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Review

The emerging role of TGF-β superfamily coreceptors in cancer

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ABSTRACT

The transforming growth factor β (TGF-β) signaling pathway plays a key role in different physiological processes such as development, cellular proliferation, extracellular matrix synthesis, angiogenesis or immune responses and its deregulation may result in tumor development. The TGF-β coreceptors endoglin and betaglycan are emerging as modulators of the TGF-β response with important roles in cancer. Endoglin is highly expressed in the tumor-associated vascular endothelium with prognostic significance in selected neoplasias and with potential to be a prime vascular target for antiangiogenic cancer therapy. On the other hand, the expression of endoglin and betaglycan in tumor cells themselves appears to play an important role in the progression of cancer, influencing cell proliferation, motility, invasiveness and tumorigenicity. In addition, experiments *in vitro* and *in vivo* in which endoglin or betaglycan expression is modulated have provided evidence that they act as tumor suppressors. The purpose of this review was to highlight the potential of membrane and soluble forms of the endoglin and betaglycan proteins as molecular targets in cancer diagnosis and therapy.

1. Introduction

Transforming growth factor β (TGF-β) is the prototypic member of a large family of evolutionarily conserved secreted cytokines that also includes activins and bone morphogenetic proteins (BMPs). Individual family members play crucial roles in development and in the regulation of tissue homeostasis during adult life [1,2]. Accordingly, alterations to the signaling pathways activated by TGF-β family members have been implicated in many human diseases, including cancer, vascular, fibrosis and autoimmune diseases [3–8].

1.1. The role of TGF-β in cancer

TGF-β plays a dual and paradoxical role in cancer [3,9–11]. On the one hand it acts as a tumor suppressor during the premalignant phase of carcinogenesis, inhibiting cell growth and inducing apoptosis or differentiation. On the other hand, cancer cells that have lost this inhibitory growth response exploit the ability of TGF-β to modulate processes such as cell invasion, angiogenesis, immune regulation, or interactions between tumor cells and their microenvironment that make them more malignant. The tumor suppressor and tumor promoting effect of TGF-β may be exerted on the tumor cells themselves or indirectly by taking advantage of the interactions between the tumor and stroma [12]. For example, TGF-β arrests the progression of epithelial cells in the G1 phase of the cell cycle by mobilizing cyclin-dependent kinase inhibitors (cdki) and downregulating c-Myc expression [13]. Yet in addition to this direct effect on tumor cells, TGF-β can restrict epithelial cell proliferation and tumor formation by preventing the production of paracrine factors in stromal fibroblasts [14], or by constraining tumorigenic inflammation [15]. Some cancer cells evade the tumor suppressor effects of TGF-β by accumulating inactivating mutations in the TGF-β receptors and Smad proteins [16]. This is particularly true for subsets of colorectal, pancreatic, ovarian, gastric, and head and neck carcinomas. Interestingly, the BMP signaling pathway may also be disrupted in cancer, and mutations in BMPR1A are associated with juvenile polyposis syndrome (see below). However, the core components of TGF-β signaling remain intact in the majority of tumors (i.e., breast and prostate carcinomas, melanomas, gliomas and hematopoietic neoplasias). Cells within these tumors avoid the tumor suppressor activity of TGF-β by a variety of mechanisms that are not fully understood and which lead to the loss of the TGF-β anti-proliferative response [17]. For example, the p15INK4b locus is a downstream target gene of TGF-β signaling that encodes a cdki involved in regulating the cell cycle, and it is homozygously deleted in a subset of gliomas [18]. Other alterations that may contribute to the loss of the anti-proliferative response in gliomas are PI3K hyperactivation or mutational inactivation of the RB locus [19]. However, in many tumors that preserve an intact TGF-β signaling pathway the precise mechanisms leading to the loss of the TGF-β cytostatic response is unknown.

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Tumor cells resistant to the suppressor effects of TGF-β may respond to this growth factor by enhancing cell motility and invasiveness. TGF-β is a potent inducer of the epithelial–mesenchymal transition (EMT) [20,21], a phenotypic conversion by which epithelial cells lose their polarity and cohesiveness (indeed, a hallmark of EMT is the loss of the adherens junction protein E-cadherin), acquiring the migratory features typical of fibroblasts. EMTs are necessary for morphogenetic processes during embryonic development but they are also involved in pathological situations, such as fibrosis, tumor invasion and metastasis [22,23]. TGF-β can also promote tumor cell growth by stimulating the production of autocrine mitogenic factors [19]. Moreover, TGF-β contributes to tumor progression in the stroma by regulating the generation of myofibroblasts or "cancer-associated fibroblasts" from mesenchymal precursors, the evasion of the immune surveillance, the stimulation of angiogenesis and the promotion of distal metastasis [11,12].

In the last decade, several studies have documented a role for the TGF-β type III receptors betaglycan and endoglin in cancer (see Gordon and Blobe [5], for a recent review). These coreceptors not only bind TGF-β, BMP and inhibin ligands and receptors, but they also regulate their function by as yet unknown mechanisms. It is generally believed that alterations of betaglycan and endoglin in cancer contribute to the deregulation of TGF-β signaling. Nevertheless, these coreceptors interact with proteins and signaling pathways other than TGF-β and thus, they may have cellular effects that are independent of TGF-β signaling.

1.2. The TGF-β receptor complex

The effects of the TGF-β family of soluble proteins in cancer are exerted through specific receptor complexes present at the cell surface. These receptor complexes contain heterodimeric type I and II receptors that constitute the core signaling complex, as well as type III receptors that include the auxiliary molecules endoglin and betaglycan (Fig. 1). Membrane bound and soluble forms of endoglin and betaglycan can modulate signaling by binding to the ligand and to the type I and II TGF-β receptors. The type I and II receptors are serine/threonine kinases involved in downstream signaling, whereas the coreceptors are proteins with no known signaling motifs. The core TGF-β signaling pathway comprises at least seven type I (also known as activin-like kinase receptors) and five type II receptors, where type I receptors act downstream of type II receptors, and the combinatorial heterodimeric association of these receptors determines the specificity of the ligand signaling [1,2]. Thus, ligand binding to the type II receptor activates and transphosphorylates the type I receptor, which subsequently propagates the signal by phosphorylating the receptor-regulated Smad (R-Smad) family of proteins. Upon activation, R-Smad proteins form heteromeric complexes with a cooperative homologue named Co-Smad (Smad4 in mammals), and they are translocated into the nucleus where they regulate the transcriptional activity of target genes (Fig. 1).

Although there are other TGF-β superfamily coreceptors such as the members of the repulsive guidance family of glycosylphosphatidylinositol-anchored proteins DRAGON, RGMa, and hemojuvelin [24–26], or the member of the α2-macroglobulin/C3, C4, C5 family of thioester-containing proteins, CD109 [27], their role in cancer is still unclear. Therefore, in this review we will focus on the role of the transmembrane protein coreceptors endoglin and betaglycan in the TGF-β system.

2. The TGF-β coreceptors endoglin and betaglycan

2.1. Structure/Function relationship

Human endoglin and betaglycan are type I integral membrane proteins with large extracellular domains (561 and 766 amino acids,

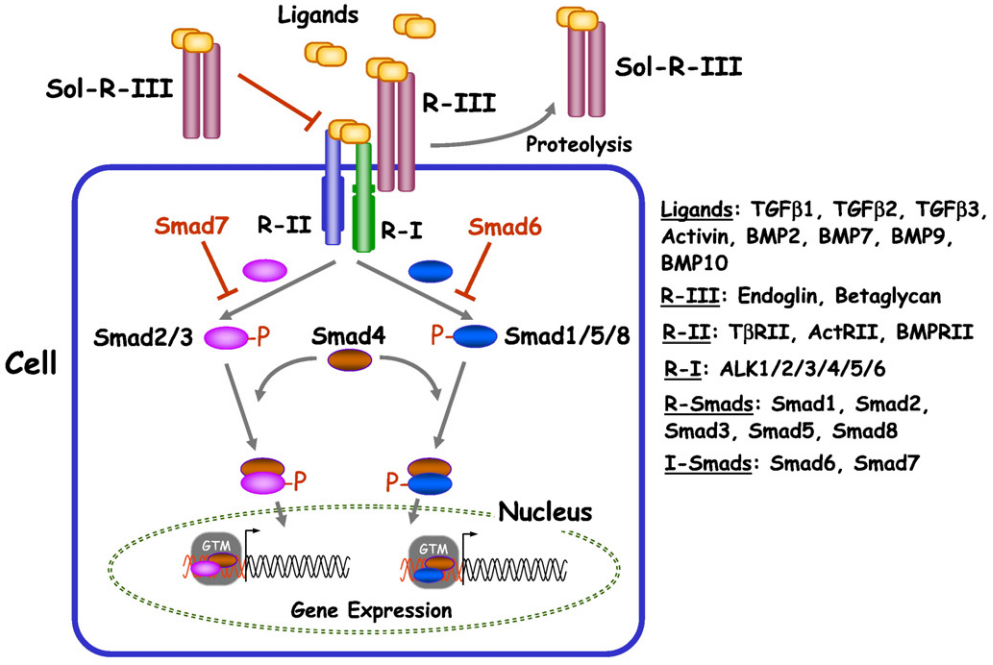

Fig. 1. The TGF-β signaling pathway. The members of the TGF-β family, which include TGF-βs, activins and BMPs, bind to specific type I (R-I) and type II (R-II) cell surface receptors that exhibit serine/threonine kinase activity. Accessory receptors endoglin and betaglycan, also known as type III (R-III) receptors, modulate TGF-β signaling via R-I and R-II. A soluble form of R-III can be generated by juxtamembrane proteolysis of the membrane bound receptor that can sequester ligands and thereby inhibit their binding to R-I/R-II. In most cells TGF-β signals via the ALK5/TβRII heterodimer. In other cell types, including endothelial cells, ALK1, another R-I, also mediates TGF-β signaling via ALK1/TβRII. Activins signal via ALK4/ActRII, whereas BMPs signal via ALK1, ALK2, ALK3 or ALK6 as the R-I, and through BMPRII or ActRII as the R-II. The R-I acts downstream of R-II and it determines the signaling specificity of the receptor complex. Thus, activated R-I phosphorylates specific R-Smads. More specifically, activation of ALK1, ALK2, ALK3 and ALK6 leads to phosphorylation of Smad1, Smad5 and Smad8, while activation of ALK4, ALK5 and ALK7 phosphorylates Smad2 and Smad3. Activated R-Smads associate with Smad4 in heteromeric complexes that are translocated to the nucleus and that regulate specific gene expression responses by binding to DNA together with other DNA binding transcription factors. Inhibitory Smads (I-Smads), Smad6 and Smad7, can interfere with R-Smad activation. ActR, activin receptor; ALK, activin receptor-like kinase; BMP, bone morphogenetic protein; BMPR, BMP receptor; GTM, general transcription machinery; Sol-R-III, soluble endoglin/betaglycan; TβR, TGF-β receptor.

respectively), regular hydrophobic transmembrane domains, and short cytoplasmic domains (47 and 42 residues, respectively) [28,29]. Endoglin and betaglycan are independently expressed as homodimeric glycoproteins, although a minor subset of heteromeric complexes of endoglin and betaglycan have been detected in endothelial cells [30]. In the case of endoglin, the monomers are disulphide-linked, whereas the betaglycan dimer is sustained by non-covalent interactions (Fig. 2). Endoglin and betaglycan are transmembrane proteins that share a degree of sequence similarity. Consequently, the initial search for functional attributes of endoglin has drawn upon previous results from the study of betaglycan in the TGF-β system. Endoglin shares a high degree of amino acid sequence homology with betaglycan in the transmembrane and cytoplasmic domains. Indeed, the cytoplasmic domain of these proteins constitutes the most highly conserved region among homologous members from different mammalian species, as well as between endoglin and betaglycan [31]. The in vivo expression of 2 different alternatively spliced isoforms, long (L)-endoglin and short (S)-endoglin, has been demonstrated in human and mouse tissues [32–34]. Both isoforms share common extracellular and transmembrane domains, but they differ from each other in the composition of their cytoplasmic domain. In humans, L-endoglin contains a cytoplasmic domain of 47 residues, whereas S-endoglin contains a cytoplasmic domain of 14 residues. Because L-endoglin is much more prevalent than S-endoglin and most functional studies have been carried out with L-endoglin, no further reference to the endoglin isoforms will be made in this review. At variance with endoglin, no alternatively spliced isoforms have so far been described for betaglycan.

