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### Transforming growth factor (TGF)-beta expression and activation mechanisms as potential targets for anti-tumor therapy and tumor imaging

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*Published in:*  
Pharmacology & Therapeutics

*DOI:*  
[10.1016/j.pharmthera.2012.05.001](https://doi.org/10.1016/j.pharmthera.2012.05.001)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2012

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Arjaans, M., Munnink, T. H. O., Timmer-Bosscha, H., Reiss, M., Walenkamp, A. M. E., Lub-de Hooge, M. N., de Vries, E. G. E., & Schroder, C. P. (2012). Transforming growth factor (TGF)-beta expression and activation mechanisms as potential targets for anti-tumor therapy and tumor imaging. *Pharmacology & Therapeutics*, 135(2), 123-132. <https://doi.org/10.1016/j.pharmthera.2012.05.001>

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Associate editor: B. Teicher

## Transforming growth factor (TGF)- $\beta$ expression and activation mechanisms as potential targets for anti-tumor therapy and tumor imaging

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### ARTICLE INFO

#### Keywords:

TGF- $\beta$   
Cancer  
TGF- $\beta$  activation  
TGF- $\beta$  inhibition  
Imaging

### ABSTRACT

Cancer remains one of the leading causes of death in the developed countries and cancer mortality is expected to rise globally. Despite encouraging developments regarding targeted drugs, the most prevalent cancer mortality remains metastatic disease. Therefore, drugs that target cancer progression, invasion and metastasis are clearly needed. One of the most interesting targets in this setting is transforming growth factor  $\beta$  (TGF- $\beta$ ). TGF- $\beta$  can promote tumor growth, invasion and metastasis. However, TGF- $\beta$  also has a physiological, opposing role: maintaining tissue homeostasis and suppression of tumor progression. The window of effective TGF- $\beta$  targeting is therefore evidently small, which poses a clear challenge in selecting patients at the right time. Despite this complexity, several TGF- $\beta$  inhibitors are currently in clinical development, modulating TGF- $\beta$  production, activation or signaling. Still, specificity and long term toxicity remain unclear, emphasizing the importance of careful monitoring of clinical trials.

Development and application of these drugs in the clinic require adequate insight and evaluation methods for the role of TGF- $\beta$  during tumor invasion and metastasis. In this review, presently available methods for clinical evaluation will be discussed, such as an ex vivo stimulation assay, TGF- $\beta$  response signature and molecular imaging techniques. Future clinical trials incorporating the validation of these evaluation methods will show which method will be most predictive and suitable for clinical application.

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*Abbreviations:* AA, Anaplastic astrocytoma; GBM, Glioblastoma multiforme; HER2, Human epidermal growth factor receptor 2; LAP, Latency associated protein; LTBP, Latent TGF- $\beta$  binding protein; MMP, Matrix metalloproteinases; NSCL, Non-small-cell lung cancer; PET, Positron emission tomography; pSMAD2, Phosphorylated SMAD 2; TBR5<sup>MSKCC</sup>, TGF- $\beta$  response gene signature of Memorial-Sloan Kettering Cancer Center; TGF- $\beta$ , Transforming growth factor  $\beta$ ; TGF- $\beta$ RII, TGF- $\beta$  receptor II; TKIs, Tyrosine kinase inhibitors.

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## 1. Introduction

Cancer remains one of the leading causes of death in the developed countries and cancer mortality is expected to rise globally (Akhurst & Derynck, 2001). Unprecedented, cancer treatment is rapidly moving toward use of targeted drugs that specifically aim at disrupting essential processes in cancer cells. Despite these encouraging developments, the most prevalent cause of cancer mortality remains metastatic disease. Therefore, drugs that target cancer progression, invasion and metastasis are clearly needed. One of the most interesting targets in this setting is the protein transforming growth factor  $\beta$  (TGF- $\beta$ ). TGF- $\beta$  signaling is involved in maintaining the balance of normal tissue homeostasis and suppression of tumor progression in healthy and premalignant tissue (Massagué et al., 2000; Shi & Massagué, 2003; Massagué, 2008). In response to injury, TGF- $\beta$  is released by blood platelets and various stromal components to prevent excessive cell proliferation and inflammation. In the surrounding environment of an oncogenic process, TGF- $\beta$  prevents malignant progression (Massagué et al., 2000; Akhurst & Derynck, 2001; Massagué, 2008). However, during malignant progression TGF- $\beta$  also functions as a factor that tumor cells use to their own advantage, by promoting tumor growth, invasion and eventually metastatic disease (Chiang & Massagué, 2008). TGF- $\beta$  is especially overexpressed in aggressive and invasive types of cancer and overexpression is associated with poor outcome in amongst others glioma, melanoma, breast, lung, colon and prostate cancer patients (Bierie & Moses, 2006; Levy & Hill, 2006).

Its overexpression and tumor growth promoting functions make TGF- $\beta$  a potential drug target for cancer treatment. However, its dual role is a major challenge in the successful clinical implementation of TGF- $\beta$  targeted drugs. To overcome this problem, and to safely intervene with TGF- $\beta$  in cancer treatment, more insight is needed in TGF- $\beta$  dynamics during oncogenesis. Much research has focused on the role of changes in the TGF- $\beta$  signaling pathway (Fig. 1), but mechanisms of TGF- $\beta$  production and activation by tumor or stromal cells have been less well clarified (Reiss & Barcellos-Hoff, 1997; Bierie &

Moses, 2006; Levy & Hill, 2006; Stover et al., 2007; Riethdorf et al., 2008). Understanding production and activation mechanisms next to cellular signaling pathways is of key significance for defining potential TGF- $\beta$  intervention strategies. Additionally, as with other targeted therapies it is well possible that only patients with specific tumor characteristics will benefit from TGF- $\beta$  directed intervention.

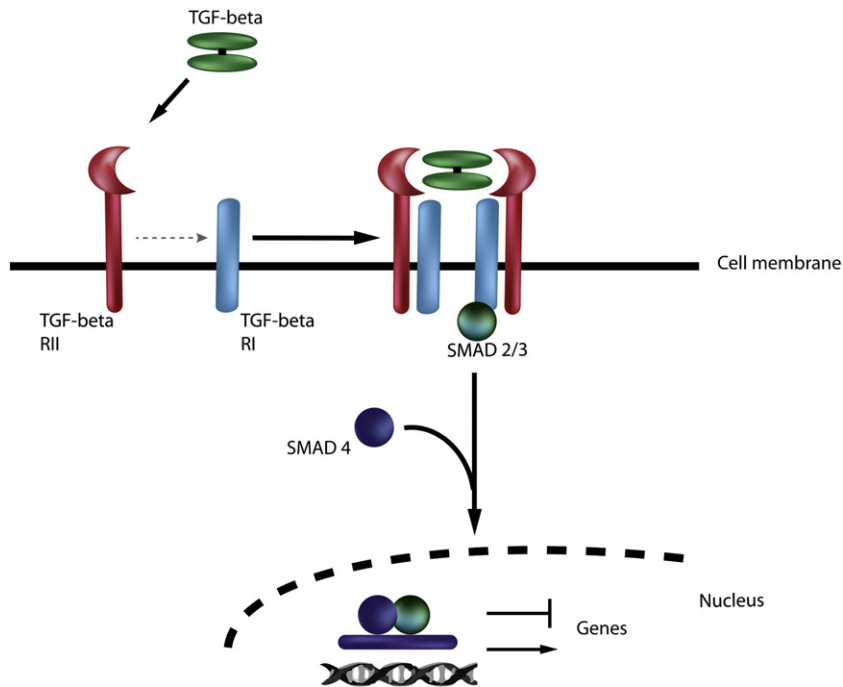
Therefore, in this review we focus on the mechanisms of TGF- $\beta$  production and activation and the currently known intervention options for modulating TGF- $\beta$  activity. Furthermore, methods for patient selection and evaluation of intervention strategies, by means of TGF- $\beta$  imaging and quantification methods will be addressed. Finally, future perspectives for the clinical implication of TGF- $\beta$  targeted therapies will be discussed.

## 2. Production of transforming growth factor $\beta$

The TGF- $\beta$  superfamily consists of TGF- $\beta$ , bone morphogenetic proteins, anti-Mullerian hormone, activin, nodal and growth and differentiation factors. TGF- $\beta$  plays a key role in maintaining homeostasis, inducing wound healing and controlling the immune system. Consequently, TGF- $\beta$  and its receptors are expressed by almost all cell types (Tucker, 2006). TGF- $\beta$  is secreted into the extracellular matrix by amongst others, endothelial cells, fibroblasts, blood platelets, B-, T- and dendritic cells and macrophages (Moustakas & Heldin, 2005). In a tumor, the main sources of TGF- $\beta$  are the cancer cells and certain cells present in the tumor stroma, such as fibroblasts. Both by healthy tissue and in malignant tumors, TGF- $\beta$  is secreted as a latent complex of high molecular weight (290 kDa) (Annes et al., 2003; Shi & Massagué, 2003).

### 2.1. Synthesis of the latent complex of transforming growth factor $\beta$

In humans, three isoforms of TGF- $\beta$  have been identified, namely TGF- $\beta$ 1, - $\beta$ 2 and - $\beta$ 3. These isoforms are encoded by the genes TGFB1, TGFB2 and TGFB3, which are located on chromosome 19, 1 and 14 respectively (Assoian et al., 1983; Derynck et al., 1998).



