Seminars in Cancer Biology 25 (2014) 15–22

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

TGF-beta in CAF-mediated tumor growth and metastasis

A. Calon[a,*], D.V.F. Tauriello[a], E. Batlle[a,b,*]

[a] Oncology Department, Institute for Research in Biomedicine, 08028 Barcelona, Spain
[b] Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain

ARTICLE INFO

Article history:
Received 23 July 2013
Received in revised form
19 December 2013
Accepted 30 December 2013

Keywords:
TGF-beta
Tumor microenvironment
Cancer-associated fibroblast
Metastasis
Stroma

ABSTRACT

TGF-beta signaling is one of the major pathways controlling cell and tissue behavior not only in homeostasis but also in disease. During tumorigenesis TGF-beta orchestrated processes are key due to its dual role as tumor suppressor and tumor promoter. Important functions of this pathway have been described in a context-dependent manner both in epithelial cancer cells and in the tumor microenvironment during tumor progression. Carcinoma-associated fibroblasts (CAFs) are one of the most abundant stromal cell types in virtually all solid tumors. CAFs favor malignant progression by providing cancer cells with proliferative, migratory, survival and invasive capacities. A complex network of signaling pathways underlying their tumor-promoting properties is beginning to take shape. In this review, we examine current evidence on the emerging mechanisms involving TGF-beta in CAF-mediated cancer progression, and discuss their potential as therapeutic targets.

1. Introduction

TGF-beta signaling emerged in early animal species and controls a variety of key functions in tissue development, homeostasis and regeneration [1]. Underlining its importance, dysfunction of the pathway is linked to severe diseases. Although first described as a secreted polypeptide hormone capable of rendering recipient cells malignant, transforming growth factor-beta was subsequently found to be able to inhibit cell growth, affect differentiation, and induce cell death as well. In fact, TGF-beta can bring about disparate responses depending on the cell type or on the cellular context [2,3]. In spite of this high level of pleiotropy, TGF-beta signaling can be simplified as a linear pathway from a ligand-receptor complex that phosphorylates an intracellular receptor-regulated effector (R-SMAD proteins SMAD2 or SMAD3), which then complexes with a co-SMAD (SMAD4) to enter the nucleus and regulate target gene transcription with the help of cofactors [2,4,5]. The diversity of outcomes depends on the regulation of these cellular components and on the prevailing transcriptional cofactors that interact with SMADs, conditions that are subject to various other signaling pathways. Three decades of study have shed light on the main parameters that modify the outcome of TGF-beta signaling in various cell types, during different stages of tissue homeostasis and disease. Nevertheless, many paradoxes, such as the role for TGF-beta signaling both as a tumor suppressor and as a driver of cancer progression, remain the subject of intensive study [6]. Here, we highlight some of the recent literature that focuses on the role of TGF-beta in tumor progression and metastasis, noting a key role for this pathway in landscaping the tumor microenvironment during these processes, with an emphasis on its impact over stromal fibroblasts.

2. Metastasis and the tumor microenvironment

The schematized route from a well-vascularized primary tumor to metastatic regrowth follows a cascade of discrete events, starting with local invasion of tumor cells through immediate barriers towards blood or lymphatic vessels. There, cancer cells intravasate and survive the voyage long enough to adhere to and extravasate from those vessels into a distant tissue. Encountering a foreign microenvironment, invading cells need to colonize this new location. Initially, micrometastatic nodules are formed, which somehow evade host defense. These then grow out into aggressive, macroscopic tumors [7,8]. The often non-random pattern of organs targeted for distant colonization by primary tumors has long ago been acknowledged and led to the famous 'seed and soil' hypothesis in 1889, the proposal that metastasis depends on the crosstalk between tumor cells and specific organ microenvironments [9]. The tumor microenvironment (TME), comprising tumor non-epithelial stroma cells including fibroblasts, endothelial cells and immune cells, as well as the extracellular matrix (ECM), is not the same as

* Corresponding authors at: Institute for Research in Biomedicine (IRB-Barcelona), c/Baldiri Reixac 10, 08028 Barcelona, Spain. Tel.: +34 934039008; fax: +34 934039960.
E-mail addresses: alexandre.calon@irbbarcelona.org (A. Calon), eduard.batlle@irbbarcelona.org (E. Batlle).