The cytoplasmic domains of both endoglin and betaglycan can be phosphorylated by serine/threonine kinases, including the TGF-β type I and II receptors [35–39]. Endoglin phosphorylation might be a Smad-independent mechanism to regulate the influence of endoglin in cell growth and adhesion. In fact, it has been shown that endoglin phosphorylation influences its subcellular localization, potentially by modulating endoglin's interactions with adhesive proteins such as zyxin and ZRP-1, thereby modifying the adhesive properties of endoglin-expressing cells [37]. The regulation and pattern of endoglin phosphorylation by the TGF-β receptors are complex. A detailed study was carried out of endoglin phosphorylation by constitutively active (ca) forms of the TGF-β receptors, caALK1, caALK5 and wild type TβRII. Site-directed mutagenesis of endoglin suggests that caALK5 and TβRII phosphorylate the ^{634}SerSer^{635} motif within endoglin's cytoplasmic domain, whereas ALK1 preferentially phosphorylates wild type endoglin at threonine residues. Interestingly, mutation of the ^{634}SerSer^{635} residues to ^{634}AlaAla^{635} strongly reduces threonine phosphorylation of endoglin, suggesting that phosphorylation of ^{634}SerSer^{635} is a prerequisite for subsequent endoglin threonine phosphorylation. This hypothesis was confirmed by replacing one mutated alanine with a phospho-mimicking aspartate residue (^{634}AspAla^{635}), which restores threonine phosphorylation by caALK1 [37].

Both endoglin and betaglycan cytoplasmic domains contain consensus PDZ-binding motifs at their carboxyl terminus (SerSerMetAla and SerSerThrAla, respectively; Fig. 2). Studies of specific mutations have been informative about the role of PDZ domains and for example, betaglycan can alter the subcellular localization of the signaling receptor complex by interacting with the PDZ domain containing proteins, GIPC [40] and β-arrestin2 [38]. Also, the removal of endoglin's putative C-terminal PDZ-binding motif results in endoglin hyperphosphorylation of distal threonine residues [37]. These data indicate that receptor-mediated phosphorylation of endoglin is a complex process involving negative regulation by the PDZ-binding motif and an unexpected sequential mechanism of serine and threonine phosphorylation.

Yeast two-hybrid and cell biology approaches identified zyxin and the zyxin-related protein 1 (ZRP-1) as the first cytosolic proteins that interact with endoglin's cytoplasmic domain [41,42]. Because these interactions occur with endoglin's cytoplasmic domain, which contains serine/threonine phosphorylation sites [37], protein–protein interactions involving this endoglin domain are likely to be regulated by phosphorylation. The interaction between endoglin and the zyxin-related proteins is exclusive since the latter does not appear to interact with betaglycan, even though their cytoplasmic domains are 70% identical [41,42]. However, endoglin does associate with proteins that also interact with betaglycan. For example, β-arrestin2 interacts with the conserved distal end of the betaglycan cytoplasmic domain and it regulates betaglycan internalization [38]. This interaction also occurs with the endoglin cytoplasmic domain, resulting in the internalization of endoglin with β-arrestin2 into endocytic vesicles [43]. Furthermore, the betaglycan/endoglin endocytosis mediated by β-arrestin2, dampens TGF-β signaling [38,43]. More recently, it has been shown that betaglycan undergoes endocytosis in a ligand- and glycosaminoglycan-independent manner, but dependent on its cytoplasmic domain [44]. Moreover, the interaction of Thr^{841} in the cytoplasmic domain of betaglycan with β-arrestin2 enhances betaglycan endocytosis. Interestingly, betaglycan undergoes both clathrin-mediated and clathrin-

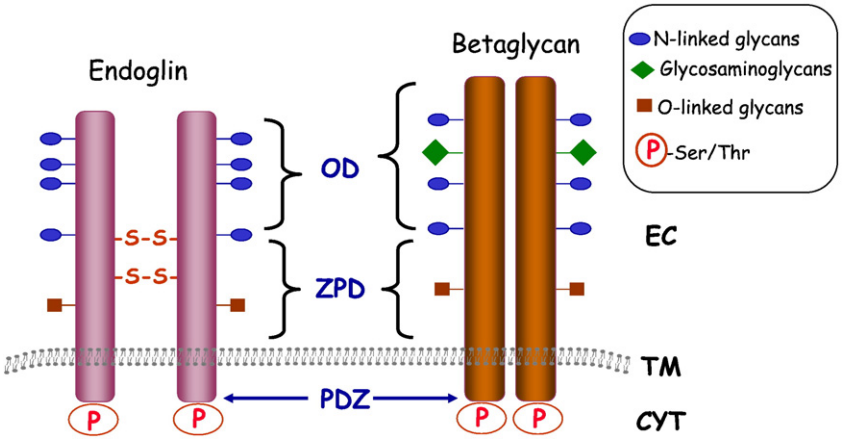

Fig. 2. Structural representation of type III TGF-β receptors. Endoglin and betaglycan are type I membrane proteins with a large extracellular domain that contains a zona pelucida domain (ZPD) of 260 amino acids in the juxtamembrane region and an N-terminal orphan domain (OD) with no known homology. Both endoglin and betaglycan form dimers. While endoglin monomers are disulphide-linked, the betaglycan dimer is sustained by non-covalent interactions. Consensus motifs to attach N-linked glycans, O-linked glycans and glycosaminoglycans to the extracellular domain have been identified. Both endoglin and betaglycan cytoplasmic domains are phosphorylated at Ser/Thr residues and they contain consensus PDZ-binding motifs present at the carboxyl terminus. The cytoplasmic (CYT), transmembrane (TM) and extracellular (EC) domains of the protein are indicated. The scheme is not to scale.

independent endocytosis. However, inhibition of the clathrin-independent, lipid raft pathway, but not that of the clathrin-dependent pathway, decreased TGF-β1 induced Smad2 and p38 phosphorylation, suggesting a specific role for clathrin-independent endocytosis of betaglycan in regulating both Smad-dependent and Smad-independent TGF-β signaling [44].

The cytoplasmic domain of endoglin also interacts with a member of the Tctex1/2 family of cytosolic dynein light chains, Tctex2β, linking endoglin to the microtubule-based transport machinery [45]. Interestingly, Tctex1 is phosphorylated by the type II BMP receptor, BMPRII [46], further supporting a functional linkage between Tctex proteins, endoglin and TGF-β receptor complexes. Together, these studies suggest the endoglin cytoplasmic domain is involved in different protein–protein interactions that modulate endoglin function.

Although endoglin and betaglycan extracellular domains share limited homology, they do share the presence of a zona pellucida (ZP) consensus motif in the juxtamembrane region (Fig. 2). Comparative analysis of their primary structure reveals that endoglin and betaglycan belong to the ZP family of extracellular proteins that share a ZP domain consisting of 260 amino acids with 8 conserved cysteine residues close to the transmembrane region [47,48]. This consensus ZP domain is divided in two ZP subdomains (ZP-N and ZP-C) that are potentially involved in receptor oligomerization [48,49]. Human endoglin, but not betaglycan, contains an RGD tripeptide located in the ZP domain of the extracellular region [28]. Although this RGD motif led to the hypothesis that endoglin binds to integrins or other RGD-binding receptors [50,51], the presence of the RGD sequence in human endoglin may be a recent adaptation, as this motif is absent from mouse [52], porcine [53], rat and canine [49] endoglin proteins. The primary structure of endoglin suggests that there are four N-linked glycosylation sites in the N-terminal domain and a probable O-glycan domain rich in Ser and Thr residues proximal to the membrane-spanning domain [28] (Fig. 2). Experimental studies using specific glycosidases confirmed that endoglin is glycosylated [54]. This post-translational modification occurs in multiple stages when endoglin is overexpressed in COS cells, giving rise to partially and fully glycosylated species that are present at the cell surface [55]. Betaglycan is an integral membrane proteoglycan with an average mass of 280–330 kDa, of which 200-kDa corresponds to glycosaminoglycans and 10 kDa to N-linked glycans attached to the extracellular domain of a heterogeneous core polypeptide of 100–120 kDa [56]. Most of the betaglycan proteins contain heparan sulphate and chondroitin sulphate in varying proportions [57]. Interestingly, the Ser^{533}Ala and Ser^{544}Ala mutations in murine betaglycan yield a betaglycan devoid of glycosaminoglycan chains [58].

The three-dimensional structure of the extracellular region of endoglin was determined at a resolution of 25 Å using single-particle electron microscopy [49]. The molecular reconstruction suggests that endoglin exists as a dome comprised of anti-parallel orientated monomers enclosing a cavity at one end (Fig. 3). Using these data, the high-resolution structure of endoglin suggests that each endoglin subunit comprises three well defined domains, the two ZP regions (ZP-N and ZP-C) and one orphan domain, which are organized into an open U-shaped monomer [49] (Fig. 3). Recently, small angle X-ray scattering experiments of soluble endoglin revealed a more elongated conformation for the dimer, suggesting that the protein may undergo conformational adaptation upon ligand binding [59]. Unfortunately, no data are available regarding the three-dimensional structure of betaglycan.

2.2. Regulation of TGF-β ligand access to receptors

Endoglin is predominantly expressed in vascular endothelial cells, while betaglycan is more ubiquitously distributed. Betaglycan, is a major TGF-β binding molecule at the cell surface and it binds multiple members of the TGF-β family with high affinity, including TGF-β1, TGF-β2, TGF-β3, Activin-A, BMP-2, BMP-4, BMP-7, and GDF-5 [56,60,61]. Betaglycan interacts with the type II TGF-β receptor and it plays a role in presenting the ligand to TβRII, either inhibiting or enhancing signaling through mechanisms that are yet to be defined [31,62–65]. TGF-β binds to the N-terminal endoglin-related region of betaglycan, and mutational analyses suggest that the remaining