**Fig. 1.** TGF- $\beta$  signaling pathway. Activated TGF- $\beta$  dimers signal by bringing together two pairs of serine/threonine kinase receptors located on the cell membrane. Activated TGF- $\beta$  binds to TGF- $\beta$ RII on the cell surface. In turn TGF- $\beta$ RII then activates TGF- $\beta$ RI, forming a complex of TGF- $\beta$  and two pairs of TGF- $\beta$  receptors I and II. SMAD proteins play an important role in the TGF- $\beta$  signaling pathway. SMAD proteins are situated in the cytoplasm and can be divided in three classes: receptor-regulated SMAD (SMAD 2/3), co-mediator SMAD (SMAD 4) and inhibitory SMAD (SMAD 7). SMAD 2/3 binds to the activated TGF- $\beta$ RI. Subsequently, SMAD 4 will bind to SMAD 2/3 and this complex will shift to the nucleus. In the nucleus the complex will bind with additional DNA-binding cofactors and co-activators or -repressors, in order to activate or repress specific target genes.

Transcription of TGF- $\beta$  mRNA is induced by different stimuli from the microenvironment, such as hyperglycemia (via activating protein-1) or hypoxia (via hypoxia-inducible factor-1) (Weigert et al., 2001; Nishi et al., 2004). Translation of TGF- $\beta$  mRNA in the nucleus results in a homodimeric protein complex consisting of two molecules of TGF- $\beta$  and the latency associated protein (LAP) each (Kanzaki et al., 1990). The following proteolytical processing of the complex, by means of the convertase family of endoproteases, results in a conformational change of TGF- $\beta$  (Dubois et al., 1995). This change is essential for subsequent activation steps. After processing, TGF- $\beta$  remains non-covalently bound to LAP, forming the small latent complex. Still in the cytoplasm, the small latent complex then binds to latent TGF- $\beta$  binding protein (LTBP) with a disulfide bond. This complex, consisting of TGF- $\beta$ , LAP and LTBP, is called the large latent complex. Finally, the large latent complex is secreted into the extracellular environment (Fig. 2) (Dallas et al., 1995; Munger et al., 1997; Annes et al., 2003).

## 2.2. Latency associated protein and latent TGF- $\beta$ binding protein in the large latent transforming growth factor $\beta$ complex

All elements of the latent form of TGF- $\beta$  have their own function. LAP blocks the binding site for the TGF- $\beta$  receptor II (TGF- $\beta$ RII), thus maintaining the TGF- $\beta$  complex in its latent form. LAP is also involved in the folding and synthesis of TGF- $\beta$ . There are three LAP isoforms, LAP-1, LAP-2 and LAP-3 (Lawrence et al., 1985; Gray & Mason, 1990; Dubois et al., 1995). LAP monomers form a dimer by joining another LAP molecule by intrachain disulfide bonds near the

C-terminus. Near the N-terminus, LAP can bind to LTBP (Munger et al., 1997).

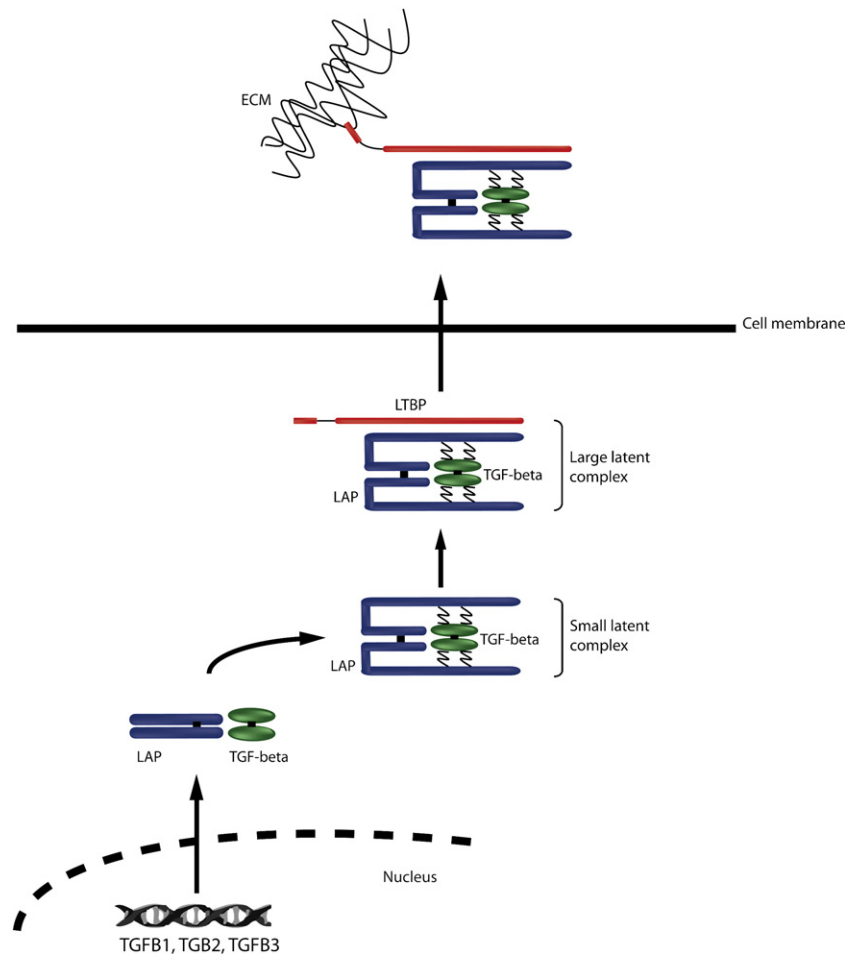
In fibroblasts and fibrosarcoma cells LTBP directs TGF- $\beta$  to the extracellular matrix. Latent TGF- $\beta$  present in the extracellular matrix serves as a reservoir of TGF- $\beta$  that can be activated via proteolytic cleavage of LTBP (Taipale et al., 1994). LTBP has four isoforms, LTBP-1, LTBP-2, LTBP-3 and LTBP-4 (Miyazono et al., 1991). For most cells in which the large latent complex is defined, TGF- $\beta$  is found as a large latent complex containing the isoform LTBP-1 (Munger et al., 1997).

Thus TGF- $\beta$  is intracellularly processed before it is secreted as the large latent complex. This complex can consist of two or three elements, which all have their own function. After secretion, the large latent complex needs to be activated before it can bind to its receptors. Therefore overexpression of the latent form does not have an effect on its own, unless the release of the active form is increased (Flaumenhaft & Rifkin, 1992).

## 3. Activation of the latent transforming growth factor $\beta$ complex

The effects of TGF- $\beta$  are predominantly controlled by local activation of the latent TGF- $\beta$  complex. TGF- $\beta$  can be activated by denaturation, radiation or interaction with the extracellular matrix.

Activation induces a conformational change of LAP, or a release of TGF- $\beta$  from LAP via proteolysis, thus revealing the binding site for the TGF- $\beta$ RII (Brown et al., 1990; Khalil, 1999). In this section, we will focus on different activators of TGF- $\beta$ .



**Fig. 2.** Production of latent TGF- $\beta$ . TGF- $\beta$  is intracellularly processed before it is secreted into the extracellular matrix as the large latent complex. The large latent complex consists of TGF- $\beta$  itself, latency associated protein and latent TGF- $\beta$  binding protein. After secretion, the large latent complex needs to be activated before it can bind to its receptors.

