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Caveolin and TGF-β Entanglements

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Transforming growth factor (TGF)-β is a multifunctional cytokine acting during development, tissue homeostasis, regeneration processes, and disease progression. Due to its pleiotropic effects, tight regulation of the induced signaling cascades is mandatory. Caveolin proteins regulate a specific endocytic pathway and modulate diverse signaling pathways and thus have been related to severe disorders, for example, cancer and fibrosis. Caveolin affects TGF-β/-Smad and non-Smad signaling in many ways and thus can determine the cellular outcome upon TGF-β challenge. Reciprocal regulation of caveolin and TGF-β is also evident, ranging from gene expression to miRNA regulation. Finally, there is in vivo evidence that this crosstalk influences disease development and progression. This review gives an overview about the multifaceted relations of caveolin and TGF-β.

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The eponym of the TGF-β family, amongst activins, bone morphogenetic proteins (BMPs) and growth differentiation factors, transforming growth factor (TGF)-β is a major regulator of developmental processes, tissue homeostasis but also several pathologies. There are three isoforms in humans, with TGF-β1 being the best studied cytokine. It is expressed and secreted as an inactive precursor covalently bound to latency associated peptide (LAP-TGF-β). Upon proteolytic cleavage of the LAP fragment, accomplished by diverse enzymes such as thrombospondin, TGF-β may initiate signaling events in target cells. To fulfill its effects, TGF-β binds to type II TGF-β receptors (TβRII) on the cell surface. In turn, type I TGF-β receptors (TβRI, mainly ALK5, but also ALK1) associate with type II receptors forming heterotetrameric complexes. The constitutive serine/threonine kinase activity of the type II receptors activate type I receptors that subsequently transduce the signal to receptor-Smad proteins (R-Smads). TGF-β triggers the phosphorylation of R-Smads Smad2 and Smad3 (also Smad1 transiently) at the C-terminal—SXS motif. This enables formation of heteromeric complexes with the common Smad, Smad4, which subsequently shuttle into the nucleus to regulate gene transcription. Hundreds of TGF-β target genes have been identified, and this large cohort is regulated by a myriad of co-transcription factors, such as p300/CBP or Snail, modulating gene expression or repression. Canonical Smad signaling itself can be regulated by diverse mechanisms, spanning from expression, activation via modulated nuclear shuttling and co-factor binding to deactivation. Tremendous data is available and has already been excellently reviewed, for example, Heldin and Moustakas (2012). To envision the complexity of the TGF-β signaling network, the TGF-β pathway interaction SnapShot is recommended (Taylor and Wrana, 2008).

Besides Smad signaling, TGF-β mediates its effects additionally via many other pathways, among those are ERK, p38, JNK, or PI3K/AKT. Also regulation of small GTPases and FAK/Src is modulated by TGF-β (Mu et al., 2012). In part, activation mechanisms have been described, for example, ShcA associates with the active TGF-β receptor complex thus leading to its serine and threonine residue phosphorylation. This in turn enables binding for Grb2 and Sos subsequently triggering Ras and downstream ERK activation. In contrast, the precise mechanism how TGF-β activates PI3K/AKT signaling has not yet been described, but will be discussed in more detail in this article.

Many efforts have been put on solving molecular mechanisms and spatial requirements of TGF-β signaling initiation, Smad phosphorylation and release from the receptor complexes. Ultimately, these questions are linked to endocytosis dependency of signaling initiation. Many groups generated data on this issue, in part with conflicting results. Thus, a general concept of endocytic regulation of TGF-β signaling is not available (Chen, 2009; Meyer et al., 2011). Furthermore, most studies only focused on canonical Smad signaling in context of endocytosis and disregarded activation of non-canonical signaling pathways. It was demonstrated that TGF-β receptors can localize in clathrin coated pits as well as cholesterol enriched lipid rafts/caveolae. The hallmark of the omega shaped structure of caveolae is the presence of the protein caveolin. Three isoforms have been described, caveolin-1, -2, and -3. The latter is only expressed in skeletal and heart muscles, whereas isoforms 1 and 2 are more ubiquitously present. There is compelling evidence, that caveolin dysregulation affects diverse severe disorders, for example, cancer and fibrotic diseases (especially in lung

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fibrosis; Burgermeister et al., 2008; Tourkina and Hoffman, 2012).