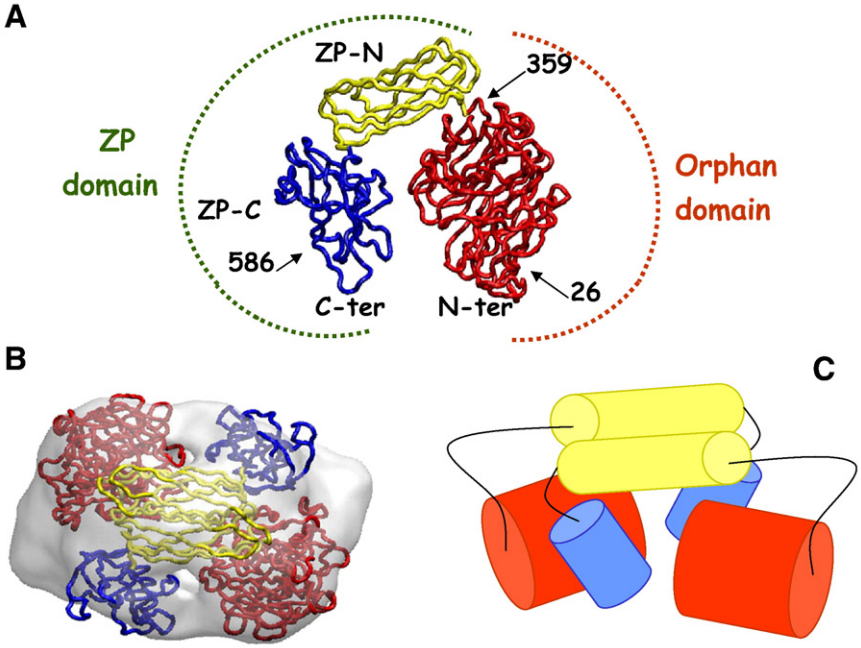

Fig. 3. Three-dimensional model of endoglin. (A) The atomic model predicted in silico shows the presence of three different subdomains in red, yellow and blue. The orphan domain contains amino acid residues Glu^{26}-Ile^{359} (red), whereas the ZP domain encompasses the fragment Gln^{360}-Gly^{586}. The ZP-N and ZP-C subdomains are colored in yellow and blue, respectively. The amino acid numbers corresponding to the border regions of the globular domains are indicated. (B) The electron microscopy density map of soluble endoglin (grey volume) allows an atomic model of dimeric endoglin to be fitted. (C) Cartoon model for the domain organization of endoglin within the dimer (domain colors as in A). Adapted from Llorca et al. [49].

carboxy-terminal half of the protein is not required for betaglycan-dependent enhancement of TGF-β binding to TβRII. However, protein anchoring to the membrane is required [66]. The betaglycan ectodomain is endowed with two bona fide and independent ligand-binding domains that can perform specific functions as coreceptors of distinct members of the TGF-β superfamily [67]. Early studies on the primary structure of betaglycan revealed a strong homology with endoglin in the cytoplasmic and transmembrane domains [31], suggesting that endoglin was also a component of the TGF-β receptor system. In fact, it was established that endoglin binds TGF-β1 and TGF-β3 but not TGF-β2 [68], distinguishing it from betaglycan. These studies provided the basis to examine endoglin's functions as a component of the TGF-β receptor system. Because endoglin differs from betaglycan in its TGF-β ligand-binding profile [68], it was not surprising to learn that both functional differences and similarities exist between these two proteins. An exclusive role for the extracellular TGF-β ligand-binding domain was evident when cytoplasmic domains of endoglin and betaglycan were switched, which had no effect on endoglin ligand binding [69]. However, in contrast to betaglycan, the binding of TGF-β1, activin-A, BMP-7 and BMP-2 to endoglin requires the presence of the corresponding type II receptor [69,70], suggesting that endoglin participates in the binding of these ligands only within the TGF-β receptor complex. This behavior would explain why only a small fraction of the total cell surface endoglin binds TGF-β1 [68]. However, there are other data supporting the independent ligand-binding capacity of the extracellular domain of endoglin and indeed BMP-9 appear to bind membrane anchored endoglin in the absence of signaling receptors [71], while a soluble form of endoglin has been shown to sequester TGF-β [72].

2.3. Endoglin and betaglycan in the TGF-β receptor complex

Endoglin bound to ligand can be isolated as a complex with type I and type II TGF-β receptors [68]. The type I receptors include ALK1, the BMP receptors ALK2, ALK3 and ALK6, as well as ALK5 and the activin receptors, ALK2 and ALK4. In addition, various type I receptors can interact with the type II TGF-β (TβRII), activin (ActRII) or BMP (BMPRII) receptors [1,4,5]. *In vitro* co-immunoprecipitation studies of endoglin with type I and type II receptors indicate that endoglin interacts with TGF-β1, TGF-β3, activin-A, BMP-7 and BMP-2 ligands [53,68–70]. These results are supported by functional experiments, at least in the case of BMP-7, demonstrating that endoglin overexpression enhances the BMP-7/Smad1/Smad5 pathway, while inhibiting the TGF-β1-induced ALK5/Smad3 signaling [73,74]. As discussed above, these interactions require co-expression of the respective ligand-binding kinase receptors [69,70]. Accordingly, endoglin binds TGF-β1 and TGF-β3 by associating with TβRII, and it interacts with activin-A and BMP-7 in association with the ActRII receptors ActRIIA and ActRIIB. Also, endoglin binds BMP-2 by interacting with the BMP ligand-binding receptors ALK3 and ALK6 [70]. Interestingly, BMP-9 binds with high affinity to endoglin in the absence of TGF-β signaling receptors [71]. Accordingly, overexpression of endoglin increases the BMP-9 response, whereas it is completely abolished by silencing both BMPRII and ActRIIA expression [75]. These studies indicate that endoglin binds to most ligand-type I/II receptor complexes, potentially reflecting a role for endoglin in the dynamics of type I/II receptor interactions and their downstream signaling pathways.

Studies of the interaction of endoglin with ALK5 and TβRII indicate that both ALK5 and TβRII interact with the extracellular and cytoplasmic domains of endoglin. However, ALK5 only interacts with the endoglin cytoplasmic domain when the kinase domain is inactive. Upon association, ALK5 and TβRII phosphorylate the endoglin cytoplasmic domain and then, ALK5 but not TβRII, dissociates from the complex [36]. Thus, phosphorylated endoglin, as a consequence of the Ser/Thr kinase activation, appears to play a regulatory role in the TGF-β receptor complex. Together, these data suggest that the extracellular and cytoplasmic domains of endoglin and betaglycan play distinct roles in receptor signaling downstream of ligand binding and receptor activation. Table 1 summarizes the proteins identified to date that interact with the extracellular and cytoplasmic domains of endoglin and betaglycan, including TGF-β superfamily members, type I and type II TGF-β receptors, and some cytoplasmic proteins.

Table 1
Endoglin and betaglycan interacting proteins*.

R-III domain	TGF-β superfamily members	R-I	R-II	Cytoplasmic proteins
Endoglin-ECD	TGF-β1, TGF-β3, activin-A, BMP-2, BMP-7, BMP-9, BMP-10	ALK1, ALK2, ALK3, ALK5, ALK6	TβRII, ActRII, BMPRII	
Betaglycan-ECD	TGF-β1, TGF-β2, TGF-β3, activin-A, inhibin-A, BMP2, BMP4, BMP7, GDF-5			
Endoglin-CD		ALK1, ALK5	TβRII	Zyxin, ZRP-1/TRIP-6, β-Arrestin2, Tctext2β
Betaglycan-CD			TβRII	GIPC, β-arrestin2

*Proteins interacting with the extracellular (ECD) and cytoplasmic (CD) domains of endoglin and betaglycan are indicated. This is a summary of different reports describing protein–protein interaction, co-immunoprecipitation, cross-linking, phosphorylation, and functional experiments, as indicated in the text.

2.4. Endoglin and betaglycan in TGF-β-dependent cell responses

Endoglin modulates TGF-β-dependent responses in several cell types. In human monocytic U-937 cells, responses to TGF-β1, but not to TGF-β2, are abrogated in the presence of endoglin [76]. Moreover, in a variety of cell types, including myoblasts vascular endothelial cells, monocytes and fibroblasts, endoglin opposes to TGF-β1-dependent responses such as the inhibition of cellular proliferation, the induction of apoptosis, cellular adhesion or homotypic cell aggregation, and the increased expression of extracellular matrix components such as plasminogen activator inhibitor-1 (PAI-1), collagen and fibronectin [69,76–81], as well as the secreted extracellular matrix-associated protein lumican [82]. Interestingly, no changes in total ligand binding were observed in endoglin cell transfectants [76], suggesting that endoglin's effects occur downstream of ligand binding. Like TGF-β receptor signaling in general, the regulatory effects of endoglin are likely to be cell type specific, subject to conditions that include the specific type I and type II TGF-β receptors present and the relative levels of endoglin.

Although TGF-β is a potent inhibitor of cell proliferation, endoglin expression counteracts this inhibitory effect in several cell types, including endothelial cells [76,80]. The positive correlation between endoglin expression and endothelial cell proliferation was confirmed in several experimental models. Indeed, endoglin is markedly upregulated in proliferating endothelium of tissues undergoing angiogenesis [83–87] and *in vitro* inhibition of its expression in endothelial cells impairs this process [80]. In addition, suppression of endoglin not only increases the TGF-β1-dependent inhibition of endothelial cell proliferation but also, endothelial cell apoptosis induced by hypoxia and TGF-β1 [81]. Furthermore, in mice bearing targeted endoglin (*Eng*) alleles, studies of $Eng^{-/-}$ and $Eng^{+/-}$ embryonic endothelial cells indicate that endoglin promotes endothelial cell proliferation via a TGF-β/ALK1 pathway [88].

How endoglin regulates these TGF-β-dependent responses is unknown. A potential mechanism of action is via endoglin-dependent effects on TGF-β receptor phosphorylation. TβRII is thought to be a

constitutively active receptor that activates the type I receptor via phosphorylation upon ligand-induced association. Betaglycan functions by selectively binding the phosphorylated TβRII through its cytoplasmic domain to promote TGF-β2 signaling [39]. Interestingly, endoglin association with TβRII alters the phosphorylation state of TβRII and provokes the loss of ALK5 from the complex [36]. Either of these events could explain the inhibitory effects of endoglin on ALK5 signaling, which requires phosphorylation by the TβRII kinase after its association with TGF-β1. Additionally, studies in primary human umbilical vein endothelial cells suggest that endoglin phosphorylation opposes the activated ALK1-dependent inhibition of cell adhesion [37]. These results suggest that endoglin might affect TGF-β responses by interacting with the signaling receptors through its extracellular and cytoplasmic domains.

As endoglin directly interacts with a variety of TGF-β type I receptors [36,70,89], endoglin may exert additive or opposing effects on TGF-β receptor signaling. Thus, although endoglin inhibits TGF-β/ALK5/Smad3 cellular responses [69,73,88–90], it enhances ALK5/Smad2 signaling [36,91,92]. In addition, endoglin may be required for TGF-β1/ALK1 signaling in some cell types, especially endothelial cells. This balance between ALK5 and ALK1 may play a role in the regulation of cell growth and differentiation in cells that express endoglin, as well as ALK1 and ALK5 [88]. The mechanism by which endoglin potentiates TGF-β/ALK1 signaling appears to involve direct association of ALK1 with the cytoplasmic and extracellular domains of endoglin, the extracellular domain mediating the enhancement of ALK1 signaling [89]. These studies suggest that the functional association of endoglin with ALK1 is critical for endothelial cell responses to TGF-β. Such a conclusion would agree with the fact that ALK1 and endoglin null mice have similar phenotypes [93–97], and that the pathogenic mutation of either human endoglin (*ENG*) or ALK1 (*ACVRL1*) genes results in hereditary hemorrhagic telangiectasia (HHT) [98,99]. HHT, also termed Osler-Weber-Rendu syndrome, is a dominant vascular dysplasia associated with frequent epistaxis, gastrointestinal hemorrhages, cutaneous telangiectasis and arteriovenous malformations in lung, liver and brain (OMIM #187300 and #600376) [100–102]. Together, these data provide additional support for endoglin acting with ALK1 in the same TGF-β pathway. At variance with this interpretation, it was reported that ALK1/Smad1/5/8 activation is enhanced in endoglin null endothelial cells, suggesting that endoglin is not required for TGF-β-dependent activation of ALK1 [103].

Endoglin stabilizes the Smad2 protein, potentially via a reduction in the levels of the Smad ubiquitination response factor 2, Smurf2 [92]. Thus, in the presence of endoglin Smad2 protein levels increase leading to TGF-β receptor-dependent induction of eNOS mRNA expression and enhanced Smad-dependent signaling. Through the endoglin-dependent regulation of eNOS [104,105], changes in nitric oxide levels alter COX-2 expression and thus underlying the role of endoglin in vascular homeostasis [106].