### 3.1. Activation by radiation

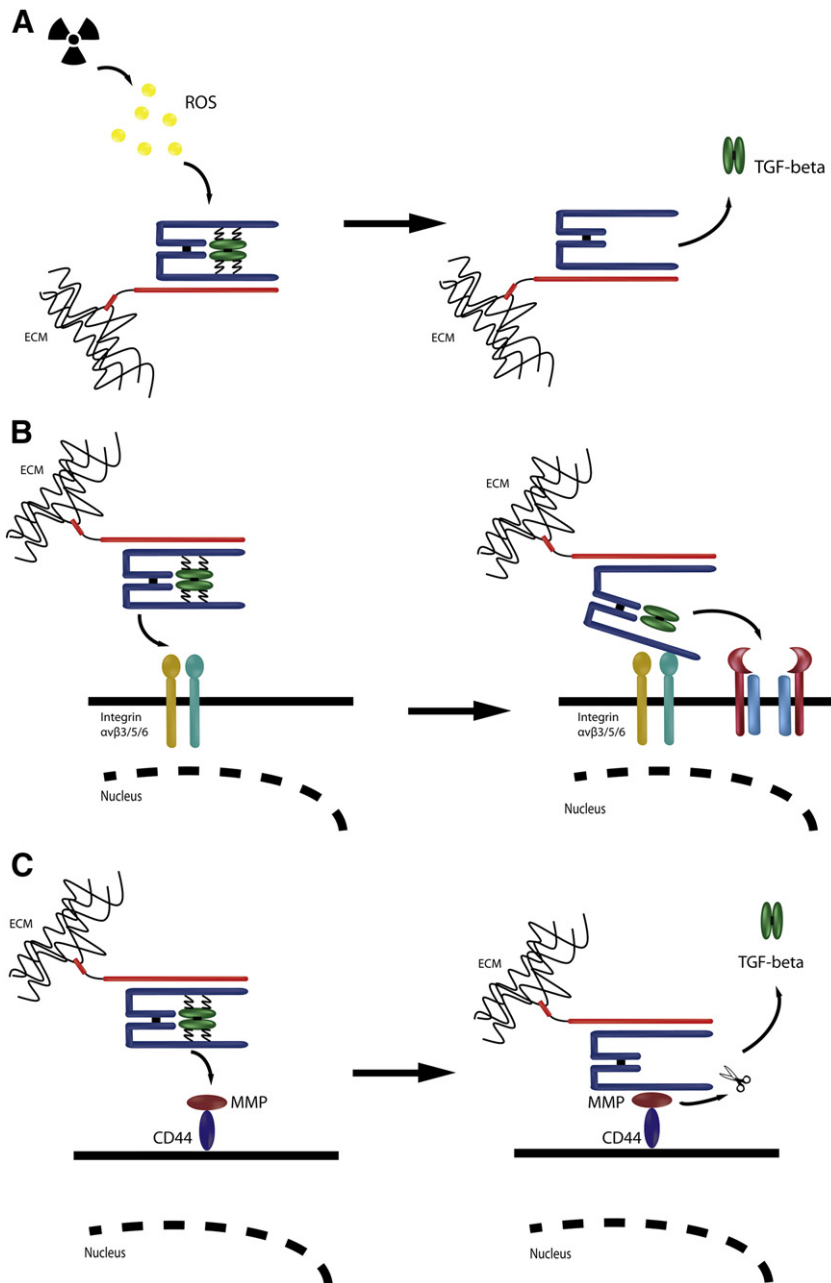
Reactive oxygen species (ROS) produced after ionizing radiation or metal-catalyzed ascorbate oxidation can activate latent TGF- $\beta$  (Fig. 3A). The hydroxyl radicals are thought to disable the counter acting LAP by scissions and side group modifications (Barcellos-Hoff & Dix, 1996). In irradiated murine mammary glands, increased active TGF- $\beta$  compared to non-irradiated mammary glands was shown by means of immunofluorescent staining (Barcellos-Hoff et al., 1994). In lung cancer, plasma TGF- $\beta$  levels can predict the risk for pulmonary injury after radiotherapy. (Vujaskovic et al., 2000; Anscher et al., 2001; Zhao et al., 2008) A study in non-small-cell lung cancer (NSCLC) patients (n=26), showed that plasma TGF- $\beta$  levels above the normal range after radiotherapy predicted significantly for pulmonary injury in these patients (Zhao et al., 2008). Another study amongst NSCLC patients (n=38) showed the feasibility of plasma TGF- $\beta$  levels to select

for patients who can be treated with higher doses of radiotherapy (Anscher et al., 2001). Thus in lung cancer patients receiving radiotherapy, plasma TGF- $\beta$  levels can serve as a predictive marker for pulmonary injury or tolerance for higher doses of radiotherapy, thereby allowing for individualization of treatment. Furthermore, TGF- $\beta$  can also be activated by genotoxic stress and DNA damage induced by ionizing radiation or cytotoxic chemotherapy. (Kirshner et al., 2006; Biswas et al., 2007) TGF- $\beta$  seems to play a role in regulating responses to genotoxic stress. Inhibition of TGF- $\beta$  could in turn possibly sensitize tumors to DNA damage and promote cell death.

### 3.2. Activation by interaction with the extracellular matrix

#### 3.2.1. Activation by integrins

Integrins are heterodimeric transmembrane glycoproteins that attach cells to extracellular matrix proteins of the basement membrane



**Fig. 3.** Activation of latent TGF- $\beta$ . A) TGF- $\beta$  can be activated by reactive oxygen species, which is produced after ionizing radiation or metal-catalyzed ascorbate oxidation. B) Integrins are glycoproteins that attach cells to extracellular matrix proteins of the basement membrane or to ligands on other cells. Integrins can also bind to LAP, thereby enabling binding of TGF- $\beta$  to its receptor on the cell membrane. C) Proteases can also activate TGF- $\beta$  via proteolytic cleavage, which releases TGF- $\beta$  from its latent complex.

or to ligands on other cells. They are composed of  $\alpha$  and  $\beta$  subunits. Four types of integrins are identified as activators of latent TGF- $\beta$ :  $\alpha$ v $\beta$ 3,  $\alpha$ v $\beta$ 5,  $\alpha$ v $\beta$ 6 and  $\alpha$ v $\beta$ 8. These integrins all activate TGF- $\beta$ 1 and TGF- $\beta$ 3 by binding to the amino acid sequence arginine-glycine-aspartate (RGD) present in LAP (Worthington et al., 2011). The integrins  $\alpha$ v $\beta$ 6 and  $\alpha$ v $\beta$ 8 fully activate TGF- $\beta$ 1 and TGF- $\beta$ 3 in vivo. (Aluwihare et al., 2009) Primarily for  $\alpha$ v $\beta$ 6, a clear role in cancer progression has been described. This integrin is located on the cell membrane of epithelial cells (Worthington et al., 2011). It can bind to the RGD sequence in LAP, which induces a conformational change in LAP. This subsequently enables TGF- $\beta$  to bind to its receptor. To create this conformational change, the cytoplasmic domain of this integrin is connected to the actin cytoskeleton, thereby pulling LAP to the cell membrane. Simultaneously, LTBP1 is still bound to the extracellular matrix, thereby pulling TGF- $\beta$  in the opposite way. These opposing forces create a conformational change in LAP (Fig. 3B), thereby allowing TGF- $\beta$  activation (Annes et al., 2004; Wipff et al., 2007; Wipff & Hinz, 2008). In normal epithelial cells, the expression of  $\alpha$ v $\beta$ 6 is very low. Physiological upregulation of  $\alpha$ v $\beta$ 6 expression is seen during injury, which in turn activates TGF- $\beta$ . Activation of TGF- $\beta$  via  $\alpha$ v $\beta$ 6 is mediated by lysophosphatidic acid (Xu et al., 2009). In  $\alpha$ v $\beta$ 6 negative mice, bleomycin treatment induced pulmonary inflammation, but protected the mice from fibrosis due to lack of TGF- $\beta$  activation (Munger et al., 1997, 1999). Thus, blocking  $\alpha$ v $\beta$ 6 could possibly prevent TGF- $\beta$  mediated fibrosis. Blocking of  $\alpha$ v $\beta$ 6 in mice indeed caused inhibition of fibrosis after radiation or bleomycin treatment, without initiating inflammation (Horan et al., 2008; Puthawala et al., 2008).

Multiple cancer types (including breast, lung, colorectal, cervical and skin carcinomas) show increased  $\alpha$ v $\beta$ 6 expression. (van Aarsen et al., 2008; Bandyopadhyay & Raghavan, 2009). Blockade of  $\alpha$ v $\beta$ 6 did not affect tumor cell proliferation in vitro, but did inhibit tumor growth in vivo (van Aarsen et al., 2008). This suggests a role for the microenvironment in this response, which should be further investigated. Since  $\alpha$ v $\beta$ 6 is hardly expressed on normal epithelial cells (Breuss et al., 1993), it has been suggested that this upregulation might be a good marker for TGF- $\beta$  activation in carcinomas, but so far evidence to support this is lacking.

### 3.2.2. Activation by proteases

Matrix metalloproteinases (MMP) are zinc-based proteolytic proteases. MMP 2 and 9 are involved in extracellular matrix degradation and can both activate latent TGF- $\beta$  (Fig. 3C) (Annes et al., 2003). MMPs can cleave LTBP at a hinge region from the extracellular matrix, thereby releasing the large latent complex. This large latent complex can be directly activated by MMPs, through proteolytic cleavage of LAP (Lyons et al., 1988; Taipale et al., 1994).

In mammary carcinoma cells and human melanoma cells, the hyaluronan receptor CD44 localizes proteolytically active MMP9 to the cell surface (Yu & Stamenkovic, 1999). A co-culture system of mammary carcinoma cells and mouse fetal myoblasts showed that TGF- $\beta$  activation by MMP-9 depends on this localization of MMP9 at the cell surface by CD44. TGF- $\beta$  activation is reduced when CD44 is only present in a soluble form and thus unable to bind to MMP-9. In contrast, activation increases when CD44 is present at the cell surface and is able to bind to MMP-9 (Yu & Stamenkovic, 2000). This is supported by xenograft mouse models with subcutaneous tumors. In tumors where TGF- $\beta$  was less activated due to soluble CD44, there was reduced angiogenesis and invasiveness (Yu & Stamenkovic, 2000). The same mechanism has been shown for MMP2, with the difference that MMP2 is localized at the cell surface by the integrin  $\alpha$ v $\beta$ 3 (Brooks et al., 1996).

In summary, TGF- $\beta$  can be activated by denaturation, radiation or interaction with the extracellular matrix. These different activators all have their own mechanism to activate TGF- $\beta$  by breaking down LAP. This induces a conformational change in the large latent complex or obliterates the non-covalent bonds between LAP and TGF- $\beta$ . The

end product is the same for all; active TGF- $\beta$ , which is subsequently able to bind to its receptor and start its signaling cascade.