Caveolin-1, but not caveolin-2 is ultimately required for caveolae formation. Functions of caveolae are not limited to regulate endocytosis, but also affect cholesterol and lipid metabolism. Caveolar endocytosis may also mediate glycosphingolipid, integrin, and membrane receptor internalization. Especially the caveolin scaffolding domain (CSD) interacts with many adaptor and signaling molecules, thus giving a platform for diverse signaling events, such as binding and inhibiting eNOS (Schwartz et al., 2005). The localization of different membrane receptors at caveolae gave rise to investigations of caveolin-1 regulating signaling events. This has been shown, among others for platelet-derived growth factor (PDGF), the epidermal growth factor receptor (EGFR), H-Ras, and TGF-β signaling (Quest et al., 2013). With respect to this review, the latter will be discussed in detail below.

Caveolin and dampening of TGF-β/Smad signaling

Many studies demonstrated that caveolin-1 acts as a negative regulator of Smad signaling. Caveolin-1 via its CSD directly interacts with the TβRI (Razani et al., 2001). Furthermore, this study convincingly demonstrated that Smad2 phosphorylation and nuclear shuttling of phospho-Smad2 was abrogated by caveolin-1 overexpression. Later on, Wrana and co-workers published a milestone paper concluding that clathrin-dependent endocytosis is promoting Smad signaling, whereas TβR internalization via lipid rafts/caveolae leads to dampening of signaling (Di Guglielmo et al., 2003). The latter is accomplished by physical interaction with Smad7-Smurf complexes. Smurfs (1 and 2) are E3 ubiquitin ligases and as such trigger the degradation of TβRs (Kavsak et al., 2000; Ebisawa et al., 2001). As a consequence, the cells are less responsive to TGF-β for further Smad activation (Fig. 1).

The relevance of lipid rafts/caveolae in regulating Smad signaling was further shown by studies manipulating cholesterol levels. Addition of cholesterols to cell cultures culminated in reduced Smad activation and signaling, likely due to increased accumulation of TβRs to lipid rafts. Supplementing cells with cholesterol depleting agents (leading to disruption of lipid rafts) on the other hand promoted Smad signaling (Chen et al., 2007). Furthermore, heparan sulfate negatively regulates TGF-β responsiveness, in part by facilitating caveolar endocytosis of TGF-β and subsequent degradation (Chen et al., 2006). Another polysaccharide, hyaluronan, acts in a similar way. Via its receptor CD44, it mediates accumulation of TβRs in caveolin-1 containing membrane fractions. This leads to elevation of interference with Smad7 and thus to a reduction in canonical signaling (Ito et al., 2004).

Two recent publications report about CD109, a co-receptor for TGF-β, to negatively regulate Smad signaling (Bizet et al., 2011, 2012). This effect is due to shifting TGF-β/receptor complex localization towards lipid rafts/caveolae compartments, and therewith increasing receptor degradation in a Smad7-Smurf2-dependent manner. PICK1 also enhances receptor degradation by forming a scaffold for TβRI and caveolin-1 interaction. PICK1 binds to the C-terminal region of the receptor and therewith facilitates binding to caveolin-1 (Zhao et al., 2012). Subsequently, it leads to ubiquitination of receptors and triggers degradation. A major role for PICK1 in breast cancer development has been suggested in the same study as there is evidence for a negative correlation of PICK1 expression and pSmad2 levels in vivo.

Cytokine crosstalk involving TGF-β signaling regulation via lipid rafts has also been documented. Interleukin (IL)-6 potently increases Smad signaling in kidney tubular cells via shifting the receptor distribution from lipid rafts to non-raft fractions. As a consequence, less TβRs are degraded via caveolar internalization (Zhang et al., 2005).