A schematic model to summarize the modulatory role of endoglin in the TGF-β signaling pathways is depicted in Fig. 4. Endoglin physically interacts with ALK1 and ALK5, functionally modulating their signaling and leading to the potentiation of Smad1 and Smad2, as well as the inhibition of Smad3, which regulate the expression of the *Id1*, *eNOS*, and *PAI-1* genes, respectively. Studies of the downstream genes affected by endoglin expression have been carried out by gene expression fingerprinting of endoglin deficient human endothelial cells from HHT patients. These studies identified hundreds of genes that were downregulated or upregulated, including genes involved in angiogenesis, cytoskeleton organization, cell guidance, intercellular connections, cell migration and proliferation, or nitric oxide synthesis [107,108].

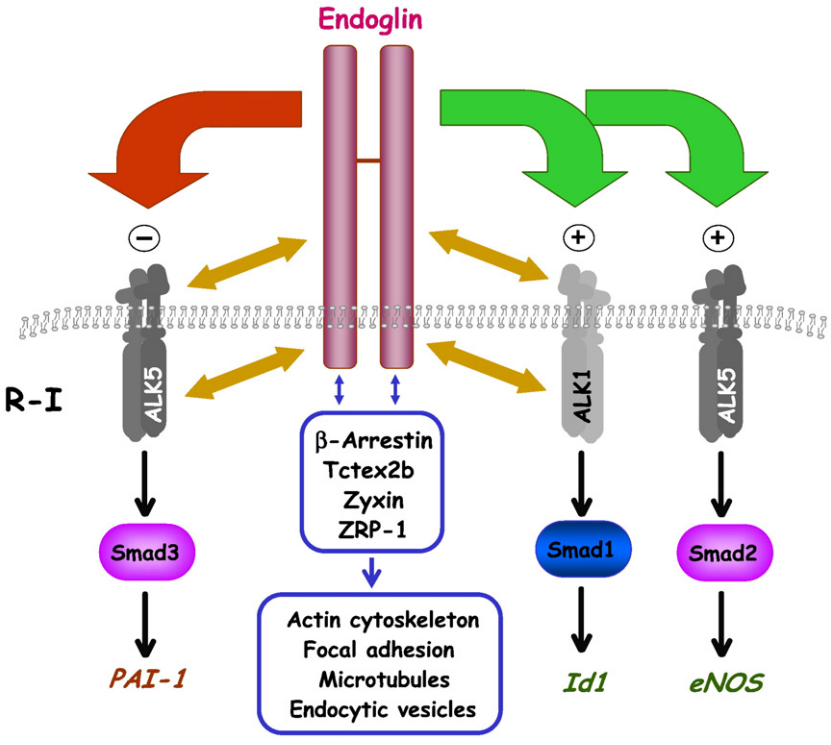

Fig. 4. Hypothetical model for downstream endoglin signaling. The endoglin extracellular and cytoplasmic domains interact with ALK1 [89] and ALK5 [36], as indicated with brown arrows. Endoglin modulates TGF-β signaling by potentiating ALK1/Smad1 and ALK5/Smad2 (green arrows), and inhibiting the ALK5/Smad3 (red arrow) pathways that lead to the regulation of *Id1* [88,89], *eNOS* [92,104] and *PAI-1* genes [69,36], respectively. The involvement of TβRII and TGF-β has been omitted for simplification. The endoglin cytoplasmic domain interacts with the β-arrestin, Tctex2b, zyxin, and ZRP-1 proteins [37,41,43,45]. These cytosolic interactions probably mediate downstream functions, including F-actin dynamics, focal adhesion composition, and protein transport via endocytic vesicles. As described in the text, some of these endoglin functions are also shared by betaglycan. Adapted from Bernabeu et al. [74,89].

3. Endoglin in cancer

In contrast to betaglycan, which is the most abundantly expressed TGF-β receptor, endoglin is primarily expressed in proliferating vascular endothelial and smooth muscle cells, and its expression increases during tumor angiogenesis and inflammation. Such properties have made endoglin a reliable marker of tumor angiogenesis and a prime target for antiangiogenic therapy [109–111]. Besides the proangiogenic role of endoglin, there is also evidence of its direct involvement in cancer through its effects on tumor cells themselves. Thus, recent studies on human cancer cell lines and experimental models of carcinogenesis have linked endoglin to malignant progression, suggesting an invasion suppressor role for this coreceptor in carcinomas. In addition, endoglin has been associated with a hereditary cancer disorder: juvenile polyposis syndrome. In this section, we will address the role of endoglin in each of these settings.

3.1. Endoglin in tumor angiogenesis

3.1.1. Endoglin staining as a marker of tumor neoangiogenesis

While endoglin is expressed at low levels in resting endothelial cells, it is more strongly expressed in vascular endothelial cells at sites of active angiogenesis during embryogenesis [112], in inflamed tissues of healing wounds [113], upon arterial injury [114,115], in infected tissues, psoriatic skin, inflamed synovial arthritis and in tumor vessels [84,112,116–120]. Endoglin is also overexpressed after ischemia and reperfusion in the kidney [121], hindlimbs [122] and heart [123]. The mechanisms involved in this enhancement of endoglin levels are probably multifactorial, hypoxia being one of the most suitable candidates. In fact, many of the pathophysiological settings where endoglin is upregulated involve hypoxic microenvironments, as is the case of tumor angiogenesis. In favor of this view, a hypoxia-responsive element (HRE) has been characterized downstream of the main transcription start site in the endoglin gene and under hypoxic conditions, the HIF-1 complex binds this functional HRE upregulating endoglin promoter activity [124]. Interestingly, TGF-β potently stimulates endoglin expression via Smads [76,125,126] and in synergy with the hypoxia pathway [124]. This transcriptional synergism appears to be mediated by a multi-protein complex that includes HIF-1α/HIF-1β, Smad3/Smad4 and Sp1, bound to their cognate DNA binding motifs.

Elevated expression of endoglin correlates with the proliferation of tumor endothelial cells [116] and it seems to be a potent marker of solid tumors vasculature in the mammary gland [109], prostate [127], cervix [128], colon and rectum [129,130], lung [131], head and neck [132], kidney [133], esophagus [134] and uterus [135]. This has been also observed in hematopoietic tumors such as multiple myeloma [136] and in hairy cell leukemia [137]. Furthermore, an active role for endoglin in the angiogenic process is supported by experiments in animal models. Thus, reduced angiogenic responses and tumor angiogenesis have been demonstrated in endoglin haploinsufficient mice [122,138].

3.1.2. Prognostic value of endoglin staining in microvessels

It has been reported that microvessel density (MVD) is an independent prognostic indicator of the outcome for several human tumors, as increased MVD was associated with shorter overall and relapse-free survival rates [139–141]. However, these findings have not always been confirmed [142,143] and some such discrepancies may be explained by the use of different antibodies against endothelial markers to determine MVD, even though not all the markers are associated with tumor neoangiogenesis. For instance, while antibodies against the von Willebrand factor (vWF) stain endothelial cells in large blood vessels, they fail to stain endothelial cells in some microvessels and thus the use of these antibodies would underestimate microvascular density in tumors. As previously described, while endoglin is expressed at low levels in endothelial cells of quiescent vasculature, it is overexpressed during angiogenesis. Thus, anti-endoglin antibodies should show greater specificity for tumor microvasculature than other endothelial markers [84,131,144–146]. Indeed, MVD measured with antibodies directed against endoglin has been compared with the MVD measured with antibodies against other endothelial markers such as CD34, CD31 or vWF. In breast carcinoma, the MVD assessed with an anti-endoglin antibody, but not with anti-CD34 was correlated with the overall and disease-free survival, and it was an independent prognostic factor in a multivariate analysis [147]. In non-small cell lung cancer patients, the MVD obtained using an anti-endoglin antibody was negatively correlated with survival [131]. Moreover, the MVD obtained in colorectal mucosa with monoclonal anti-endoglin antibodies (mAbs) was higher in the carcinoma than high-grade dysplasia, and it was also higher in high-grade dysplasia than in low-grade dysplasia, suggesting that endoglin-associated MVD predicted the risk of progressing from dysplasia to carcinoma [130]. In prostate cancer, the MVD score obtained with anti-endoglin antibodies was associated with the Gleason score, metastasis, tumor stage, tumor cell proliferation index and survival, although these associations were not observed when antibodies against vWF were used [148]. In patients with colorectal cancer, the MVD was measured using antibodies against CD34 and endoglin, and Kaplan-Meier survival analysis revealed that only MVD values obtained with anti-endoglin antibodies were significantly correlated with survival. Furthermore, patients with a higher MVD showed the worst prognosis [149], as later confirmed in a study where endoglin was a more specific and sensitive marker for tumor angiogenesis in colon carcinoma than the commonly used pan-endothelial markers, since it stained more proliferating vessels [150]. Endoglin staining was also significant in terms of prognosis due to its positive correlation with angiolymphatic invasion and metastases to the lymph nodes and liver [150]. In patients with early oral cancer, strong expression of endoglin was significantly correlated with positive nodal metastasis and with a lower survival rate [151]. In patients with Barrett's esophagus, a pre-tumor dysplasia, endoglin staining gave a significantly higher MVD in Barrett's esophagus with high-grade dysplasia than in Barrett's esophagus low-grade dysplasia, whereas the MVD obtained by CD31 staining showed no such significant differences [134]. Moreover, in patients with esophageal or prostatic adenocarcinoma, endoglin but not CD31 staining had prognostic value and it was positively correlated with the presence of angiolymphatic invasion, lymphatic nodes metastases, tumor stage and survival [134,152]. The expression of endoglin is also a useful predictive prognostic factor in early tongue cancer, as stronger expression of endoglin in the tumor bed implicates a more aggressive potential for T1 and T2 tongue cancers [153]. Similar results were obtained in squamous cell carcinomas of the hypopharynx [154]. In head and neck squamous carcinoma, the disease-free and overall survival was significantly shorter in patients with a high MVD when assessed by endoglin staining. By contrast, MVD assessed by CD34 staining was not associated with survival in these same patients. Similarly, in the subset of lymph node negative patients, higher endoglin-assessed MVD values were significantly associated with both disease-free and overall survival. A multivariate analysis showed that a high MVD when assessed by endoglin staining was the only independent marker of tumor recurrence or death [155]. In patients with breast carcinoma, and unlike staining with anti-CD31 antibody on paraffin sections, staining with an anti-endoglin antibody had prognostic significance in terms of overall survival [156]. In stage IB non-bulky (under 5 cm) cervical cancer subjects, MVD assessed with anti-endoglin antibodies was more sensitive than MVD using factor VIII antibodies to stain capillaries in neoplastic tissues, and better predicted lymph node metastases

[157]. Furthermore, in pretreatment biopsies from breast cancer patients, weak immunohistochemical endoglin expression predicts a favorable clinical response to chemotherapy [158].

The better prognostic value of endoglin when compared with CD34 staining of microvessels has not been confirmed for lymphopoietic tumors. However, in patients with multiple myeloma, the staining with anti-endoglin mAb was significantly more sensitive than the staining with anti-CD34 mAb in visualizing blood vessels both in control and multiple myeloma samples, and the MVD was significantly higher in multiple myeloma than in controls with both anti-CD34 and anti-endoglin mAbs. Moreover, patients with low $CD34^+$ MVD survived longer than patients with a higher $CD34^+$ MVD, whereas there was no difference in the survival of patients with low and high $endoglin^+$ MVD. Multivariate analysis confirmed the independent significant association between $CD34^+$ MVD and survival, unlike $endoglin^+$ MVD [136].