## 4. Modulation of transforming growth factor $\beta$

The overexpression, tumor growth promoting functions and facilitation of an immunosuppressive environment by TGF- $\beta$  have drawn attention to this protein as a potential target for anti-cancer drugs. There are a number of pharmacological ways to exert an anti-TGF- $\beta$  effect. A variety of TGF- $\beta$  inhibitors have been developed, and are in preclinical studies or already in clinical trials. In this section, we will discuss the TGF- $\beta$  targeting drugs known at present. These drugs will be presented by mechanism of action, including: 1) modulation of TGF- $\beta$  production, 2) modulation of TGF- $\beta$  activation and 3) modulation of TGF- $\beta$  signaling (Fig. 4).

### 4.1. Modulation of transforming growth factor $\beta$ production

#### 4.1.1. Antisense oligonucleotides

TGF- $\beta$  production can be decreased by means of antisense oligonucleotides, through silencing of the corresponding mRNA sequence. Trabedersen (AP-12009) is an antisense oligonucleotide against the mRNA of TGF- $\beta$ 2 and is of interest for treating TGF- $\beta$ 2 overexpressing tumors. In a randomized phase IIb study in patients with recurrent/refractory glioblastoma multiforme (GBM) or anaplastic astrocytoma (AA), 10 or 80  $\mu$ M of trabedersen was administered intratumorally through a subcutaneous port access system for 7 days every other week. In the total study population ( $n = 134$ ), trabedersen showed no significant benefit compared to chemotherapy. In the 39 AA patients however, trabedersen increased median- and overall survival rates compared to chemotherapy (Bogdahn et al., 2011). The main toxicity observed was neurotoxicity. Procedure related serious adverse events were around 30% in the trabedersen groups. Currently, a phase III study with trabedersen (SAPPHIRE) in AA patients is ongoing [clinicaltrials.gov: NCT00761280]. Also a phase I/II study in pancreatic carcinoma, metastatic melanoma and metastatic colorectal carcinoma patients has started. Another antisense oligonucleotide against TGF- $\beta$ 1 (AP-11014) is in preclinical development (Saunier & Akhurst, 2006).

Clinical results of antisense oligonucleotides targeting TGF- $\beta$  seem to be favorable for recurrent/refractory AA but not for GBM patients. Still, these conclusions are based on a very small subgroup of only 39 patients and should be confirmed in future phase III trials.

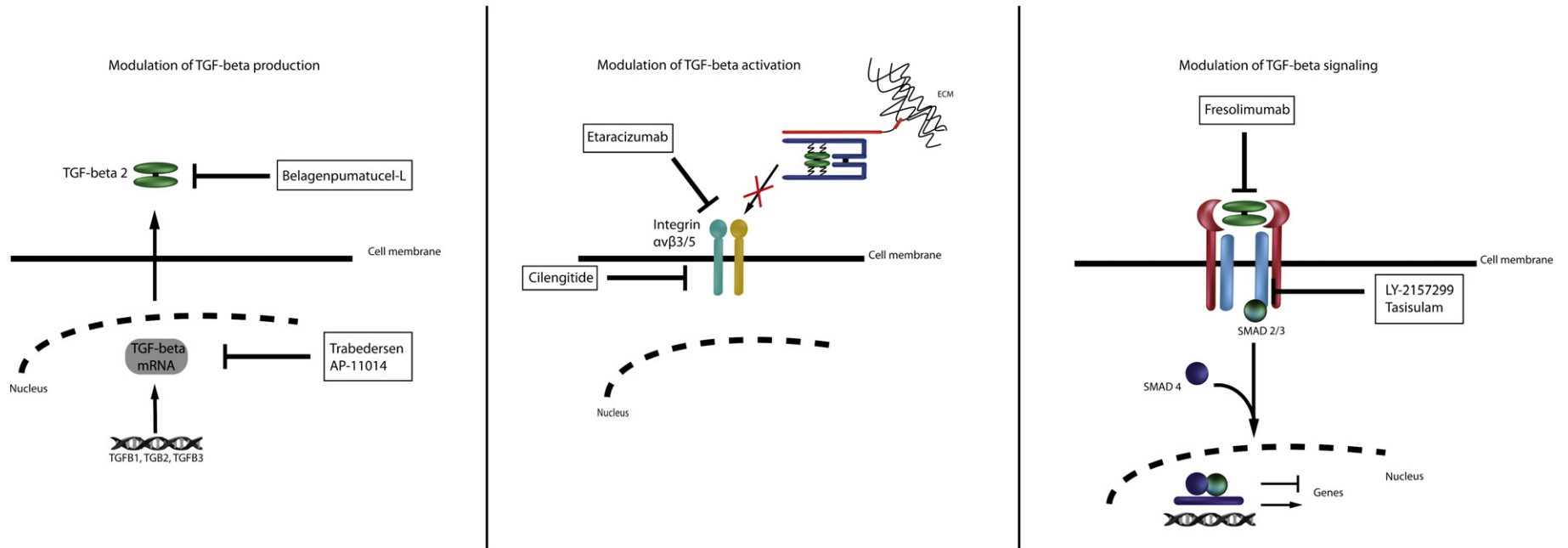
#### 4.1.2. Tumor cell vaccines

Increased expression of activated TGF- $\beta$ 2 induces immunosuppression, favoring tumor progression. Belagenpumatucel-L (Lucanix®) is a gene-based allogeneic tumor cell vaccine, transfected with a TGF- $\beta$ 2 antisense plasmid vector. By inhibiting TGF- $\beta$ 2 expression, the tumor cell vaccine can increase tumor antigen recognition. In a randomized phase II study amongst 75 NSCLC patients, who completed or refused conventional therapy, a single intradermal injection of 1.25, 2.5 or  $5 \times 10^7$  cells given every month or every other month was safe and well tolerated with low toxicity (Nemunaitis et al., 2006, 2009). An injection of  $\geq 2.5 \times 10^7$  cells in NSCLC patients ( $n = 60$ ) led to an estimated 2 year survival of 47%, compared to 18% in NSCLC patients ( $n = 25$ ) who received fewer cells, suggesting a dose-related survival benefit (Nemunaitis et al., 2006). At the moment, a phase III study in patients with advanced stage of NSCLC, comparing belagenpumatucel-L with placebo, is ongoing [clinicaltrials.gov: NCT00676507].

### 4.2. Modulation of transforming growth factor $\beta$ activation

#### 4.2.1. Integrins

Integrins are widely expressed on tumor cells and also on tumor vasculature. Ligand binding to integrins leads, amongst others, to



**Fig. 4.** Modulation of TGF-β. TGF-β can be modulated at several levels: production, activation or signaling. Production can be modulated by means of antisense oligonucleotides like trabedersen or AP-11014 or by tumor cell vaccines like belagenpumatucel-L. Activation of TGF-β can be modulated by blocking integrin αvβ3 with the antibody etaracizumab or by using cilengitide, a small molecule inhibitor against αvβ3 and αvβ5. Modulation of TGF-β signaling can be achieved by directly blocking TGF-β with the human monoclonal antibody fresolimumab or with a tyrosine kinase inhibitor such as LY-2157299.

angiogenesis. Blocking integrin activity can impede angiogenesis, raising interest for integrins as a potential target for anti-cancer therapy. Integrins can also activate TGF- $\beta$ , and indirect modulation of TGF- $\beta$  may ensue when integrins are blocked. Etaracizumab is a monoclonal antibody against the  $\alpha v\beta 3$  integrin. In a phase II study in patients with metastatic melanoma, the addition of etaracizumab to dacarbazine did not show any benefit (Hersey et al., 2010). Currently, clinical development of etaracizumab is on hold. Cilengitide is a small molecule inhibitor against the  $\alpha v\beta 3$  and  $\alpha v\beta 5$  integrins. In a phase II study in patients with newly diagnosed GBM, cilengitide was added to radiotherapy with concomitant and adjuvant temozolomide. Compared to historical data for chemoradiotherapy, the addition of cilengitide modestly increased the median- and overall survival (Stupp et al., 2010). At the moment cilengitide is the first anti-integrin agent to enter a phase III study called CENTRIC (Tabatabai et al., 2010). Furthermore, phase I/II studies with cilengitide combined with standard therapy in recurrent and/or metastatic squamous cell carcinoma of head and neck cancer and in NSCLC are ongoing [clinicaltrials.gov: NCT00842712 and NCT00705016].

### 4.3. Modulation of transforming growth factor $\beta$ signaling

#### 4.3.1. TGF- $\beta$ receptor II kinase inhibitors

Tyrosine kinase inhibitors (TKIs) inhibit signaling cascades by binding to a specific tyrosine kinase domain, located on the receptor of interest (Ge et al., 2006; Ehata et al., 2007; Melisi et al., 2008). At the moment, two TKIs targeted against the kinase domain of the TGF- $\beta$ RI are being evaluated in the clinic. A small phase I study including 7 patients with advanced or metastatic tumors showed that daily oral administration of 40 or 80 mg of LY-2157299 was safe and well tolerated (Calvo-Aller et al., 2008). In another phase I study ( $n = 28$ ), glioma patients were treated with 160, 240 or 300 mg of LY-2157299 per day with intermittent dosing (14 days on, 14 days off). Again, treatment with LY-2157299 was safe and well tolerated (Rodon Ahnert et al., 2011). An ongoing phase I/II study in GBM patients aims at evaluating safety, tolerable dose and pharmacodynamics of LY-2157299 combined with radiotherapy and temozolomide [clinicaltrials.gov: NCT01220271]. A phase II study to determine progression free survival after LY-2157299 monotherapy in patients with hepatocellular carcinoma is ongoing [clinicaltrials.gov: NCT01246986].