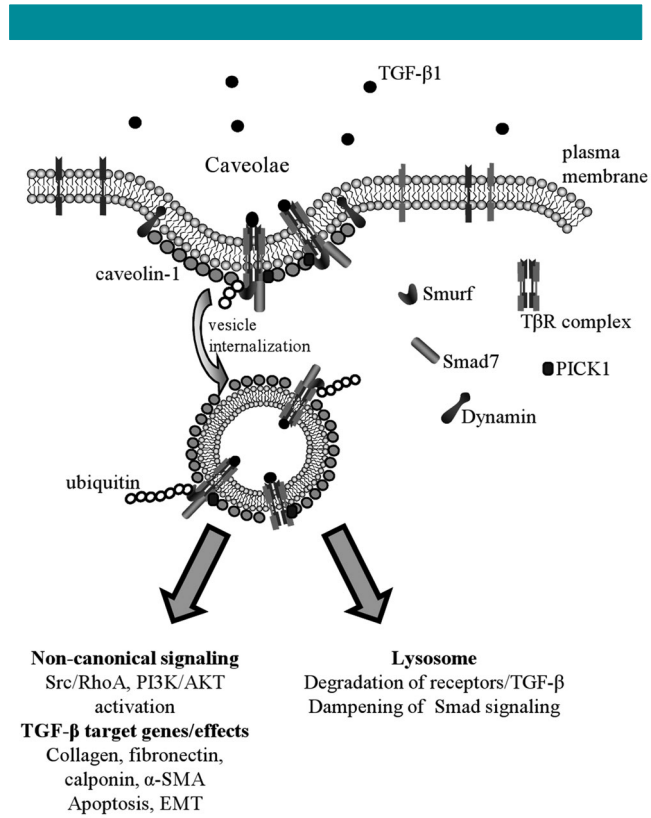

Fig. 1. Caveolin and TGF-β signaling. This scheme illustrates the effects of caveolin on the TGF-β signaling cascade. Non-Smad signaling can be initiated in caveolae and may influence EMT and apoptosis. However, Smad signaling is dampened and TGF-β receptors are directed to degradation. See text for details.

To conclude, there is compelling evidence for caveolin-1 being a negative regulator of TGF-β Smad signaling. It needs to be mentioned, that the data published does not give a black and white picture. Focusing on TβR internalization for example, it is likely that also the clathrin-dependent, caveolin-1 independent internalization pathway of TβRs contributes to receptor degradation and thus to a reduction in signaling (Mitchell et al., 2004).

Recently, a role for caveolin-2 was proposed to interfere with TGF-β effects in endothelial cells (EC; Xie et al., 2011). In presence of tyrosine phosphorylated caveolin-2, EC proliferation was reduced due to downregulated phospho-Smad signaling. Noteworthy, this inhibitory effect on TGF-β function was independent of caveolin-1. Nevertheless, it still needs to be defined whether caveolin-2 exerts its effect via the same mechanism as caveolin-1.

Caveolin and non-Smad signaling

Evidence has emerged that caveolin-1 is also capable of modulating the cell response upon TGF-β challenge besides dampening of Smad signaling. TGF-β's profibrotic function is enhanced by overexpression of caveolin-1 in terms of increased type I procollagen expression in dermal fibroblasts (Kim et al., 2008). Although by overexpression of caveolin-1, Smad3 activation was reduced, a hyperactivation of AKT phosphorylation was obtained. Further experiments then

confirmed that caveolin-1/AKT signaling is responsible for the increase of collagen expression. This gives rise to hypothesize that caveolin-1 is not only a factor for inhibiting TGF-β signaling and effects, but rather a modulator of the TGF-β pathway, balancing Smad and non-Smad signaling events. Similarly, in primary hepatocytes, TGF-β activates the AKT pathway which is, in concert with Smad3, required for CTGF expression. In the absence of caveolin-1, the non-Smad AKT pathway failed to be activated by TGF-β resulting in reduced CTGF expression (Meyer et al., 2011). Furthermore, TGF-β is capable of inducing either epithelial to mesenchymal transition (EMT) or apoptosis in primary hepatocytes. An siRNA approach to block caveolin-1 expression further revealed that the TGF-β response is shifted towards apoptosis via abrogated AKT activation. In this scenario, caveolin-1 knockdown further led to a reduction of endogenous expression of anti-apoptotic factors, BCL2 and BCL-xL (Meyer et al., 2013a). Therewith, loss of caveolin-1 sensitizes hepatocytes for TGF-β mediated pro-apoptotic effects, whereas no effects on EMT processes could be determined (based on Snail1, collagen 1α1, E-cadherin, and vimentin expression).