All these studies suggest that in solid tumors, MDV measurements using anti-endoglin antibodies are more sensitive and have better prognostic value than those using antibodies against other endothelial markers such as CD34, CD31 or vWF.

3.1.3. Soluble endoglin as a prognostic marker

A number of laboratories have reported increased levels of a soluble, circulating form of endoglin (Sol-Eng) in serum, plasma or other fluids from cancer patients as a marker of poor prognosis (reviewed by Fonsatti et al. [85]). The serum level of Sol-Eng was significantly elevated in patients with metastatic solid malignancies, mainly breast and colorectal carcinomas, when compared with healthy individuals or patients with no metastasis [129,159] (see Table 2). These high levels of serum Sol-Eng decreased in patients receiving chemotherapy, which restricts the utility of Sol-Eng as a marker of metastasis and tumor recurrence to the long-term follow-up of cancer survivors who are not receiving chemotherapy [129]. Recently, Sol-Eng was shown to have independent prognostic value in serum as an indicator of prostate cancer metastasis to the pelvic lymph nodes and of biochemical recurrence after radical prostatectomy [160,161]. Also, soluble endoglin is a useful urinary marker for the diagnosis of prostate cancer. Indeed, Sol-Eng increased significantly in urine collected after digital rectal examination (which enriches urine with prostatic secretions) from patients with prostate cancer when compared to that from healthy men [162]. In the same study, increased levels of serum Sol-Eng were correlated with an advanced stage of tumor progression. Elevated levels of Sol-Eng have also been found in myeloid hematopoietic malignancies, such as acute myeloid leukemia and chronic myeloproliferative disorders [163].

The Sol-Eng form is released after proteolytic shedding of the extracellular domain of membrane-associated endoglin, although the enzymes involved in the cleavage of the endoglin ectodomain remain unknown. The origin of the soluble endoglin detected in cancer patients with metastasis and/or an advanced stage of disease is somewhat intriguing. The preferential expression of endoglin in endothelial cells of the tumor vasculature versus neoplastic cells, has led to the suggestion that Sol-Eng originates from the neovasculature [85,129,159]. However, our recent data on mouse skin carcinogenesis (see below) suggest that tumor cells may also contribute to Sol-Eng [164]. In addition, Sol-Eng is involved in the pathogenesis of severe vascular diseases, such as the pregnancy-specific hypertensive syndrome known as pre-eclampsia [72,165,166] and systemic sclerosis [167], preventing the binding of TGF-β to its type I and type II signaling receptors and impairing downstream signaling activity. Moreover, soluble recombinant endoglin has been shown to modulate ALK1- and ALK5-dependent signaling [59,89]. Whether Sol-Eng influences tumor development through a similar molecular mechanism remains to be elucidated.

3.1.4. Diagnostic value of endoglin in imaging techniques

As endoglin is preferentially expressed in the active, angiogenic endothelium of tumors, the expression of endoglin by the tumor endothelium may have diagnostic value if it can be imaged *in vivo*. The localization of endoglin to angiogenic tissue could potentially be used to select patients who would benefit from antiangiogenic therapies and to measure the response to such a therapy. Optimization of imaging an endothelial target requires the background blood pool and ligand extravasation to be minimized. This can be achieved by using small doses of ligand to ensure that the high-affinity receptors on the endothelial cells are not saturated and so that images are only

Table 2
Alterations to TGF-β coreceptors found in human cancer.

TGF-β coreceptor	Tumor type	Alteration type	Correlation with	Observations	References
Endoglin	JP	Germline mutation	Early onset of disease	Low frequency	[205]
	Prostate carcinoma	Downregulation	Metastasis	Cell lines	[213]
		High levels in plasma (Sol-Eng)	Lymph node metastasis	–	[160–161]
		High levels in urine (Sol-Eng)	–	Post-DRE	[162]
	Esophageal (SCC)	Downregulation	Tumor progression	Primary tumors and cell lines	[214]
	Breast carcinoma variants	Overexpression	Metastasis	MDA-MB-231 cell	[222]
		High levels in serum/plasma (Sol-Eng)	Metastasis	Sol-Eng levels decrease in patients receiving chemotherapy	[129,159]
	Colorectal carcinoma	High levels in serum (Sol-Eng)	Metastasis	Sol-Eng levels decrease in patients receiving chemotherapy	[129]
	AML	High levels in serum (Sol-Eng)	–	–	[163]
	CML	High levels in serum (Sol-Eng)	–	–	[163]
Betaglycan	Non-Hodgkin's lymphoma	Overexpression	Tumor progression	QRT-PCR (no protein analyzed)	[241]
	BCLL	Overexpression	–	Microarray hybridization, RT-PCR	[242,243]
	Neuroblastoma	Downregulation	Tumor progression	–	[244]
	Ovarian granulosa	Downregulation	–	Primary tumors and cell lines	[247]
	Ovarian carcinoma	Downregulation	Tumor progression	Epigenetic silencing	[246]
	Endometrial carcinoma	Downregulation	–	–	[248]
	Prostate carcinoma	Downregulation	Tumor progression	LOH	[250]
	Breast carcinoma	Downregulation	Invasion and metastasis	LOH	[253]
	Renal cell carcinoma	Downregulation	Early stages of carcinogenesis	Subsequent loss of TβRII correlates with metastasis	[251]
	NSCLC	Downregulation	Tumor progression	LOH	[252]
	Pancreatic carcinoma	Downregulation	Tumor grade	–	[254,255]

JP, juvenile polyposis; DRE, digital rectal examination; SCC, squamous cell carcinoma; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; BCLL, B-cell chronic lymphocytic leukemia; LOH, loss-of-heterozygosity; NSCLC, non-small cell lung cancer.

obtained for areas with high endoglin density. Furthermore, images must be obtained shortly after injection of the labeled ligand, before extravasation into the extracellular space has occurred. When fresh nephrectomy specimens from patients with renal cell carcinoma were perfused with technetium-99m-labeled anti-endoglin mAbs, the radiographic hot spots corresponded to tumors previously identified by preoperative magnetic resonance in all seven cases studied. However, in one case technetium-99m-imaging identified a malignant mass that was missed on preoperative MRI [168]. These findings suggest that $^{99}Tc^{m}$-labeled anti-endoglin mAb could be useful to detect metastatic tumors that conventional imaging modalities are unable to visualize. Similarly, ^{125}I-labeled anti-endoglin mAb was injected into two dogs with spontaneous mammary carcinoma and in images obtained 8 h later, the tumors had rapidly and efficiently taken up radioactivity, with a high signal-to-noise ratio. A diagnosis of ductal adenocarcinoma was verified by surgical excision 10 days later and the dogs showed no systemic side effects during the 3-month follow-up period [117]. The ^{111}In-labeled rat anti-mouse endoglin mAb injected intravenously into tumor-bearing mice led to accumulation of radioactivity in the tumors. Autoradiography and immunohistochemistry showed the anti-endoglin antibody accumulated at the tumor edge where the highest density of blood vessels was found, whereas the tumor's center was heterogeneously stained [169]. Conjugated microbubbles bearing anti-endoglin mAbs have also been studied and these conjugates were injected into mice bearing orthotopic human pancreatic tumors, which were then visualized by ultrasonography due to their binding to the antibody. The excised tumors were examined using fluorescent immunohistochemistry after pancreatectomy and found to correlate quantitatively with preoperative ultrasound findings [170]. These studies suggest that endoglin is a good target for tumor imaging and that it may be useful in other angiogenic diseases. It may be particularly valuable to monitor the response to antiangiogenic therapies and in situations where conventional imaging techniques do not give clear results (e.g., to discriminate between tumor recurrence and postoperative/radiotherapeutic changes in tissues).

3.1.5. Endoglin as a target for antiangiogenic therapy

Antiangiogenic therapies in tumors theoretically adopt two different approaches: *vascular targeting* is a therapy directed at preexisting blood vessels, whereas *antiangiogenic therapy* aims to prevent the development of new vessels in the tumor. Vascular targeting of agents to treat cancer is designed to cause a rapid and selective destruction of tumor blood vessels. Unlike antiangiogenic drugs that inhibit the formation of new vessels, vascular targeting drugs occlude the pre-existing blood vessels of tumors to cause tumor cell death by ischemia and extensive hemorrhagic necrosis. Such approaches have several potential advantages over conventional chemotherapeutic agents [171,172]. However, a major obstacle in vascular targeting is the difficulty in finding the appropriate target that allows us to selectively destroy tumor vasculature without causing major damage to the normal vasculature of an organ. If endoglin is specifically overexpressed in tumor vasculature, endoglin-expressing cells can be therapeutically targeted as for antitumoral therapy. However, it has been demonstrated that even though the staining for endoglin was weaker in normal tissues, all tissues express endoglin in microvessels at least. Thus, vascular targeting using endoglin as a target should be carefully re-evaluated as it could also damage normal vessels [173].

Incubation of human endothelial cells with anti-endoglin mAbs *in vitro* significantly inhibits growth, mainly in the presence of TGF-β1 [174]. *In vivo* studies revealed that injecting anti-endoglin mAbs to mice with breast and colon cancer xenografts was associated with significantly smaller tumors and greater survival rates than in controls [175,176]. Interestingly, a synergistic effect was reported between naked anti-endoglin antibodies and conventional chemotherapeutic schedules in a human skin/SCID mouse chimera model [177]. Furthermore, anti-metastatic activity of endoglin specific antibodies was observed in lung and liver from mice injected with murine mammary and colorectal carcinoma cells [178].

Several mechanisms may potentially contribute to the vascular targeting activity of anti-endoglin mAbs. First, the binding of anti-endoglin mAbs to membrane endoglin in proliferating endothelial cells of angiogenic vessels in tumors could block endoglin function, modifying downstream signaling events, suppressing growth and even causing the death of the endothelial cells [179]. It should be noted that cells devoid of endoglin undergo apoptosis in the presence of TGF-β and hypoxia [81]. A second mechanism could involve the interaction of the Fc region of the mAb bound to membrane endoglin and the Fcγ receptors on effector cells, leading to antibody-dependent cell mediated cytotoxicity or Fc-mediated complement activation. Such events would result in complement-dependent cytotoxicity and the subsequent lysis of the target cell. A further possibility is that soluble endoglin/antibody complexes may be recognized by antigen presenting dendritic cells leading to antigen-specific T cell immunity through a cross-presentation pathway. It was recently reported that several anti-endoglin mAbs, termed SN6 series mAbs, could suppress proliferation of human endothelial cells *in vitro* without any accessory cells [174]. However, little is known about the mechanisms of this suppression. These *in vitro* studies show that one mAb member of this series, SN6j, induces apoptosis of human endothelial cells in culture, and this could be a mechanism by which these antibodies suppress the growth of proliferating endothelial cells. It is also possible that T cell immunity may be important for antibody-based antiangiogenic therapy *in vivo*. This hypothesis was conceived on the basis that anti-endoglin mAbs were more effective for tumor suppression in T cell immunocompetent BALB/c mice than in T cell immunodeficient SCID mice [179].

The use of naked anti-endoglin mAbs as a tumor vascular targeting agent appears to be clinically relevant since a multicenter phase I clinical trial using an anti-endoglin mAb (TRC105) was recently approved by the United States Food and Drug Administration (FDA) and it is now in progress in patients with advanced and/or metastatic cancer (http://clinicaltrials.gov/archive/NCT00582985/2008_10_01). Early results from 17 patients with advanced refractory cancer in this clinical trial suggest clinical activity and good tolerability of the TRC105 antibody at doses up to 1 mg/kg every 2 weeks [180].