1044-579X/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.semcancer.2013.12.008

normal tissue stroma. TGF-beta-mediated tumor–stroma interactions are thought to provide factors that help accomplish the steps required for metastasis [10–12].

3. Fibroblasts in cancer progression

TME-resident fibroblasts have been termed with a variety of names including peritumoral (myo-)fibroblasts, reactive stromal fibroblasts, cancer-associated fibroblasts (CAFs) or tumor-associated fibroblasts (TAFs). In general, these cells, differing from normal fibroblasts [13,14], can be identified by expression of smooth muscle actin (alpha-SMA), fibroblast surface protein (FSP1) and fibroblast-activated protein (FAP) [15]. Several additional CAF markers have been discovered, including NG2 chondroitin sulfate proteoglycan (NG2), PDGFR-beta [16,17] or podoplanin (PDPN) [18]. The involvement of CAFs in tumor initiation was analyzed by comparing the effect of normal fibroblasts and CAFs isolated from the primary tumor site. For instance, immortalized prostate epithelial cells gave rise to massive tumors in the presence of CAFs, whereas tumors did not form in the presence of normal fibroblasts [19]. Furthermore, CAFs in non-small lung cell carcinoma (NSCLC) have a greater ability to enhance tumorigenicity of lung cancer cells than normal fibroblasts. Accordingly, the gene expression signature derived from protumorigenic CAFs shows prognostic value for disease relapse both in NSCLC [14] and in colorectal cancer (CRC) [20]. This effect is in large part driven by the secretion of cytokines and growth factors that affect proliferation, survival, adhesion, and migration of cancer cells [21,22]. Mitogenic factors secreted by fibroblasts include hepatocyte growth factor (HGF), EGF family members, chemokine (C-X-C) ligand 12 (CXCL12), several fibroblast growth factors (FGFs), and stanniocalcin-1 (STC1) [12,23–26]. In CRC, TGF-beta activated CAFs express several known pro-metastatic factors that include angiopoietin-like 4 (ANGPTL4), connective tissue growth factor (CTGF) and vascular endothelial growth factor (VEGFA) [27].

Perhaps the best-understood function of CAFs is to promote tumor cell invasion. Fibroblasts promote epithelial-to-mesenchymal transition (EMT) by secreting either matrix metalloproteases (MMPs) or cytokines including tumor necrosis factor alpha (TNF-α). TNF-α induces stabilization of the Snail protein through nuclear factor NFkB and Akt signaling pathways which in turn enhances migratory and invasive phenotype of cancer cells [28]. Also, CAFs facilitate the invasiveness of originally non-invasive cancer cells [29], possibly through a PAR1-dependent Ca2+ signals and MMP1 upregulation [30]. More recently, studies on gastric cancer development suggested that CAFs could promote cancer cell migration and invasion via transgelin (TAGLN) upregulation. TAGLN is an extracellular matrix protein which induces MMP2 production [31]. MMPs promote the degradation of ECM and cleave cell adhesion molecules such as cadherins, thus potentiating cancer cell motility [28].

Additional studies suggest a role for activated fibroblasts at the metastatic site, similar to CAFs in the primary tumor, promoting the survival and proliferation of cancer cells. Formation of liver metastases is associated with the activation of local hepatic stellate cells (HSC), the major cell type involved in liver fibrosis in response to liver damage [32]. In this scenario, PDGF-C promotes tumor growth by stimulating the proliferation of HSCs [33]. In addition, tumors and metastasis derived from engrafted mouse mammary carcinoma cells are significantly reduced when injected in mice deficient for the CAF marker FSP1 [34]. Co-inoculation of wild-type fibroblasts into these mice partially reverses the phenotype, providing further evidence for the involvement of metastasis-associated fibroblasts in the metastatic process [34]. It has also been suggested that CAF-produced factors enhance stem cell-like properties of cancer cells [35,36], and therefore may constitute a niche for this important cell type.