The question how TGF-β is capable of inducing AKT activation has insufficiently been addressed so far, especially in context of caveolin requirement. However, a detailed explanation has been provided by the group of Fabregat (Murillo et al., 2005). In foetal rat hepatocytes, TGF-β activates TACE (a metalloprotease) that likely leads to activation of EGF ligands. EGF receptors subsequently activate Src that finally mediates AKT activation. This mechanism had effects on TGF-β's apoptotic function, but did not affect the EMT process. However, Yi et al. (2005) have shown that the TβRI can directly bind and activate PI3K. It has yet to be explored what role caveolin-1 plays in these signaling events.

A controversial finding concerning AKT activity and caveolin-1 expression was reported in pancreatic cancer cells. Caveolin-1 is not expressed in Panc 10.05 and a subsequent restoration of caveolin-1 expression not only led to reduction in activated AKT, but also decreased TGF-β/Smad signaling. In contrast to dermal fibroblasts—as above, in that case caveolin-1 expression negatively regulates AKT activity. Therefore, the role of caveolin-1 on non-Smad signaling can be considered as highly cell type specific. Further studies will need to elucidate whether there are alterations in TGF-β non-Smad signaling in healthy and cancer cells from the same origin. With respect to pancreatic cancer, enhanced caveolin-1 levels were accompanied by a more epithelial phenotype (more membraneous E-cadherin and β-catenin) with reduced migration and invasion capacity. These in vitro findings were validated in vivo by transplanting Panc10 cells overexpressing caveolin-1. Tumor weight and volume were significantly reduced compared to control Panc10 tumors. Also, differentiation status as measured by upregulated E-cadherin and downregulated Snail expression was improved (Salem et al., 2011).

Besides activating the AKT cascade, TGF-β induces RhoA activation in diverse cell types. Using mesangial cells, the Krepinsky lab could nicely document that fibronectin production upon TGF-β challenge requires caveolin-1. This is mediated by Src phosphorylation of caveolin-1 at Y14 and subsequent RhoA activation (Peng et al., 2008). Another caveolin-1/RhoA dependent mechanism was described recently in vascular smooth muscle cells. TGF-β stimulated the generation of reactive oxygen species leading to activation of Src kinases. In turn, in a caveolin-1 dependent manner, RhoA was activated and this led to reduction of PPM1A levels. Reduced levels of the Smad2/3 C-terminal phosphatase PPM1A enabled sustained Smad2/3 phosphorylation and transcriptional activity (with focus on PAI-1 expression). Hence, caveolin-1 indirectly can also enhance phospho-Smad signaling activity (Samarakoon et al., 2011). Another study in NIH3T3 fibroblasts however, showed that TGF-β triggered PAI-1 induction is reduced in cells overexpressing caveolin-1 (Lee et al., 2007).

In HaCaT cells, TGF-β rapidly induces an EMT phenotype. Disrupting caveolae/lipid raft formation by cholesterol depletion blunted epithelial to mesenchymal transition (for illustration, see Fig. 1). However, AKT and Smad2/3 activation were not affected, whereas signaling via ERK and p38 was modulated, being the reason for the change in the TGF-β outcome (Zuo and Chen, 2009). Although this study does not give evidence that caveolin-1 is directly involved in regulating ERK and p38 activation, its role in caveolae formation and modulating diverse signaling events (including p38 and ERK) is supportive for such hypothesis. In contrast to the positive regulation of caveolin-1 on ERK, another study reports on a different effect. In MRC-5 cells (a human pulmonary fibroblast cell line), overexpression of caveolin-1 abrogates TGF-β mediated activation of ERK and JNK. Subsequently, induction of collagen I and fibronectin is abolished (Wang et al., 2006), indicating that caveolin directly affects TGF-β mediated activation of fibroblasts. Similarly, in airway smooth muscle cells, caveolin-1 is required for TGF-β mediated induction of calponin and α-smooth muscle actin, both being markers for the contractile cell (and thus activated) phenotype. Based on this, the presence of caveolin-1 and TGF-β in concert contributes to allergic asthma (Gosens et al., 2011).