One alternative to the use of naked anti-endoglin mAbs in vascular targeting therapy is to conjugate the antibody with toxic molecules in order to kill the endothelial cell that binds the antibody. Anti-endoglin mAbs have been conjugated to Auger-electron emitters, which damage DNA after internalization, and they have been injected into mice bearing human breast cancers. The treatment reduced tumor growth in the absence of significant toxicity, weight loss or organ damage [181]. Moreover, when mice bearing human breast cancer xenografts were treated with anti-endoglin mAbs conjugated to deglycosylated ricin A, tumor regression was evident in half of the mice and no progression was seen even after 100 days with no further therapy [175]. Similarly, when anti-endoglin mAbs conjugated to deglycosylated ricin A were administered to SCID mice with a human breast cancer (MCF-7) tumor; the immunotoxin completely inhibited tumor growth in all the treated mice, with no significant side effects [182]. In addition, the non-toxic type II ribosome-inactivating protein ebulin l has been conjugated to anti-endoglin mAbs, producing a complex capable of specifically killing L929 mouse fibroblasts, as well as L6E9 rat myoblasts ectopically expressing endoglin, with an efficacy much higher than ebulin I alone [183]. This conjugate had almost no effect on cells lacking endoglin expression. Similar results were obtained by linking anti-endoglin mAbs to the non-toxic type II ribosome-inactivating protein nigrin b. Immunofluorescence analysis indicated that the immunotoxin accumulates in a perinuclear region whereas the antibody was localized at the cell surface [184]. These

studies suggest that immunotoxins containing non-toxic type II ribosome-inactivating proteins such as ebulin 1 or nigrin b are promising tools for anticancer therapy, especially due to the very low toxicity of these proteins *in vivo* when compared to ricin or other toxins commonly employed as immunotoxins.

Another approach is to conjugate anti-endoglin mAbs into packaging molecules, such as adenovirus or cationic liposomes, to more specifically deliver their contents to activated endothelial cells, causing significant antitumor activity [185,186]. In addition to the standard mouse mAb to endoglin, the generation of novel types of antibodies such as fully synthetic human scFv [187,188] or high-affinity camelid nanobodies [189] against human endoglin opens up new and interesting avenues of applications for antiangiogenesis reagents.

A different therapeutic approach is to sensitize the immune system of the host against endoglin so that angiogenic vessels are recognized as an antigen and targeted by the immune system of the host. Using this approach, a double-attenuated *Salmonella*-based oral vaccine containing a murine endoglin-expressing plasmid was administered to mice bearing a mouse mammary carcinoma [190]. Unvaccinated mice had significantly more lung metastases and their tumors were more disseminated than in mice receiving the vaccine. Furthermore, tumors in vaccinated mice were less angiogenic and the vaccinated mice had a longer overall survival than unvaccinated animals [190]. The immune response against the host endoglin may also be elicited by using xenogeneic endoglin as a vaccine [191]. Thus, immunotherapy with porcine endoglin was effective in inducing both protective and therapeutic antitumor immunity in several mouse tumor models. The immune response was associated with IgG1 and IgG2b autoantibodies against mouse endoglin and the antitumor activity could be reproduced by the adoptive transfer of the purified immunoglobulins. Accordingly, endothelial deposition of immunoglobulins and the inhibition of angiogenesis were observed within the tumors. Interestingly, the antitumor activity and the production of autoantibodies against mouse endoglin could be abrogated by the depletion of $CD4^+$ T lymphocytes [191]. Further refinement of the xenogeneic endoglin protocol includes the combination of recombinant xenogeneic endoglin DNA and protein vaccination [192], as well as the combination of low-dose cisplatin and recombinant xenogeneic endoglin [193], in both cases producing synergistic cooperation with enhanced antiangiogenic and antitumor activities.

Taken together, these findings, suggest that manipulating the immune response against endoglin appears to be an excellent strategy for antitumor therapies.

3.2. Endoglin in tumor cells

3.2.1. Endoglin: a susceptibility gene for juvenile polyposis?

Juvenile polyposis (JP) is an autosomal dominant syndrome characterized by the presence of multiple hamartomatous polyps that usually appear before 20 years of age [194,195]. Polyps develop in the colon and rectum, and less often in the upper gastrointestinal tract. Individuals with JP are at risk of developing malignancies of the gastrointestinal system at the age of 40–50 years old [196,197]. There are several predisposing genes for JP, all encoding proteins involved in TGF-β signaling. Germline mutations of *BMPR1A* and *SMAD4* have been described in JP patients, each accounting for 20% of cases [198–200]. *SMAD4* mutations appear to predispose to massive upper gastrointestinal polyps more than *BMPR1A* mutations [201,202]. Interestingly, some families carrying *SMAD4* mutations develop both JP and HHT [203], an observation that led to the identification of *SMAD4* as the third HHT predisposing locus after *ENG* and *ALK1* [204]. Indeed, *ENG* germline mutations were found in 2 of 14 JP patients who did not have *BMPR1A* and *SMAD4* mutations [205]. The disease had an early onset in both patients with respect to those without *ENG* mutations and no sign of HHT, suggesting *ENG* as a potential novel susceptibility gene for JP. The vast majority of the *ENG* missense mutations in HHT occur between exons 1 and 9, compared with the novel JP-related *ENG* mutations that occur in exons 11 and 12. It remains unclear whether these *ENG* mutations lead to haploinsufficiency, like the cause of HHT [206], or to a gain of function. Nevertheless, while this association of germline *ENG* mutations with the development of JP in early childhood was later confirmed, *ENG* was not thought to be a susceptibility gene for JP [207]. Accordingly, *Eng* heterozygous knockout ($Eng^{+/-}$) mice exhibit features of HHT [93], but gastrointestinal polyps were not evident in these mice, nor in $Bmpr1a^{+/-}$ mice [208]. In contrast, $Smad4^{+/-}$ mice develop gastric polyposis and adenocarcinoma foci [209].

3.2.2. Endoglin in human sporadic cancer

Endoglin expression has been detected in tumor cells of human sarcomas melanomas, carcinomas and leukemias [210,211]. In acute leukemias, endoglin protein is always expressed in the most immature subtypes and not in more differentiated ones [210]. In tumors of the melanocytic lineage, endoglin protein was present to a varying degree in both benign nevi and malignant melanoma, as well as in metastatic melanoma cell lines [212]. A summary of the alterations to endoglin expression found in a variety of human tumors and their potential correlation with tumor progression and metastasis is shown in Table 2. In prostate cancer, endoglin protein levels are reduced in malignant or metastatic cell lines compared to their non-tumorigenic or less malignant counterparts [213]. In prostate primary tumors, endoglin was detected in both prostatic intraepithelial neoplasia and malignant areas by immunohistochemistry [127]. Nevertheless, a recent study of esophageal cancer detected a downregulation of endoglin expression in primary esophageal squamous cell carcinomas (SCC), as well as in SCC cell lines [214]. Both epigenetic promoter methylation and loss of heterozygosity (LOH) appear to contribute to endoglin downregulation in tumor cells. Indeed, the endoglin gene (*ENG*) maps to chromosome 9 at 9q34-qter [215], a region (9q33–34) frequently deleted in esophageal cancer [216,217]. Moreover, overexpression of endoglin in esophageal squamous carcinoma cells reduced invasiveness and tumorigenicity, evidence of a role for endoglin as a tumor suppressor in esophageal cancer [214]. Similar results were obtained in prostate cancer cell lines where reduced endoglin levels enhanced cell migration and invasion [213]. Importantly, these studies point to endoglin as a key regulator of adhesion, motility and invasion in human prostate cells. It is worthy of note that among 4,000 genes evaluated, *ENG* expression alone was downregulated during the detachment of metastatic prostate cancer cells [218]. Apparently, endoglin suppresses prostate cancer cell motility by a TGF-β-dependent mechanism involving activation of the type I TGF-β receptor ALK2 and Smad1 [219]. Furthermore, genistein, a soy isoflavone and potential anti-cancer therapeutic agent, inhibited the invasion of endoglin-deficient prostate cancer cells by activating ALK2/Smad1 signaling [220].

Besides epithelial cells, endoglin has also been found to modulate adhesion and migration in fibroblasts [78], endothelial cells [43] and myoblasts [42]. In endothelial cells, endoglin inhibits cell migration by antagonizing TGF-β-mediated ERK signaling in a manner dependent on its ability to interact with the scaffolding protein β-arrestin2 [43]. In myoblasts, endoglin expression also inhibits TGF-β-induced collagen synthesis by a mechanism involving decreased ERK activation [221]. In addition, endoglin regulates the organization of the actin cytoskeleton and focal adhesion sites by interacting with LIM domain-containing proteins and associated adapter proteins [41,42]. The physiological relevance of these findings is unknown at present, but they reveal the involvement of endoglin in other non-TGF-β related functions.

Surprisingly, a pro-invasive rather than a suppressor role of endoglin was recently reported in metastatic breast cancer cells [222]. High levels of endoglin expression were found in metastatic

breast tumor cell variants derived from the MDA-MB-231 cell line with respect to the parental cells. In breast cancer cells weakly expressing endoglin, ectopic endoglin overexpression enhanced migration/invasiveness and induced the formation of invadopodia [222]. Invadopodia are finger-like actin-rich protrusions that localize matrix-degrading metalloproteinases to cell-substratum contact points and they are related to cancer invasion and metastasis [223]. However, it is unclear whether these effects of endoglin expression in breast cancer cells are TGF-β-dependent or if they involve other processes not strictly dependent on TGF-β signaling.

3.2.3. Endoglin in mouse skin chemical carcinogenesis

Multistage mouse skin chemical carcinogenesis is the most intensively studied *in vivo* model of carcinogenesis used in almost all fields of cancer biology, biochemistry and genetics, to analyze the nature of tumor initiation, promotion and progression [224,225]. This model was developed at the beginning of the past century when it became evident that environmental factors caused sporadic cancer. Since then, it has been utilized to test chemicals that may cause, prevent or cure cancer [226], and more recently, to validate the involvement of genetic events in tumor development through the use of genetically engineered mice [227,228]. The tumor induction protocol is quite simple, versatile and reproducible. It involves treating the dorsal skin of mice with a single dose of a carcinogen (e.g., 7,12-dimethylbenz(*a*)anthracene; DMBA), followed by repeated applications of the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA). This treatment provokes the appearance of benign papillomas, more than 90% of which have a specific oncogenic mutation in the *H-Ras* gene [229]. Early papillomas are small and consist of a series of folded epidermal and/or follicular hyperplasias that protrude from the skin surface [230]. Papillomas progress to malignant squamous cell carcinomas (SCC) that break through the basement membrane and progressively invade the underlying dermis, subcutaneous tissue and muscle. Eventually, these SCCs may result in regional and distant metastasis. SCCs are characterized by a disorderly proliferation of keratinocytes with variable degrees of differentiation, and they can be classified into three different groups according to the degree of squamous differentiation: well differentiated (grade I); moderately differentiated (grade II); and poorly differentiated (grade III/IV). Some pathologists include spindle cell carcinomas (SpCC) in the group of poorly differentiated SCCs [230]. SpCC are highly anaplastic tumors predominantly formed by elongated or spindle-shaped cells that are considered the latest event in mouse skin carcinogenesis. The transition from SCC to SpCC correlates with an increase in the ratio of oncogenic versus normal *H-Ras* (reviewed by Akhurst and Balmain [231]), and with the loss or misregulation of the *Ink4* locus encoding the cell cycle regulators p16INK4a, p15INK4b and p19ARF [232,233].