#### 4.3.2. Monoclonal antibodies

The use of monoclonal antibodies appears to be a very effective approach in inhibiting TGF- $\beta$  signaling. Both antibodies against mouse and human active TGF- $\beta$  have been developed. Treatment of mice with 2G7 or 1D11, antibodies against mouse TGF- $\beta$ , suppressed tumor formation and tumor burden of both bone and lung metastases. This illustrated the potential of targeting TGF- $\beta$  with monoclonal antibodies (Nam et al., 2006; Pinkas & Teicher, 2006). Fresolimumab (GC1008) is a fully human monoclonal IgG4 antibody against mammalian TGF- $\beta$  isoform 1, 2 and 3. Recently, a phase I/II multi-center trial of fresolimumab in patients with advanced malignant melanoma or renal cell carcinoma who failed prior therapy was completed. Patients received 0.1, 0.3, 1, 3, 10 or 15 mg/kg fresolimumab intravenously. If no dose-limiting toxicity was induced, patients received three additional doses given 2 weeks apart. If stable disease, partial- or complete response was reached, fresolimumab treatment was extended. No dose-limiting toxicity was observed in any of the 22 patients (21 malignant melanoma, 1 renal cell carcinoma). In one patient with a history of skin cancer, fresolimumab treatment possibly led to the development of a squamous cell carcinoma. The most common adverse event was the development of reversible crops cutaneous lesions that histologically resembled benign keratoacanthomas. Interestingly, these appear to phenocopy the self healing epitheliomas, a genetic disorder that is caused by germline mutations in the TGF $\beta$ R1 gene (Goudie et al., 2011). Five patients achieved stable disease; three of these patients had mixed responses with shrinkage of liver metastases and at other sites. One patient with

malignant melanoma obtained a partial response with over 75% reduction of target lesions (Morris et al., 2008). At the moment, a phase II study of fresolimumab for patients with mesothelioma has been initiated [clinicaltrials.gov: NCT01112293]. Furthermore, in another study the addition of fresolimumab to local radiotherapy in metastatic breast cancer patients is investigated [clinicaltrials.gov NCT01401062].

In conclusion, several different approaches to modulate TGF- $\beta$  are in clinical development, but still many hurdles have to be overcome. Long-term toxicity still remains mainly unknown for all these different approaches. Since TGF- $\beta$  exerts multiple functions in both healthy tissue and during cancer, unwanted side effects are therefore conceivable. Upcoming clinical trials should therefore monitor this carefully. Future phase III trials will clarify whether antisense oligonucleotides show a survival benefit for recurrent AA patients. Tumor cell vaccines have a favorable efficacy/toxicity profile, but again the survival benefit compared to a placebo group needs to be investigated. Targeting TGF- $\beta$  by blocking integrins and thereby TGF- $\beta$  activation could be an interesting approach. However, compared to the other approaches this could be too indirect and unspecific for proper targeting of TGF- $\beta$ . Thus far, targeting TGF- $\beta$  with TKIs seems to be nontoxic. A prominent advantage of using TKIs is the possibility of oral- instead of intravenous administration. Targeting TGF- $\beta$  by means of ligand trapping with a monoclonal antibody shows encouraging results. Targeting all three isoforms of TGF- $\beta$  may be an advantage for the use of fresolimumab.

## 5. Evaluation of transforming growth factor $\beta$ targeting intervention strategies

As stated previously, it is clear that the dual role of TGF- $\beta$  in cancer complicates the clinical implementation of TGF- $\beta$  targeted drugs. Therefore, there is a need for adequate evaluation methods to select patients most likely to benefit from TGF- $\beta$  targeted therapies and to predict clinical response in these patients to guide treatment. Furthermore, these methods may be used to gain insight in the role of TGF- $\beta$  during tumor progression. Immunohistochemistry, although predominantly used to evaluate TGF- $\beta$  expression, is not suitable for detection of the dynamics of TGF- $\beta$  signaling *in vivo*. In this setting, *in vivo* imaging could be a very attractive alternative. In this section, an overview of developments for evaluating TGF- $\beta$  signaling and expression, with particular emphasis on their potentially clinical application, will be given.

### 5.1. Optical imaging

Disseminating cells demonstrate a certain motile behavior, which can be divided in collective slow moving cells or single fast moving cells. These motile cells are heterogeneously distributed throughout the tumor and count for only 5% of all the cells in the primary tumor (Wyckoff et al., 2000). Because of its role during tumor invasion and the forming of metastases, TGF- $\beta$  could also influence the motile behavior of tumor cells.

Rat mammary carcinoma cells, transfected with TGF- $\beta$  dependent reporter constructs, were injected in the mammary fat pad of female nude mice. Intravital imaging showed that TGF- $\beta$  signaling can influence the motile behavior of the tumor cells, favoring single fast moving cells. Then again, TGF- $\beta$  signaling alone seemed to be insufficient in driving cancer cell motility, since active TGF- $\beta$  signaling was also seen in non-moving cells. Furthermore, activated TGF- $\beta$  signaling enabled these single moving cells to disseminate into the blood, whereas cells lacking activated TGF- $\beta$  signaling were moving collectively and could not enter the blood. Conversely, persistent TGF- $\beta$  signaling did not lead to an increase in lung metastases, suggesting that TGF- $\beta$  signaling should be transient to permit efficient forming of blood borne metastasis (Giampieri et al., 2009).



In a mouse model for bone metastases, TGF- $\beta$  signaling dynamics and therapeutic response were evaluated with the help of bioluminescent imaging (Korpal et al., 2009). The triple negative breast cancer cell line SCP28 was transfected with a dual-luciferase reporter system, allowing imaging of both metastatic tumor growth and TGF- $\beta$  signaling. Preventive blocking of TGF- $\beta$  signaling with either TKI LY2109761 or a bisphosphonate, evidently reduced the number of bone metastases, illustrating TGF- $\beta$ 's role in the promotion of bone metastases. On the other hand, blocking TGF- $\beta$  signaling when bone metastases were already established was less effective. This again illustrates a transient role for TGF- $\beta$  signaling in the development of blood borne metastases (Welch et al., 1990; Giampieri et al., 2009).

In a second study using a similar human metastatic basal-like breast cancer model, the effects of two types of TGF- $\beta$  pathway antagonists (antibody 1D11 and TKI LY2109761) were examined on sub-lines of basal cell-like MDA-MB-231 human breast carcinoma cells that preferentially metastasize to lungs or bones. Both 1D11 and LY2109761 significantly reduced the metastatic burden to either lungs or bones in vivo. Besides inhibiting metastasis in a tumor cell autonomous manner, the TGF- $\beta$  antagonists inhibited angiogenesis associated with lung metastases as well as osteoclast number and -activity associated with lytic bone metastases. In aggregate, these studies support the notion that TGF- $\beta$  plays an important role in both bone- and lung metastases of basal-like breast cancer, and that inhibiting TGF- $\beta$  signaling results in a therapeutic effect independently of the tissue-tropism of the metastatic cells (Ganapathy et al., 2010).

In vivo visualization of TGF- $\beta$  activity by means of optical imaging has clearly led to improved insight in the role of TGF- $\beta$  during tumor invasion and metastasis. However, optical imaging with its poor tissue penetration depth is at present not applicable in the clinical setting.

### 5.2. Transforming growth factor $\beta$ and positron emission tomography

Of all presently available imaging techniques for TGF- $\beta$ , positron emission tomography (PET) imaging is the one potentially applicable in the clinical setting. Fresolimumab is a human monoclonal antibody neutralizing all mammalian isoforms of TGF- $\beta$  and has been evaluated in a phase I/II multi-center trial in patients with advanced metastatic melanoma or renal cell carcinoma (Morris et al., 2008). Imaging with this antibody, and thereby the presence of TGF- $\beta$ , with PET technique would be a non-invasive approach to ascertain tumor overexpression of TGF- $\beta$ . This might allow patient selection for TGF- $\beta$  targeted therapies and pharmacodynamic evaluations of these therapies. This approach, of in vivo imaging of a tumor related soluble factor with a radiolabeled antibody, was previously shown to be feasible for vascular endothelial growth factor (VEGF). In pre-clinical and clinical studies, tumor VEGF levels were visualized with zirconium-89 ( $^{89}\text{Zr}$ ) -bevacizumab-PET (Nagengast et al., 2007, 2010).  $^{89}\text{Zr}$ -fresolimumab was developed, in order to visualize and quantify in vivo TGF- $\beta$  expression, uptake of the antibody in the tumor and organ distribution, by means of PET imaging. Tumor uptake and organ distribution of  $^{89}\text{Zr}$ -fresolimumab were evaluated in different xenograft models. Clear uptake of  $^{89}\text{Zr}$ -fresolimumab in tumors of all xenograft models was observed from 72 hour post tracer injection and onwards. Distribution throughout all different organs of  $^{89}\text{Zr}$ -fresolimumab was comparable to the distribution of the control  $^{111}\text{In}$ -IgG, except for high uptake in the liver and kidney (Oude Munnink et al., 2011).

Imaging of TGF- $\beta$  with  $^{89}\text{Zr}$ -fresolimumab could be useful in the further clinical development of fresolimumab and help identify patients most likely to benefit from fresolimumab therapy. In the future  $^{89}\text{Zr}$ -fresolimumab will be used to quantify tumor uptake of fresolimumab in patients with high grade gliomas [clinicaltrials.gov NCT01472731].