4. TGF-beta signaling in cancer

TGF-beta generally restrains epithelial cells, the origin for a large number of frequently occurring cancers. Its signaling controls the growth within normal epithelial cell layers in e.g. the skin [37,38] and the intestine [39–41]. To overcome this cytostatic activity, many tumors bear gene alterations that inactivate critical TGF-beta signaling components [42]. In CRC for instance, TGF-beta receptor 2 (*TGFBR2*) or *SMAD4* are frequently mutated or inactivated by other means, indicating a tumor suppressing role for TGF-beta signaling [43–45].

Although TGF-beta signaling inhibits epithelial proliferation, this pathway is also frequently linked to tumor progression. In colorectal and prostate tumors, increased TGF-beta1 expression correlates with poor prognosis [46,47]. Indeed, positive TGF-beta immunostaining or elevated mRNA levels in breast and CRC predict metastases [27,48,49]. Moreover, experimental models in the skin, colon and breast support a biphasic function of TGF-beta; i.e. it inhibits the onset of tumorigenesis in these tissues yet once tumors are formed, TGF-beta signaling enhances malignant progression and metastasis [27,50–52]. This differential activation status of TGF-beta signaling during cancer progression depends on whether the cell retains or loses a functional pathway. In CRC, epithelial cells are markedly less stained for p-SMAD2/3 compared to adjacent stromal cells or to the epithelial compartment of pre-malignant tissue. This indicates a shift from primarily epithelial TGF-beta signaling in normal and adenoma tissues to a stromal and CAFs signaling in tumors [27,53].

TGF-beta activity is controlled at many levels including the conversion of the latent secreted form to its active state. Together with its latency-associated peptide (LAP), TGF-beta binds to the latent TGF-beta-binding protein (LTBP), which is part of the ECM [54]. TGF-beta latency represents a crucial step for active TGF-beta release. In fact, mice deficient for LTBP/TGF-beta association exhibits inflammation and tumorigenesis associated with decreased levels of active TGF-beta and decreased TGF-beta signaling [55]. In CRC patients, total TGF-beta levels increase during the mucosa-adenoma-carcinoma transition whereas active TGF-beta release is only up regulated in carcinomas where it correlates with increased alpha-smooth muscle actin (SMA) expression, desmoplastic reaction and poor prognosis [56].

5. TGF-beta signaling in the TME and during metastasis

A large number of breast cancers, melanomas and gliomas retain a functional pathway and display TGF-beta signaling in tumor cells [2]. These tumors frequently develop genetic alterations in downstream tumor suppressor genes such as p15 (*CDKN2B*), which bypass the cytostatic effects of TGF-beta signaling. Additional driver mutations can further abrogate TGF-beta-mediated inhibition of proliferation. Moreover, residual epithelial TGF-beta signaling can be rewired to promote the expression of pro-metastatic factors such as *JAGGED1*, angiopoietin-like 4 *(ANGPTL4*) and interleukin-11 (*IL11*), associated with breast to bone and lung metastasis [57–59]. In addition, TGF-beta signaling is a key driver of epithelial-to-mesenchymal transition (EMT), which favors invasion and metastatic seeding of cancer cells [60–62].

A contrasting scenario involves those tumor types that evaded TGF-beta-mediated growth arrest by completely abolishing the pathway, such as a large proportion of colorectal and pancreatic cancers [42]. The apparent paradox that high levels of TGF-beta are nevertheless linked to poor prognosis in these tumors can be explained by the fact that the tumor microenvironment remains