The analysis of endoglin expression in mouse skin tumors and cell lines revealed that the shedding of membrane endoglin, permitting the secretion of a Sol-Eng form, is a late event of carcinogenesis associated with progression from SCCs to SpCCs [164]. Immunohistochemical analysis demonstrated the scattering of a soluble form of endoglin throughout the stroma and inside the blood vessels of poorly differentiated SCCs, while a truncated form of endoglin of about 65 kDa was detected in these tumors and in the conditioned medium of spindle carcinoma cell lines by Western blotting. These studies showed that the shorter endoglin isoform (S-endoglin) is neither expressed in normal skin nor in skin tumors, and that it is refractory to

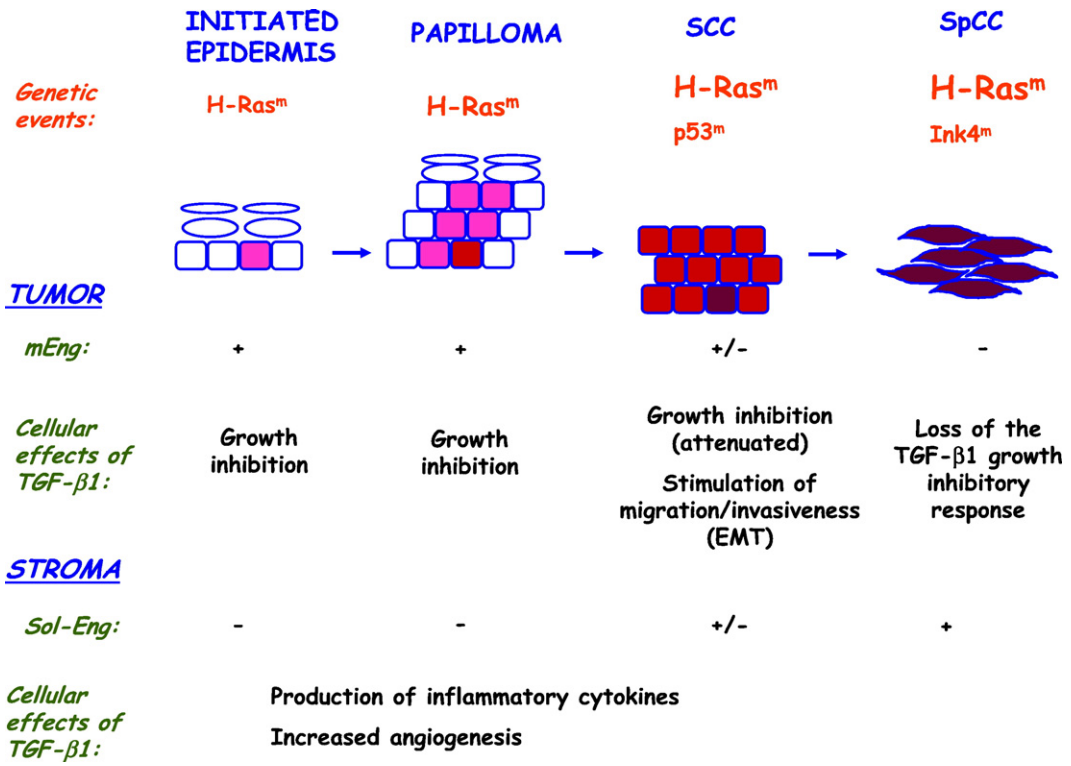

Fig. 5. Schematic representation of tumor development during mouse skin chemical carcinogenesis. The keratinocyte in the epidermis colored in pink represents a cell with H-Ras mutation initiated by 7,12-dimethylbenz(a)anthracene (DMBA). Mutant H-Ras gene dosage increases during tumor progression (indicated by font size). Additional genetic alterations that increase tumor growth and malignancy (indicated by the increasing intensity of the cell color) are associated with different stages of tumor progression. The key cellular effects of TGF-β1 on the tumor and the stroma are indicated. TGF-β1 exerts contradictory effects on proliferation depending on the cell target. It directly inhibits keratinocyte cell growth by activating ALK5/Smad2,3 in the epidermis and thus, TGF-β1 behaves as a tumor suppressor at early stages of carcinogenesis. By contrast, TGF-β1 stimulates keratinocyte cell growth indirectly by mediating the production of inflammatory cytokines in the stroma, and thus, it also exhibits a tumor promoting effect even at early stages of carcinogenesis. The relative expression of membrane (mEng) and soluble (Sol-Eng) endoglin is also indicated. Sol-Eng accumulates in the stroma and vessels at late stages of progression. The effects of Sol-Eng on tumor angiogenesis and stromal cells are unknown. The mEng behaves as a suppressor of malignancy at late stages of carcinogenesis and its shedding potentiates TGF-β1 stimulation of cell migration/invasiveness and EMT.

shedding, highlighting the importance of the cytoplasmic tail of endoglin for the recognition and activity of specific proteases. The functional consequence of the inactivation of membrane-associated endoglin in squamous carcinoma cells was addressed by using short hairpin RNA (shRNA) technology. Knockdown of endoglin in SCC cells activated the ALK5/Smad2,3 signaling, resulting in cell growth inhibition, delayed tumor latencies and an SCC–SpCC transition [164]. These results suggest that by attenuating TGF-β/ALK5/Smad2,3 signaling in keratinocytes endoglin behaves as a suppressor of malignancy in mouse skin carcinogenesis. Moreover, we propose that loss-of-function of membrane endoglin must exert strong selective pressure during tumor progression by priming carcinoma cells that have lost the TGF-β growth inhibitory response. However, whether the inactivation of membrane endoglin in SCC cells affects other TGF-β-independent events remains unexplored, as does the influence of soluble endoglin in the evolution and homeostasis of carcinomas.

These conclusions reached are in accordance with data obtained from *Eng*$^{+/-}$ mice. These mice developed less skin tumors than control *Eng*$^{+/+}$ mice, but progression to spindle cell carcinomas was vastly accelerated upon chemical carcinogenesis [33,234], an identical phenotype to that observed in transgenic mice overexpressing TGF-β1 in suprabasal layers of the epidermis [235]. These observations fit with the widely accepted notion of a dual role for TGF-β1 in carcinogenesis as a tumor suppressor at early stages and a promoter of malignancy at later stages [9,231]. Nevertheless, this putative suppressor role of TGF-β1 has been challenged in skin carcinogenesis. Epidermal hyperproliferation was accompanied by inflammation in mice in which TGF-β1 expression was targeted to the basal layer of the epidermis [236,237]. Moreover, there was a dramatic reduction in inflammation in knockout mice for Smad3 (*Smad3*$^{+/-}$ and *Smad3*$^{-/-}$) subjected to skin chemical carcinogenesis, and they were resistant to papilloma formation and malignant progression [238,239]. These observations suggest that TGF-β1 has a tumor promoting effect even at early stages of carcinogenesis, which mediates the inflammatory response produced by TPA during skin promotion [240]. Thus, TGF-β1 mediated production of inflammatory cytokines in the stroma may overcome its growth suppressor effect on epidermal keratinocytes. In the chemical carcinogenesis studies with *Smad3*$^{-/-}$ and *Eng*$^{+/-}$ mice, Smad3 and endoglin are knocked out in both the epithelia and stroma. The results with Smad3 knockout mice suggest that this member of the Smad family is a key mediator of TGF-β1 induced inflammation [239]. In the case of *Eng*$^{+/-}$ mice, endoglin haploinsufficiency in the epithelial compartment facilitates enhanced Smad2,3 signaling and it appears to have a stronger effect on tumor growth than endoglin haploinsufficiency in stromal cells.

Fig. 5 summarizes the role of endoglin in tumor development during mouse skin chemical carcinogenesis, where TGF-β behaves as a tumor suppressor at early stages and it exhibits a tumor promoting effect at late stages of carcinogenesis.

4. Betaglycan in cancer

Altered betaglycan expression has been detected in a variety of human tumors and the potential correlation with tumor progression and metastasis is shown in Table 2. Increased expression of betaglycan and of TGF-β type I and II receptors has been seen in high-grade non-Hodgkin's lymphomas with respect to low-grade lymphomas, suggesting a tumor promoting role for these receptors [241]. Betaglycan expression was also upregulated in B-cell chronic lymphocytic leukemia with respect to normal B cells [242,243]. However, betaglycan levels are generally downregulated in different types of cancers. Such reduced betaglycan expression has been associated with advanced stage neuroblastomas [244], ovarian carcinomas [245,246], ovarian granulosa cell tumors [247], endometrial carcinomas [248], prostate cancer [249,250], renal cell carcinomas [251], non-small cell lung cancer [252], breast carcinomas [253] and pancreatic carcinomas [254,255]. Indeed, downregulation or loss of betaglycan in cancer is a more frequent event than alterations to any other component of the TGF-β pathway [5]. Several mechanisms may account for the downregulation of betaglycan during tumor progression. The betaglycan locus (*TGFBR3*) is located on the short arm of chromosome 1 at 1p32, a region frequently deleted in breast, stomach, colorectal, endometrial, kidney, lung, ovarian and testicular cancer [256]. LOH at the *TGFBR3* locus has been found in about 38–50% of patients with prostate, breast and lung cancer [250,252,253] pointing to *TGFBR3* as a tumor suppressor gene. Nevertheless, several other potential suppressors' loci, such as *TP73* and *RUNX3*, also reside in this region [257,258]. In the majority of tumor samples, the low levels or complete absence of betaglycan protein does not match the genomic data, suggesting that other mechanisms to downregulate betaglycan expression must be involved. Combined treatment of ovarian carcinoma cell lines with inhibitors of methyltransferase and histone deacetylase increased betaglycan mRNA and protein expression [246], indicating that epigenetic silencing may play a role, at least in this type of cancer. Nevertheless, studies directly examining this matter have not yet been reported. In normal tissues, the expression of TGF-β is relatively low, sufficient to maintain homeostasis. This situation changes under conditions of tissue injury and in tumors where the concentration of TGF-β released by the tumor cells themselves increases, as well as that released by mesenchymal and inflammatory cells of the tumor stroma [11]. The elevated TGF-β levels in the tumor microenvironment may provoke the downregulation of betaglycan expression since TGF-β1 negatively regulates betaglycan expression at the transcriptional level in breast and ovarian cancer cell lines [259].

4.1. Functional studies of betaglycan

The functional role of betaglycan in tumor cells has been assessed by loss- and gain-of-function approaches. Expression of betaglycan in MCF-7 and MDA-MB-231 breast cancer cells, both of which synthesize low amounts of the proteoglycan, restored TGF-β1-induced cell growth inhibition and reduced either anchorage-independent growth or tumorigenicity in athymic nude mice [260,261]. Moreover, increasing betaglycan expression in a murine tumor mammary cell line inhibited cell invasiveness *in vitro* and tumor invasion, angiogenesis and metastasis *in vivo* [253]. Similar results were obtained in human prostate and ovarian cancer cell lines although these enhanced betaglycan levels did not affect either cell proliferation or cellular responsiveness to TGF-β1, but merely decreased cell motility and invasiveness [246,250]. Conversely, betaglycan knockdown in human RWPE-1 immortalized prostate epithelial cells led to the acquisition of transforming capacity in a focus formation assay, although these cells did not produce tumors in athymic nude mice [249]. The downregulation of betaglycan expression in RWPE-1 cells also diminished the expression of the potent endogenous inhibitor of angiogenesis, pigment epithelium derived growth factor (PEDGF). Furthermore, the expansion of the number of cells expressing CD133, a cell surface stem cell marker, suggested a role for betaglycan in stem cell maintenance [249]. Together, these findings are consistent with a suppressor role for betaglycan in epithelial carcinogenesis.