### 5.3. Ex vivo stimulation assay

Within the development of potential new drugs, determination of pharmacodynamics is essential. To obtain sufficient tissue samples for such an assay directly from a solid tumor is (although potentially relevant) clinically usually unfeasible. Instead, frequently surrogate markers are used, such as measurements in peripheral blood mononuclear cells of cancer patients. Although it is clear that these surrogate markers are not necessarily reflecting the true events in the tumor, for evaluation of pharmacodynamics they may be of use. With regard to TGF- $\beta$ , measuring phosphorylated SMAD 2 (pSMAD2) was tested as a potential biomarker for tumor response to a TGF- $\beta$ R kinase inhibitor. Ex vivo change in pSMAD2 levels of peripheral blood mononuclear cells (determined with a sandwich ELISA), correlated with the in vivo response of the tumor, in a xenograft model (Farrington et al., 2007). The assay was further validated within a study of 49 patients with bone metastases of prostate- or breast cancer. In blood samples from these patients, the ex vivo pSMAD2 response assay again correlated well with the TGF- $\beta$  plasma levels (Baselga et al., 2008).

This ex vivo measurement of pSMAD2 is of particular interest, as it allows evaluation of pharmacodynamics of both large and small molecule inhibitors in one assay.

### 5.4. Transforming growth factor $\beta$ response signature

Investigators at Memorial-Sloan Kettering Cancer Center (MSKCC) recently defined a TGF- $\beta$  response gene signature consisting of 153 genes (TBRSM<sup>MSKCC</sup>) that may be used to identify tumors with high levels of TGF- $\beta$  activity (Padua et al., 2008; Padua & Massague, 2009). In 368 primary breast tumors in TBRSM<sup>MSKCC</sup> positive tumors, mRNA levels for TGF- $\beta$ 1, TGF- $\beta$ 2 and LTBP1 were higher. Also in estrogen receptor (ER) negative tumors, a positive TGF- $\beta$  response signature correlated with lung metastases. This preference for lung metastases is possibly caused by an increase in angiopoietin-like 4 expression in the cancer cells, induced by TGF- $\beta$  signaling. Angiopoietin-like 4 in turn disrupts the morphology of the lung capillaries and thereby improves seeding of tumor cells into the lung.

Another study (Tan et al., 2009) determined whether the TBRSM<sup>MSKCC</sup> and a similar 92-gene signature developed at The Cancer Institute of New Jersey (TBRSC<sup>CNJ</sup>) (Kareddula et al., 2008) correlated with any particular breast cancer subset. This was validated across three independent publicly available breast cancer expression data sets. These subsets were classified as described by Alexe et al. (Alexe et al., 2007) in human epidermal growth factor receptor (HER) 2 positive tumors with or without lymphocyte infiltration, HER2 and ER negative tumors (basal-like type I and II) or HER2 negative and ER positive tumors (luminal type I and II). (Lvshina et al., 2006; Desmedt et al., 2007; Padua et al., 2008) Both signatures positively correlated with the HER2 positive tumors with no lymphocyte infiltration, basal-like type II and luminal type I subsets. Furthermore, expression of TBRSM<sup>MSKCC</sup> correlated with poor overall survival in patients with lymph node negative breast cancer, who did not receive adjuvant systemic therapy. Patients with HER2 positive tumors with lymphocyte infiltration have a significantly better outcome than patients with HER2 positive tumors with no lymphocyte infiltration (Buck et al., 2002). Also a positive signature predicted for very poor survival in HER2 positive tumors with no lymphocyte infiltration, while patients with a negative signature had a survival comparable to HER2 positive tumors with lymphocyte infiltration. This suggests that active TGF- $\beta$  signaling may be mainly responsible for the poor prognosis of these patients and that these patients will benefit from TGF- $\beta$  inhibition.

In another study, gene expression profiles of early stage cervical cancer patients with or without positive lymph nodes were compared to several pathway signatures. The TGF- $\beta$  pathway was enriched in patients without lymph node involvement. Immunohistochemical

staining for TGF- $\beta$  on tissue microarrays confirmed this negative association for TGF- $\beta$  with lymph node metastasis (Noordhuis et al., 2011).

The TGF- $\beta$  gene signatures may support patient selection for TGF- $\beta$  targeted therapy and for further identification of new biomarkers.

## 6. Conclusion and future perspectives

For targeting cancer metastasis, TGF- $\beta$  is both of interest and utterly challenging, for targeting cancer metastasis. Several TGF- $\beta$  inhibitors are under clinical development. Amongst these inhibitors, monoclonal antibodies and TKIs are likely to be the most useful agents. In comparison with other TGF- $\beta$  targeting agents, both TKIs and fresolimumab may be applicable to a broader range of tumors, since they target TGF- $\beta$  relatively specifically, without discriminating between isoforms.

The fact that TGF- $\beta$  can act both as a tumor promoter and a tumor suppressor underlines the importance of adequate evaluation methods of TGF- $\beta$  activation in the clinical setting. Several evaluation methods are under development. With the pSMAD2 response assay in peripheral blood mononuclear cells, the effect of both large and small molecule inhibitors targeting TGF- $\beta$  may be assessed in one assay. TGF- $\beta$  gene signatures may be useful for patient selection and further identification of new biomarkers. The only technique that potentially allows in vivo imaging in patients at present is the TGF- $\beta$  PET. The currently conducted trial with TGF- $\beta$  PET will assess whether this technique is indeed suitable for evaluating pharmacodynamics, patient selection and monitoring of response to TGF- $\beta$  targeted therapies. Obviously, the ideal evaluation method will allow the identification of patients who will benefit from TGF- $\beta$  targeted therapies, and select out those in whom targeting of TGF- $\beta$  will do more harm than good.

Future clinical trials should implement these evaluation methods, to show which method will be most predictive and suitable for clinical application. This could guide accurate timing of TGF- $\beta$  targeted therapy and support the clinical development of TGF- $\beta$  inhibitors, bringing us a step closer to the clinical implementation of TGF- $\beta$  targeted therapies in the future.

## Acknowledgments

This study was supported by the Dutch Cancer Society grants 2010-4739.