Another interesting aspect of caveolin-1 on regulating TGF-β signaling is related to liver regeneration. In the partial hepatectomy model, livers of caveolin-1 knockout mice displayed reduced Smad activation. This was in part due to elevated levels of SnoN, a negative regulator of TGF-β signaling. Although, not a direct interaction of caveolin-1 and TGF-β signaling components was reported here, the lack of caveolin-1 does not always necessarily promote canonical Smad signaling, especially when investigating functions in a complex environment like a whole organ or organism (Mayoral et al., 2010).

Integrin effects

Another not yet in detail investigated connection focuses on the role of integrins in the caveolin/TGF-β signaling crosstalk. Integrins (especially αvβ6 and αvβ8) can activate latent TGF-β and furthermore modulate expression of TGF-β and TβRs. In relation to the TGF-β mediated signaling effects, integrins support p38 activation and influence EMT (Wipff and Hinz, 2008; Margadant and Sonnenberg, 2010). On the other hand, caveolin can regulate integrin turnover and signaling and vice versa, integrins possess a regulatory function on caveolin (Salanueva et al., 2007). Therefore, caveolins may modulate integrin patterns and subsequently influence TGF-β activation and signaling. Especially in the context of fibrotic diseases (and in vivo studies), it will be exciting to unravel new mechanisms of this crosstalk.

TGF-β regulates caveolin expression

Primary pulmonary fibroblasts exhibit downregulated caveolin-1 upon TGF-β stimulation in a dose- and time-dependent manner (Wang et al., 2006). Therewith, the inhibiting effect of caveolin-1 on Smad signaling is reduced and the cells increase the fibrotic response in terms of fibronectin, collagen I, and α-SMA protein expression. Several other publications reported that caveolin-1 is downregulated by TGF-β, for example, in monocytes and dermal fibroblasts (Del Galdo et al., 2008a,b; Tourkina et al., 2010).

On the other hand, caveolin-1 is induced during TGF-β driven EMT in NMUMG cells (Bailey and Liu, 2008). Here,

TGF-β activated FAK/Src signaling which enabled induction of caveolin-1. In contrast, in primary hepatocytes undergoing TGF-β initiated EMT, caveolin-1 expression is attenuated. However, it could be shown that in epithelial-like hepatocellular carcinoma (HCC) cell lines, TGF-β gained the potential to induce caveolin-1 via FAK/Src signaling. On the other hand, dedifferentiated HCC cell lines displayed high basal caveolin-1 expression levels that were not altered by TGF-β signaling (Meyer et al., 2013b). In prostate cancer cells, a positive autoregulatory feedback loop was determined where TGF-β in concert with VEGF and FGF2 upregulate caveolin-1 expression (Li et al., 2009). In turn, elevated caveolin-1 levels subsequently increased production of these growth factors. This loop enhanced the metastatic capacity of the tumor cells. To conclude, under certain circumstances TGF-β can modulate its signaling activity by regulating caveolin-1 abundance.

Caveolin and modulation of TGF-β pathway component expression

In primary rat mesangial cells, there is evidence for caveolin-1 triggering upregulation of TGF-β1 by glucose challenge. This is mediated by RhoA-dependent activation of the AP-1 transcription factor complex (Zhang et al., 2012). In NIH3T3 fibroblasts, ectopic expression of caveolin-1 led to a repression of TβRII gene expression. Subsequently, this offers an alternative mechanism of dampening the TGF-β signaling cascade via modulating availability of membrane receptors (Lee et al., 2007).

MicroRNA and caveolin/TGF-β signaling relations

Another level of crosstalk between caveolin and TGF-β could be mediated by microRNAs (miRNAs). miRNAs are short non-coding RNA molecules that regulate gene expression post-transcriptionally. Thus, miRNAs are likely to be a fine-tuning mechanism to regulate protein expression and may allow rapid adaptation to changes of the cell environment (Mattick and Makunin, 2005). miRNAs can modulate the TGF-β pathway on several levels. First, miRNAs can directly affect expression of pathway components. Second, miRNAs fine-tune expression of TGF-β target genes. Third, miRNA expression can be triggered by the TGF-β signaling pathway. Fourth, TGF-β signaling might directly affect targets that are related in miRNA processing and thus modulate miRNA function. An exhaustive review is given by Butz et al. (2012).

