such as inflammation [40, 41], Ras activation [42, 43], and loss-of-function mutations in TBRs [44-46], will be predisposed to cancer develop and cancer progression.

TGF- β mediates auto-induction of TGF- β in cancer cells

TGF- β overproduction is a universal event in cancer cells and is a poor prognostic marker [26, 35, 47-50]. The mechanism, through which TGF- β regulates its own production, is different between benign and cancer cells. Under the normal physiological conditions, the level of TGF- β is tightly regulated within the microenvironment through a negative feedback loop to maintain a relatively constant level of TGF- β . Too little or too much TGF- β will have an unfavorable consequence [37, 51, 52]. However, this principle does not apply to cancer. Cancer cells, especially the advanced cases, are capable of evading the immune surveillance program due to the well-known phenomenon of auto-induction of TGF- β by cancer cells (Yu et al, 2010), resulting in an elevated TGF- β in the microenvironment through a positive feedback loop [53]. As a result, there is an accumulation of TGF- β in the microenvironment, which further promotes tumor progression [26, 35, 49].

Conclusion: vicious cycle of TGF- β signaling in tumor progression and metastasis

With regard to TGF- β signaling, a characteristic feature of cancer cells, as oppose to the benign cells, is a suppressed TBRs (the cause) and an elevated TGF- β (the effect). TGF- β signaling in

cancer cells with a compromised level of TBRs will result in Erk activation and auto-production of TGF- β in a positive feedback loop, which is the basis of the vicious cycle of TGF- β signaling in tumor progression and metastasis. The term “vicious cycle” (**Figure 4**), implies that, with each advancing cycle of TGF- β signaling, the tumor will accumulate more TGF- β and will be more “aggressive” than that of the previous cycle. The term “aggressive-

ness” can be defined by the tumor volume, the extent of metastatic spread, the pathological grading, the clinical staging, and the degree of resistant to therapy. This feature applies to all cancer cells and can be used as a biomarker for prediction of aggressiveness and outcome of therapy for cancer [23, 35].

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Disclosure of conflict of interest

None.

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