## References

- Akhurst, R. J., & Derynck, R. (2001). TGF- $\beta$  signaling in cancer — a double-edged sword. *Trends Cell Biol* 11, S44–S51.
- Alexe, G., Dalgin, G. S., Ganesan, S., Delisi, C., & Bhanot, G. (2007). Analysis of breast cancer progression using principal component analysis and clustering. *J Biosci* 32, 1027–1039.
- Aluwihare, P., Mu, Z., Zhao, Z., Yu, D., Weinreb, P. H., Horan, G. S., et al. (2009). Mice that lack activity of  $\alpha$ 5 $\beta$ 1 and  $\alpha$ 8 $\beta$ 1 integrins reproduce the abnormalities of Tgfb1- and Tgfb3-null mice. *J Cell Sci* 122, 227–232.
- Annes, J. P., Chen, Y., Munger, J. S., & Rifkin, D. B. (2004). Integrin  $\alpha$ 3 $\beta$ 6-mediated activation of latent TGF- $\beta$  requires the latent TGF- $\beta$  binding protein-1. *J Cell Biol* 165, 723–734.
- Annes, J. P., Munger, J. S., & Rifkin, D. B. (2003). Making sense of latent TGF $\beta$  activation. *J Cell Sci* 116, 217–224.
- Anscher, M. S., Marks, L. B., Shafman, T. D., Clough, R., Huang, H., Tisch, A., et al. (2001). Using plasma transforming growth factor  $\beta$ 1 during radiotherapy to select patients for dose escalation. *J Clin Oncol* 19, 3758–3765.
- Assoian, R. K., Komoriya, A., Meyers, C. A., Miller, D. M., & Sporn, M. B. (1983). Transforming growth factor-beta in human platelets. Identification of a major storage site, purification, and characterization. *J Biol Chem* 258, 7155–7160.
- Bandyopadhyay, A., & Raghavan, S. (2009). Defining the role of integrin  $\alpha$ 3 $\beta$ 6 in cancer. *Curr Drug Targets* 10, 645–652.
- Barcellos-Hoff, M. H., Derynck, R., Tsang, M. L., & Weatherbee, J. A. (1994). Transforming growth factor-beta activation in irradiated murine mammary gland. *J Clin Invest* 93, 892–899.
- Barcellos-Hoff, M., & Dix, T. (1996). Redox-mediated activation of latent transforming growth factor-beta 1. *Mol Endocrinol* 10, 1077–1083.
- Baselga, J., Rothenberg, M. L., Taberero, J., Seoane, J., Daly, T., Cleverly, A., et al. (2008). TGF- $\beta$  signalling-related markers in cancer patients with bone metastasis. *Bio-markers* 13, 217–236.
- Bierie, B., & Moses, H. L. (2006). Tumour microenvironment: TGF $\beta$ : the molecular jekyll and hyde of cancer. *Nat Rev Cancer* 6, 506–520.
- Biswas, S., Guix, M., Rinehart, C., Dugger, T. C., Chytil, A., Moses, H. L., et al. (2007). Inhibition of TGF-beta with neutralizing antibodies prevents radiation-induced acceleration of metastatic cancer progression. *J Clin Invest* 117, 1305–1313.
- Bogdahn, U., Hau, P., Stockhammer, G., Venkataramana, N. K., Mahapatra, A. K., Suri, A., et al. (2011). Targeted therapy for high-grade glioma with the TGF- $\beta$ 2 inhibitor trabedersen: results of a randomized and controlled phase IIb study. *Neuro Oncol* 13, 132–142.
- Breuss, J., Gillett, N., Lu, L., Sheppard, D., & Pytela, R. (1993). Restricted distribution of integrin beta 6 mRNA in primate epithelial tissues. *J Histochem Cytochem* 41, 1521–1527.
- Brooks, P. C., Strömblad, S., Sanders, L. C., von Schalscha, T. L., Aimes, R. T., Stetler-Stevenson, W. G., et al. (1996). Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin  $\alpha$ 3 $\beta$ 3. *Cell* 85, 683–693.
- Brown, P. D., Wakefield, L. M., Levinson, A. D., & Sporn, M. B. (1990). Physicochemical activation of recombinant latent transforming growth factor-beta's 1, 2, and 3. *Growth Factors* 3, 35–43.
- Buck, M., der Fecht, J. V., & Knabbe, C. (2002). Antiestrogenic regulation of transforming growth factor beta receptors I and II in human breast cancer cells. *Ann N Y Acad Sci* 963, 140–143.
- Calvo-Aller, E., Baselga, J., Glatt, S., Cleverly, A., Lahn, M., Arteaga, C. L., et al. (2008). First human dose escalation study in patient with metastatic malignancies to determine safety and pharmacokinetics of LY2157299, a small molecule inhibitor of the transforming growth factor factor-beta receptor kinase I. *J Clin Oncol* 26 (Abstract#14554).
- Chiang, A. C., & Massagué, J. (2008). Molecular basis of metastasis. *N Engl J Med* 359, 2814–2823.
- Dallas, S. L., Miyazono, K., Skerry, T. M., Mundy, G. R., & Bonewald, L. F. (1995). Dual role for the latent transforming growth factor-beta binding protein in storage of latent TGF-beta in the extracellular matrix and as a structural matrix protein. *J Cell Biol* 131, 539–549.
- Derynck, R., Lindquist, P. B., Lee, A., Wen, D., Tamm, J., Graycar, J. L., et al. (1998). A new type of transforming growth factor-beta, TGF-beta 3. *EMBO J* 7, 3737–3743.
- Desmedt, C., Piette, F., Loi, S., Wang, Y., Lallemand, F., Haibe-Kains, B., et al. (2007). Strong time dependence of the 76-gene prognostic signature for node-negative breast cancer patients in the TRANSBIG multicenter independent validation series. *Clin Cancer Res* 13, 3207–3214.
- Dubois, C. M., Laprise, M., Blanchette, F., Gentry, L. E., & Leduc, R. (1995). Processing of transforming growth factor 1 precursor by human furin convertase. *J Biol Chem* 270, 10618–10624.
- Ehata, S., Hanyu, A., Fujime, M., Katsuno, Y., Fukunaga, E., Goto, K., et al. (2007). Ki26894, a novel transforming growth factor- $\beta$  type I receptor kinase inhibitor, inhibits in vitro invasion and in vivo bone metastasis of a human breast cancer cell line. *Cancer Sci* 98, 127–133.
- Farrington, D. L., Yingling, J. M., Fill, J. A., Yan, L., Qian, Y., Shou, J., et al. (2007). Development and validation of a phosphorylated SMAD ex vivo stimulation assay. *Bio-markers* 12, 313–330.
- Flaumenhaft, R., & Rifkin, D. B. (1992). The extracellular regulation of growth factor activation. *Mol Biol Cell* 3, 1057–1065.
- Ganapathy, V., Ge, R., Grazioli, A., Xie, W., Banach-Petrosky, W., Kang, Y., et al. (2010). Targeting the transforming growth factor-beta pathway inhibits human basal-like breast cancer metastasis. *Mol Cancer* 9, 122.
- Ge, R., Rajeev, V., Ray, P., Lattime, E., Rittling, S., Medicherla, S., et al. (2006). Inhibition of growth and metastasis of mouse mammary carcinoma by selective inhibitor of transforming growth factor- $\beta$  type I receptor kinase in vivo. *Clin Cancer Res* 12, 4315–4330.
- Giamperi, S., Manning, C., Hooper, S., Jones, L., Hill, C. S., & Sahai, E. (2009). Localized and reversible TGF $\beta$  signalling switches breast cancer cells from cohesive to single cell motility. *Nat Cell Biol* 11, 1287–1296.
- Goudie, D. R., D'Alessandro, M., Merriman, B., Lee, H., Szevevényi, I., Avery, S., et al. (2011). Multiple self-healing squamous epithelioma is caused by a disease-specific spectrum of mutations in TGFBR1. *Nat Genet* 43, 365–369.
- Gray, A. M., & Mason, A. J. (1990). Requirement for activin A and transforming growth factor- $\beta$ 1 pro-regions in homodimer assembly. *Science* 247, 1328–1330.
- Hersey, P., Sosman, J., O'Day, S., Richards, J., Bedikian, A., Gonzalez, R., et al. (2010). A randomized phase 2 study of etaracizumab, a monoclonal antibody against integrin  $\alpha$ 3 $\beta$ 3,  $\pm$  dacarbazine in patients with stage IV metastatic melanoma. *Cancer* 116, 1526–1534.
- Horan, G. S., Wood, S., Ona, V., Li, D. J., Lukashev, M. E., Weinreb, P. H., et al. (2008). Partial inhibition of integrin  $\alpha$ (v) $\beta$ 6 prevents pulmonary fibrosis without exacerbating inflammation. *Am J Respir Crit Care Med* 177, 56–65.
- Kanzaki, T., Olofsson, A., Morén, A., Wernstedt, C., Hellman, U., Miyazono, K., et al. (1990). TGF- $\beta$ 1 binding protein: a component of the large latent complex of TGF- $\beta$ 1 with multiple repeat sequences. *Cell* 61, 1051–1061.
- Kareddula, A., Zachariah, E., Notterman, D., & Reiss, M. (2008). Transforming growth factor- $\beta$  signaling strength determines target gene expression profile in human keratinocytes. *J Epithelial Biol Pharmacol* 1, 40–94.
- Khalil, N. (1999). TGF- $\beta$ : from latent to active. *Microbes Infect* 1, 1255–1263.
- Kirshner, J., Jobling, M. F., Pajares, M. J., Ravani, S. A., Glick, A. B., Lavin, M. J., et al. (2006). Inhibition of transforming growth factor- $\beta$ 1 signaling attenuates ataxia telangiectasia mutated activity in response to genotoxic stress. *Cancer Res* 66, 10861–10869.
- Korpal, M., Yan, J., Lu, X., Xu, S., Lerit, D. A., & Kang, Y. (2009). Imaging transforming growth factor- $\beta$  signaling dynamics and therapeutic response in breast cancer bone metastasis. *Nat Med* 15, 1960–1966.