Noteworthy, Smad proteins can directly influence the miRNA processing machinery via binding to the p68 RNA helicase, a component of the Drosha microprocessor complex (Davis et al., 2008). A prerequisite is that the R-Smads are phosphorylated and thus in an active state (Davis et al., 2010). Subsequently, TGF-β is modulating a certain set of miRNAs.

A very recent study from Mari and Pottier et al. could demonstrate in idiopathic pulmonary fibrosis, that miR-199a-5p is upregulated and a target of TGF-β signaling (Lino Cardenas et al., 2013). miR-199a-5p alone was able to induce fibroblast proliferation and activation. Intriguingly, the authors could demonstrate that the mechanism is depending on repressing caveolin-1 which has been shown to be a critical regulator of lung fibrosis. Even more, also in other fibrotic disorders, such as kidney fibrosis or CCl_4 induced liver fibrosis, miR-199a-5p expression is dysregulated. However, it has yet to be determined whether this regulation plays a role during cancerogenesis, as for example, in hepatocellular carcinoma, high caveolin-1 expression is linked to bad prognosis for the patient. Additionally, it is evident that caveolins can also be targeted directly by other miRNAs. In kidney, miRNA-802 can suppress caveolin-1, in HEK293, adipocytes and murine liver, miRNAs-103 and -107 suppressed caveolin expression, respectively (Lin et al., 2011; Trajkovski et al., 2011). Several more miRNAs were described to regulate caveolin expression, amongst miRNA-375, miRNAs-133a and -203 (Basu et al., 2011; Nohata et al., 2011; Orom et al., 2012). miRNA-128 has been shown to be involved in ovarian cancer progression. In a screen for miRNA-128 targets in ovarian cancer cells, Shahab et al. (2012) identified caveolin-1 as a prominent target. This study enables another link to the TGF-β signaling pathway as Smad2 expression was also affected by miRNA-128. Hence, miRNAs modulate TGF-β/caveolin entanglements via regulation of expression. Research needs to be fostered to elucidate the relevance for diseases, as miRNA expression is cell type dependent and often deregulated during disease progression, especially cancer (Garzon et al., 2009).

Point mutations in caveolin genes

It may not be sufficient to screen for caveolin-1 expression during disease progression, additionally it is worth investigating point mutations or single-nucleotide polymorphisms (SNPs) in caveolins. Fanzani and colleagues reported about a mutation (P104L) in Cav3 in myoblasts affecting AKT, p38, and TGF-β/Smad signaling (Stoppani et al., 2011). Thus, a deregulation of TGF-β signaling during disease might derive not only from caveolin transcriptional regulation, but also caveolin gene integrity. In skeletal muscle, the P104L mutation leads to atrophy in mice and this is accompanied with increased TGF-β signaling (Ohsawa et al., 2012). Other mutations have been described in breast cancer for caveolin-1 (Hayashi et al., 2001). Here, the characterized P132L mutation leads to cellular transformation, amongst hyperactivation of ERK signaling. Another in depth analysis of the caveolin-1 gene identified more breast cancer associated point mutations, amongst W128Stop, Y118H, and Y148H (Li et al., 2006). Several other point mutations of the caveolin-1 gene have been reported in human oral squamous cell carcinomas that do not overlap with the findings in breast cancers (Han et al., 2004). However, concerns about abundance and frequency of caveolin-1 gene mutations arised recently (Koike et al., 2010; Patani et al., 2012a). The group of Bau screened for SNPs in the caveolin-1 gene that might correlate with cancer development and progression of disease, for example, in liver, colorectal or bladder cancer (Bau et al., 2010; Yang et al., 2010; Hsu et al., 2013). And indeed, they found single polymorphisms that significantly correlate with cancer risk. However, the underlying mechanisms still need to be unraveled. Potential consequences on TGF-β signaling events by modulating responses to TGF-β also need clarification. Future studies need to reveal how frequent caveolin mutations occur and whether they are implicated in certain cancer types with TGF-β function having switched from a tumor suppressor towards a promoter.