Betaglycan has also been associated with EMT in development and cancer. It is required for the EMT events occurring during cardiac development to form the valves and septa [262], as well as during palate fusion [263,264]. Accordingly, *TGFBR3* null mouse embryos die due to heart and liver defects [265,266]. It appears that betaglycan is required in these morphogenetic processes to sustain TGF-β signaling in the cells undergoing EMT [262,264], whereas in neoplastic cells the loss of betaglycan facilitates EMT. Thus, upon TGF-β1-induced EMT in human immortalized keratinocytes and pancreatic carcinoma cell lines, betaglycan expression is downregulated [255,267], consistent

with the repression of the *TGFBR3* promoter by TGF-β signaling [259]. Interestingly, maintaining betaglycan expression in pancreatic cancer cells did not block the TGF-β1-induced loss of E-cadherin or the cytoskeletal reorganization but rather, stimulation of motility and invasiveness was suppressed [255]. These results are intriguing and suggest that: i) downregulation of betaglycan expression is dispensable for TGF-β1-induced EMT but not for TGF-β1-induced cell migration and invasiveness; and ii) EMT and cell migration, two closely related processes, are regulated by distinct mechanisms. A reduction in betaglycan levels has also been found during the EMT promoted by HMGI(Y) architectural transcription factors in breast epithelial cells [268]. Moreover, betaglycan knockdown in preneoplastic mouse mammary cells led to decreased expression of E-cadherin and increased cell growth, motility and invasiveness [269]. More recently, however, a correlation between high levels of betaglycan and invasiveness was seen in breast cancer cell lines

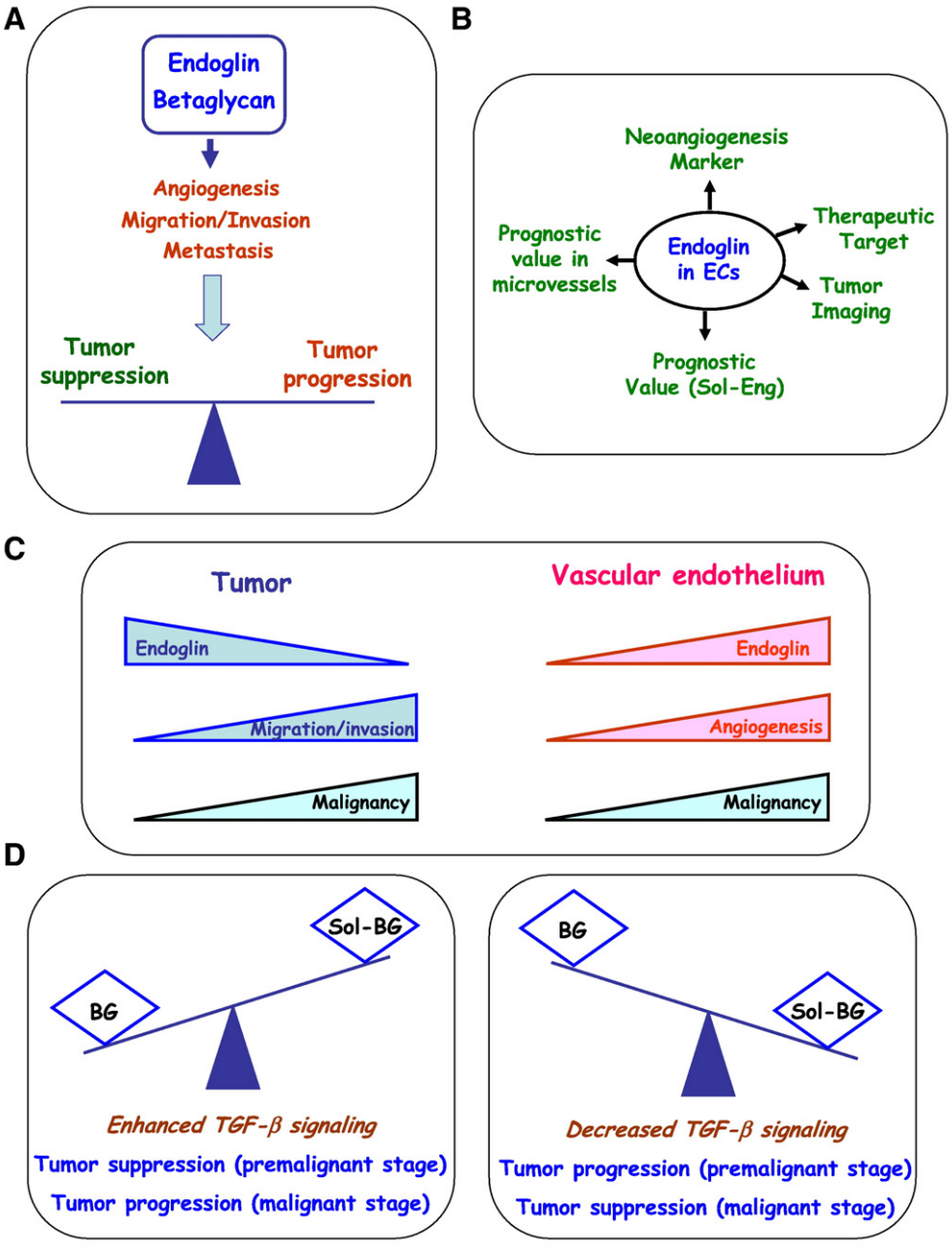

Fig. 6. Role of endoglin and betaglycan in cancer. (A) The in vivo and in vitro studies referred to in this review support the involvement of endoglin and betaglycan in tumor suppression and in tumor progression, modulating tumor proliferation, angiogenesis, migration, invasion and metastasis. (B) Endoglin is expressed in endothelial cells (ECs) and it is highly upregulated during tumor neoangiogenesis. As a marker of neoangiogenesis, endoglin may have diagnostic (tumor imaging), prognostic (microvessels staining and in terms of the soluble form in the serum) and therapeutic (anti-endoglin antibodies and endoglin protein as a vaccine) value. (C) Increased endoglin expression correlates with increased tumor angiogenesis, probably due to the proangiogenic role of endoglin in endothelial cells. In addition, endoglin expression is generally downregulated in tumor cells during progression allowing increased tumor cell migration, invasion and EMT. Therefore, changes in endoglin expression in both the tumor and the vascular endothelium modulate malignancy. (D) The betaglycan paradox. Most experimental evidence suggests that membrane betaglycan (BG) behaves as a tumor suppressor at early stages of cancerogenesis by enhancing TGF-β signaling as well as by TGF-β-independent mechanisms. However, BG is shed to release a soluble form (Sol-BG) that antagonizes TGF-β signaling. Thus, the effect of BG in tumor cells may depend on the extent of shedding, a fact that may explain some discrepancies reported in the literature. High levels of Sol-BG at early stages of tumorigenesis might contribute to tumor development by attenuating TGF-β-induced growth inhibition. On the contrary, enhanced levels of Sol-BG at later stages suppress malignancy, as suggested in a variety of human cancer xenograft models.

[270]. Furthermore, betaglycan knockdown in these highly invasive breast cancer cells impaired the cellular response to TGF-β and resulted in decreased motility and invasion.

The mechanism by which betaglycan regulates tumor cell growth, invasion and metastasis has not been fully elucidated. Betaglycan enhances the binding of all three TGF-β ligands to the TGF-β signaling receptors and it is essential for the high-affinity cell surface binding of TGF-β2 [64,271]. Betaglycan also acts as a coreceptor for inhibin [60] and it mediates signaling of different members of the BMP subfamily [61]. Obviously, this function of betaglycan as a ligand-presenting coreceptor resides in the ectodomain. In addition, a role for the betaglycan cytoplasmic domain in modulating TGF-β signaling has been highlighted. The betaglycan endodomain can interact with the autophosphorylated form of TβRII and it is required for TGF-β2-induced signaling [39]. It also interacts with Gα-interacting protein which stabilizes betaglycan on the cell surface [40], and with β-arrestin2 that is involved in the internalization and degradation of betaglycan and TβRII [38]. The selective advantage of cancer cells that downregulate betaglycan expression early in carcinogenesis may be due to an attenuated growth inhibition in response to TGF-β. This has been shown for breast and renal cancer cell lines in which restoration of betaglycan expression re-established TGF-β-mediated signaling [251,260]. In endometrial, ovarian and prostate cancer, loss of betaglycan may contribute to increase the biological influence of activin as a promoter of tumor growth. Inhibin and activin are gonadal expressed members of the TGF-β superfamily that are crucial for maintaining normal function in many tissues, particularly those of the reproductive axis [272]. Inhibin is thought to elicit its antagonistic action on activin by displacing it from the ActRII, thereby impairing ActRII from dimerizing with ActRI, although inhibin must bind to betaglycan to antagonize activin activity [60]. Inhibin has been proposed to have an antagonistic and physiologically complementary role to androgens, acting as a negative regulator of prostate growth. Thus, betaglycan downregulation in prostate cancer results in resistance to inhibin-mediated tumor suppression [273]. Inhibin resistance as a result of the loss of betaglycan expression has also been associated with a more aggressive behavior in epithelial-derived ovarian cancer cell lines [246,274]. Moreover, targeted deletion of the inhibin α-subunit in mice results in sex-cord stromal tumors at an early age [275], suggesting that inhibin acts as a suppressor in ovarian cancer of stromal origin. On the other hand, the interaction of betaglycan with cytoplasmic scaffolding proteins, such as Gα-interacting protein and β-arrestin2, suggests a signaling role that may be independent of Smad proteins and even of TGF-β. Thus, betaglycan was found to modulate NFκB activity in association with invasiveness [269,270]. Betaglycan can also activate p38 MAPK signaling in the absence of TGF-β ligand [276], and recent evidence suggests that the p38 stress MAPK pathway may suppress tumor progression [277].

4.2. Soluble betaglycan

Like endoglin, the extracellular domain of betaglycan can be proteolytically cleaved to produce a soluble form of betaglycan (Sol-BG) that sequesters TGF-β ligand antagonizing TGF-β activity [66,278,279]. Thus, betaglycan may act as a dual modulator of TGF-β signaling: as a membrane protein it enhances TGF-β activity and as a soluble form it acts as an inhibitor [66,279]. Indeed, systemic administration of Sol-BG in human cancer xenograft models antagonizes the tumor promoting activities of TGF-β and suppresses tumor cell growth, invasion and metastasis, as well as tumor angiogenesis [280–282]. These observations suggest that at least part of the tumor suppressor effects of betaglycan in human cancer cell lines may be mediated by Sol-BG [252,253]. In this setting, betaglycan downregulation in tumors would stimulate malignant progression due to increased TGF-β activity.

Its role as an inhibitor of tumor growth, invasion and metastasis makes Sol-BG a promising agent to be used in cancer therapy. Several approaches to inhibit TGF-β signaling have been explored including antisense oligonucleotides, drugs that inhibit the kinase activity of TβRI and antagonists that block the binding of ligands to TGF-β receptors [283]. This latter strategy seems to be the most favorable approach, as inhibiting TGF-β extracellularly avoids the need for the internalization of the agent, and it can block TGF-β signaling that is independent of TβRI. Thus, methods to increase the ligand affinity of Sol-BG and its ability to inhibit the activity of all three TGF-β isoforms are currently being designed in order to improve the efficacy of Sol-BG in cancer therapy [284].

5. Concluding remarks

The TGF-β signaling pathway plays a key role in a plethora of physiological processes such as development, cellular proliferation, extracellular matrix synthesis, angiogenesis or immune response, whose dysregulation may result in tumor development. The TGF-β coreceptors endoglin and betaglycan are modulators of the TGF-β response with important roles in cancer (Fig. 6). Endoglin upregulated expression in tumor-associated vascular endothelium has revealed its prognostic and diagnostic value in solid tumors, as well as its potential as a vascular target for antiangiogenic cancer therapy. In addition, functional studies on the role of endoglin and betaglycan in the tumor cells themselves have demonstrated their critical role as tumor suppressors during cancer progression. While much more work must be done to fully understand the molecular mechanisms that underlie the role of endoglin and betaglycan in tumor angiogenesis and malignancy, the recent advances provide new research avenues for better diagnosis, prognosis and therapy in cancer.

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