- Lawrence, D. A., Pircher, R., & Jullien, P. (1985). Conversion of a high molecular weight latent  $\beta$ -TGF from chicken embryo fibroblasts into a low molecular weight active  $\beta$ -TGF under acidic conditions. *Biochem Biophys Res Commun* 133, 1026–1034.
- Levy, L., & Hill, C. S. (2006). Alterations in components of the TGF- $\beta$  superfamily signaling pathways in human cancer. *Cytokine Growth Factor Rev* 17, 41–58.
- Lvshina, A. V., George, J., Senko, O., Mow, B., Putti, T. C., Smeds, J., et al. (2006). Genetic reclassification of histologic grade delineates new clinical subtypes of breast cancer. *Cancer Res* 66, 10292–10301.
- Lyons, R. M., Keski-Oja, J., & Moses, H. L. (1988). Proteolytic activation of latent transforming growth factor- $\beta$  from fibroblast-conditioned medium. *J Cell Biol* 106, 1659–1665.
- Massagué, J. (2008). TGF $\beta$  in cancer. *Cell* 134, 215–230.
- Massagué, J., Blain, S. W., & Lo, R. S. (2000). TGF $\beta$  signaling in growth control, cancer, and heritable disorders. *Cell* 103, 295–309.
- Melisi, D., Ishiyama, S., Scwab, G. M., Fleming, J. B., Xia, Q., Tortora, G., et al. (2008). LY2109761, a novel transforming growth factor  $\beta$  receptor type I and type II dual inhibitor, as a therapeutic approach to suppressing pancreatic cancer metastasis. *Mol Cancer Ther* 7, 829–840.
- Miyazono, K., Olofsson, A., Colosetti, P., & Heldin, C. H. (1991). A role of the latent TGF- $\beta$  1-binding protein in the assembly and secretion of TGF- $\beta$  1. *EMBO J* 10, 1091–1101.
- Morris, J., Shapiro, G., Tan, A. R., Lawrence, D. P., Olencki, T. E., Dezube, B. J., et al. (2008). Phase 1/2 study of GC1008: a monoclonal antibody (Mab) in patients with advanced malignant melanoma or renal cell carcinoma. *J Clin Oncol* 26 (Abstract#9028).
- Moustakas, A., & Heldin, C. (2005). Non-smad TGF- $\beta$  signals. *J Cell Sci* 118, 3573–3584.
- Munger, J. S., Harpel, J. G., Gleizes, P. E., Mazziari, R., Nunes, I., & Rifkin, D. B. (1997). Latent transforming growth factor-beta: structural features and mechanisms of activation. *Kidney Int* 51, 1376–1382.
- Munger, J. S., Huang, X., Kawakatsu, H., Griffiths, M. J. D., Dalton, S. L., Wu, J., et al. (1999). A mechanism for regulating pulmonary inflammation and fibrosis: the integrin  $\alpha$ v $\beta$ 6 binds and activates latent TGF  $\beta$ 1. *Cell* 96, 319–328.
- Nagengast, W. B., de Korte, M. A., Oude Munnink, T. H., Timmer-Bosscha, H., den Nunnen, W. F., Hollema, H., et al. (2010).  $^{89}\text{Zr}$ -bevacizumab PET of early anti-angiogenic tumor response to treatment with HSP90 inhibitor NVP-AUY922. *J Nucl Med* 51, 761–767.
- Nagengast, W. B., de Vries, E. G., Hospers, G. A., Mulder, N. H., de Jong, J. R., Hollema, H., et al. (2007). In vivo VEGF imaging with radiolabeled bevacizumab in a human ovarian tumor xenograft. *J Nucl Med* 48, 1313–1319.
- Nam, J., Suchar, A. M., Kang, M., Stuelten, C. H., Tang, B., Michalowska, A. M., et al. (2006). Bone sialoprotein mediates the tumor cell-targeted prometastatic activity of transforming growth factor  $\beta$  in a mouse model of breast cancer. *Cancer Res* 66, 6327–6335.
- Nemunaitis, J., Dillman, R. O., Schwarzenberger, P. O., Senzer, N., Cunningham, C., Cutler, J., et al. (2006). Phase II study of belagenpumatucel-L, a transforming growth factor beta-2 antisense gene-modified allogeneic tumor cell vaccine in non small-cell lung cancer. *J Clin Oncol* 24, 4721–4730.
- Nemunaitis, J., Nemunaitis, M., Senzer, N., Snitz, P., Bedell, C., Kumar, P., et al. (2009). Phase II trial of belagenpumatucel-L, a TGF- $\beta$ 2 antisense gene modified allogeneic tumor vaccine in advanced non small cell lung cancer (NSCLC) patients. *Cancer Gene Ther* 16, 620–624.
- Nishi, H., Nakada, T., Hokamura, M., Osakabe, Y., Itokazu, O., Huang, L. E., et al. (2004). Hypoxia-inducible factor-1 transactivates transforming growth factor- $\beta$  3 in trophoblasts. *Endocrinology* 145, 4113–4118.
- Noordhuis, M. G., Fehrmann, R. S. N., Wisman, G. B. A., Nijhuis, E. R., van Zanden, J. J., Moerland, P. D., et al. (2011). Involvement of the TGF- $\beta$  and  $\beta$ -catenin pathways in pelvic lymph node metastasis in early-stage cervical cancer. *Clin Cancer Res* 17, 1317–1330.
- Oude Munnink, T. H., Arjaans, M. E. A., Timmer-Bosscha, H., Schröder, C. P., Hesselink, J. W., Vedelaar, S. R., et al. (2011). PET imaging with  $^{89}\text{Zr}$  labeled transforming growth factor (TGF)- $\beta$  antibody fresolimumab in tumor models. *J Nucl Med* 52, 2001–2008.
- Padua, D., & Massagué, J. (2009). Roles of TGF- $\beta$  in metastasis. *Cell Res* 19, 89–102.
- Padua, D., Zhang, X. H., Wang, Q., Nadal, C., Gerald, W. L., Gomis, R. R., et al. (2008). TGF $\beta$  primes breast tumors for lung metastasis seeding through angiopoietin-like 4. *Cell* 133, 66–77.
- Pinkas, J., & Teicher, B. A. (2006). TGF- $\beta$  in cancer and as a therapeutic target. *Biochem Pharmacol* 72, 523–529.
- Puthawala, K., Hadjiangelis, N., Jacoby, S. C., Bayongan, E., Zhao, Z., Yang, Z., et al. (2008). Inhibition of integrin  $\alpha$ (v) $\beta$ 6, an activator of latent transforming growth factor- $\beta$ , prevents radiation-induced lung fibrosis. *Am J Respir Crit Care Med* 177, 82–90.
- Reiss, M., & Barcellos-Hoff, M. H. (1997). Transforming growth factor-beta in breast cancer: a working hypothesis. *Breast Cancer Res Treat* 45, 81–95.
- Riethdorf, S., Wikman, H., & Pantel, K. (2008). Review: biological relevance of disseminated tumor cells in cancer patient. *Int J Cancer* 123, 1991–2006.
- Rodon Ahnert, J., Baselga, J., Calvo, E., Seoane, J., Brana, I., Sicart, E., et al. (2011). First human dose (FHD) study of the oral transforming growth factor- $\beta$  receptor I kinase inhibitor LY2157299 in patients with treatment-refractory malignant glioma. *J Clin Oncol* 29 (Abstract #3011).
- Saunier, E. F., & Akhurst, R. J. (2006). TGF beta inhibition for cancer therapy. *Curr Cancer Drug Targets* 6, 565–578.
- Shi, Y., & Massagué, J. (2003). Mechanisms of TGF- $\beta$  signaling from cell membrane to the nucleus. *Cell* 113, 685–700.
- Stover, D. G., Bierie, B., & Moses, H. L. (2007). A delicate balance: TGF- $\beta$  and the tumor microenvironment. *J Cell Biochem* 101, 851–861.
- Stupp, R., Hegi, M. E., Neyns, B., Goldbrunner, R., Schlegel, U., Clement, P. M. J., et al. (2010). Phase I/IIa study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma. *J Clin Oncol* 28, 2712–2718.
- Tabatabai, G., Weller, M., Nabors, B., Picard, M., Reardon, D., Mikkelsen, T., et al. (2010). Targeting integrins in malignant melanoma. *Target Oncol* 5, 175–181.
- Taipale, J., Miyazono, K., Heldin, C., & Keski-Oja, J. (1994). Latent transforming growth factor-beta 1 associates to fibroblast extracellular matrix via latent TGF-beta binding protein. *J Cell Biol* 124, 171–181.
- Tan, A. R., Alexe, G., & Reiss, M. (2009). Transforming growth factor-beta signaling: emerging stem cell target in metastatic breast cancer? *Breast Cancer Res Treat* 115, 453–495.
- Tucker, G. C. (2006). Integrins: molecular targets in cancer therapy. *Curr Oncol Rep* 8, 96–103.
- van Aarsen, L. A., Leone, D. R., Ho, S., Dolinski, B. M., McCoon, P. E., LePage, D. J., et al. (2008). Antibody-mediated blockade of integrin  $\alpha$ v $\beta$ 6 inhibits tumor progression in vivo by a transforming growth factor-beta-regulated mechanism. *Cancer Res* 68, 561–570.
- Vujaskovic, Z., Marks, L. B., & Anscher, M. S. (2000). The physical parameters and molecular events associated with radiation-induced lung toxicity. *Semin Radiat Oncol* 10, 296–307.
- Weigert, C., Brodbeck, K., Häring, H. U., Gambaro, G., & Schleicher, E. D. (2001). Low-molecular-weight heparin prevents high glucose- and phorbol ester-induced TGF- $\beta$  1 gene activation. *Kidney Int* 60, 935–943.
- Welch, D. R., Fabra, A., & Nakajima, M. (1990). Transforming growth factor beta stimulates mammary adenocarcinoma cell invasion and metastatic potential. *Proc Natl Acad Sci* 87, 7678–7682.
- Wipff, P., & Hinz, B. (2008). Integrins and the activation of latent transforming growth factor  $\beta$  1 – an intimate relationship. *Eur J Cell Biol* 87, 601–615.
- Wipff, P., Rifkin, D. B., Meister, J., & Hinz, B. (2007). Myofibroblast contraction activates latent TGF- $\beta$ 1 from the extracellular matrix. *J Cell Biol* 179, 1311–1323.
- Worthington, J. J., Klementowicz, J. E., & Travis, M. A. (2011). TGF $\beta$ : a sleeping giant awoken by integrins. *Trends Biochem Sci* 36, 47–54.
- Wyckoff, J. B., Jones, J. G., Condeelis, J. S., & Segall, J. E. (2000). A critical step in metastasis: in vivo analysis of intravasation at the primary tumor. *Cancer Res* 60, 2504–2511.
- Xu, M. Y., Porte, J., Knox, A. J., Weinreb, P. H., Maher, T. M., Violette, S. M., et al. (2009). Lysophosphatidic acid induces  $\alpha$ v $\beta$ 6 integrin-mediated TGF- $\beta$  activation via the LPA2 receptor and the small G protein G  $\alpha$ (q). *Am J Pathol* 174, 1264–1279.
- Yu, Q., & Stamenkovic, I. (1999). Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. *Genes Dev* 13, 35–48.
- Yu, Q., & Stamenkovic, I. (2000). Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF- $\beta$  and promotes tumor invasion and angiogenesis. *Genes Dev* 14, 163–176.
- Zhao, L., Sheldon, K., Chen, M., Yin, M. S., Hayman, J. A., Kalemkerian, G. P., et al. (2008). The predictive role of plasma TGF- $\beta$ 1 during radiation therapy for radiation-induced lung toxicity deserves further study in patients with non-small cell lung cancer. *Lung Cancer* 59, 232–239.