Caveolin and TGF-β in vivo

It is now obvious, that the connection between caveolin and TGF-β exists on many regulatory levels. Especially TGF-β's pro-fibrogenic function has been shown to be responsible for diverse fibrotic disorders, for example, in lung and liver. Furthermore, TGF-β can switch from a tumor suppressor to a tumor promoter for epithelium-derived cancers (Padua and Massague, 2009). Caveolins themselves also underlie gene regulation in diverse diseases. Especially caveolin-1 is deregulated in fibrotic diseases and cancers (Zou et al., 2011). Both downregulation and elevated expression of caveolin-1 has been linked to cancer progression. In liver, caveolin-1 expression correlates with cancer progression and poor prognosis (Cokakli et al., 2009; Zhang et al., 2009; Tang et al., 2012). On the other hand, loss of caveolin-1 in mammary tumors can

worsen the outcome of disease and increases the metastatic potential of the tumor (Hayashi et al., 2001; Williams et al., 2004; Patani et al., 2012b). A comprehensive overview about cancer types and caveolin regulation is provided by Williams and Lisanti (2005).

Studies in breast cancer have strongly expanded our knowledge about caveolin function and disease progression. Cancer associated fibroblasts with reduced expression of caveolin-1 have undergone a metabolic reprogramming and thus generate nutrients (as lactate) that support the growth of neighboring tumor cells. This reprogramming of associated fibroblasts can be triggered by TGF-β (Guido et al., 2012).

With respect to wound healing and fibrosis, studies have been published that give evidence for the in vivo TGF-β/caveolin-1 connection. Upon myocardial injury, caveolin-1 knockout animals exhibit increased TGF-β signaling that was accompanied by increased collagen deposition in the heart (Miyasato et al., 2011). In lung fibrosis, lack of caveolin-1 increases collagen and matrix deposition, mediated by enhanced ERK signaling (Tourkina et al., 2005). It still needs to be elucidated whether TGF-β signaling might account for this effect via downregulation of caveolin that has been shown in human pulmonary fibroblasts. Caveolin-1 furthermore suppresses TGF-β mediated extracellular matrix production in fibroblasts via the JNK pathway (Wang et al., 2006; Del Galdo et al., 2008a). Very recently, the relevance of TGF-β/myostatin signaling in muscular dystrophy has been shown (Ohsawa et al., 2012). A mutation in caveolin-3 leads to elevated Smad signaling. This was owing to a lack of negative regulation of type I myostatin receptors (Alk4/5) by caveolin-3 (Ohsawa et al., 2008). Using a new compound to block TβRI kinase activity, Ki26894, the disease was successfully improved (Ohsawa et al., 2012).

Future studies need to focus on analyzing the crosstalk of TGF-β signaling and caveolin expression in vivo to gather better insights into the role in disease progression. A better understanding of the mechanisms might pave the way for more focused therapeutic approaches to medicate such kind of diseases.

Perspectives/Outlook

It is evident that caveolin can modulate TGF-β pathway and functions in many different ways. Even more, different regulatory loops between TGF-β signaling and caveolin exist (amongst TGF-β signaling components as targets of caveolin and vice versa, miRNA regulations) that enable the description of a signaling network. Whereas caveolins' dampening functions on TβRs turnover and Smad activation have been well described, the picture on non-Smad signaling is far from being complete. Especially the observed cell type dependent differences do not allow the drawing of a simple scheme. Therefore, a more detailed analysis of the signaling effects are required to complement our knowledge, from a mechanistic perspective as well as focusing on the outcome of signaling (cell effects, e.g. role on apoptosis, EMT or other cancer progression-related phenotypes).

In particular, the complexity of the reciprocal effects on each other needs to be further assessed in vivo. Although many aspects of caveolin-1 on the TGF-β signaling cascades are known, the relevance in vivo is not well understood. Furthermore, more information is required whether the regulatory functions are implicated in diverse diseases as for example can be speculated in fibrotic lung diseases. Especially, the relevance about the reciprocal influence during disease progression is of interest—like progression of diverse cancers with TGF-β switching from a tumor suppressor to a promoter fostering metastasis formation—is caveolin deregulation participating?

Hence, a better understanding of how caveolin regulates TGF-β signaling cascades and cellular outcome may be invaluable knowledge to enable development of targeted therapeutic approaches to treat specific TGF-β related diseases, with focus on fibrotic disorders and cancers.

Acknowledgments

We are aware that this review is not entirely covering all aspects of caveolin and TGF-β interactions. We apologize to all authors whose work is not included in this review.

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