Molecular Science

AIMS Molecular Science, 4(1): 41-61.
DOI: 10.3934/molsci.2017.1.41
Received 19 October 2016,
Accepted 25 January 2017,
Published 13 February 2017

http://www.aimspress.com/journal/Molecular

*Review*

# The potential role of transforming growth factor beta family ligand interactions in prostate cancer

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**Abstract:** The transforming growth factor beta (TGF-β) family plays an important role in embryonic development and control of the cell cycle. Members of the TGF-β family have pleiotropic functions and are involved in both the inhibition and progression of various cancers. In particular, deregulation of the TGF-β family has been associated with prostate cancer, as both a mechanism of disease progression and a possible therapeutic target. This review concentrates on the TGF-βs, activins and inhibins, bone morphogenetic proteins and NODAL and their connection to prostate cancer. Whilst most studies examine the family members in isolation, there are multiple interactions that may occur between members which can alter their function. Such interactions include ligand competition for receptor binding and shared intracellular pathways such as the Mothers against decapentaplegic (SMAD) proteins. Another mechanism for interaction within the TGF-β family is facilitated by their dimeric structure; heterodimers can form which exhibit different functional capabilities to their homodimeric counterparts. The potential formation of TGF-β family heterodimers has not been well examined in prostate cancer. The multiple methods of interrelations between members highlights the need for gross analysis of the TGF-β family and related factors in association with prostate cancer, in order to discover possible future avenues for TGF-β based diagnosis and treatments of the disease. This review describes the role of the TGF-β family of proteins in cancer and, in particular, prostate cancer. After a brief overview, the role of individual members of the family is considered and how these members may be involved in prostate cancer growth is discussed. The review highlights the complex interactions that occur between family members and that may contribute to the progression of prostate cancer.

**Keywords:** transforming growth factor beta family; transforming growth factor beta; activin; inhibin; bone morphogenetic protein; growth and differentiation factor; nodal growth factor; prostate; prostate cancer; heterodimers

## 1. Introduction

The transforming growth factor beta (TGF-β) family consists of 33 proteins which includes the TGF-βs, activins and inhibins, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) and Nodal growth factor (NODAL). The family members share a similar structure, and functions that include roles in development, wound healing, differentiation and control of the cell cycle [1-6]. Members of the TGF-β family usually exist as dimers and are often secreted in an inactive form which is cleaved by proprotein proteases to produce a mature active region [7]. It is the activated mature fragment that normally binds to the receptor, although there is evidence that the proregion fragment may also affect signalling [8-10]. Although there are 33 members of the family there are considerably fewer receptors available for these ligands to bind. There are five known type-2 receptors and seven known type-1 receptors often referred to as activin-like kinase receptors (ALK1-7) but in this review will be referred with their correct nomenclature (ACVRL1, ACVR1A, BMPR1A, ACVR1B, TGFBR1, BMPR1B, and ACVR1C) [11,12]. Within the mature protein is an active region that is formed of a cysteine knot that commonly binds first to a type-2 receptor, though some are able to bind directly to the type-1 receptor [13,14]. As a result of ligand binding, the type-2 receptor recruits and phosphorylates a type-1 receptor. This receptor complex then activates intracellular signalling proteins, Mothers against decapentaplegic (SMADs), which translocate to the nucleus to alter gene transcription. These components of the canonical TGF-β family signalling pathway depend on phosphorylation to activate them. However, 'non-canonical' pathways also exist and include the utilization of signalling molecules such as the kinases; the mitogen activated kinase (MAPK) pathways including ERK, JNK and p38 and protein kinase B (Akt).

*The role of the TGF-β family in cancer*

The role of the TGF-β family in cancer development and progression is context dependent, and members of the family have been shown to be both cancer suppressive and cancer promoting at different stages of the disease. Some members of the TGF-β family are associated with cancer inhibition in the early stages of the disease, being generally associated with cell cycle arrest and apoptosis. However as the disease progresses and becomes metastatic, resistance to these growth inhibitory effects have been noted, with some TGF-β family members promoting tumour progression and invasion as the functions of the TGF-β family start to become more akin to their roles in early development, in contrast to their roles of tissue maintenance in mature tissue [15,16]. For example, functions such as epithelial mesenchymal transition (EMT) [17,18], fibrosis, myofibrosis, angiogenesis [19] and osteoclast differentiation [20,21] are normal actions of the TGF-β family and are important in the correct context. However, in cancer, aberrant expression of the proteins may exacerbate the condition by allowing the tumour to invade or by altering the microenvironment to favour metastasis [22]. Many TGF-β family related proteins have been shown to be up-regulated in cancer, and have potential use as diagnostic markers, or to monitor disease progression [3,23,24]. An example of a TGF-β family related target in prostate cancer progression is plasminogen urokinase A (PLAU), which cleaves plasminogen in the extracellular matrix and allows mass migration of cells. Cleaving of plasminogen when expressed normally assists with wound healing, but in cancer aids invasion [25-27]. Another TGF-β family target gene, that when expressed aberrantly results in the advancement of cancer, is homeobox protein goosecoid (GSC) which is involved with the Spemann

organiser and induces the migration of cells [28]. Likewise, the association of various TGF-β family members with bone formation and control, makes bone a target for the aberrant expression of TGF-β family members observed in cancer. Target genes of the TGF-β family in bone production and metastasis include receptor activator of nuclear factor κ B (RANK), which may be promoted with increased TGF-β production. This aids metastasis to bone as RANK promotes osteoclast differentiation allowing for the breakdown of bone tissue and for the invasion of the cancer cells into bone. Other TGF-β associated genes that may aid bone metastasis include connective growth tissue factor (CGTF), which may act as an extracellular mediator of invasion and angiogenesis, and interleukin 11 (IL11) which stimulates receptor activator of nuclear factor κ B ligand (RANKL) to activate osteoclastic activity [29,30]. In healthy mature bone tissue, the TGF-β family is associated with the formation and absorption of bone by osteoblasts and osteoclasts. In prostate cancer metastasis this relationship and balance is lost, resulting in the promotion of metastasis and tumor growth in bone [31,32]. The relationship between TGF-β family members, prostate cancer and metastasis to the bone is complex due to the large number of ligands involved in bone growth, their pleiotrophy, and the heterogeneous nature of prostate cancer.

The relationship between the TGF-β family and prostate cancer may be examined at three possible levels: the expression of the TGF-β family ligands, the receptors utilized and also the signalling pathways activated. Complexity is derived from the number of possible ligands, their structure and multiple signalling pathways. An inter-ligand relationship is evident in that members of the TGF-β family are shown to interact with each other, either directly, through shared receptors, or in the form of heterodimerisation and cleaved fragment interactions. As a consequence, the signalling pathways, canonical and non-canonical, may be affected by competition, or inhibition by other TGF-β family proteins as well as the formation of heterodimers that have different functions [33-35]. Therefore, this review proposes that an important avenue for further investigation is how interactions between members of the TGF-β family may affect prostate cancer development and progression.

## 2. TGF-β

In mammals the TGF-β proteins comprise of three main isoforms: TGF-β1, TGF-β2 and TGF-β3. These isoforms share approximately 75% sequence homology, differing mostly in their extracellular interacting domains [36]. All have 9 cysteine residues that form disulphide bonds between subunits. TGF-β is secreted *in vivo* as a latent complex (pre-pro-TGF-β). The precursor molecule is then cleaved by the proprotein cleavage protein, furin [2] to release the mature signalling portion. The three isoforms of TGF-β, have a conserved protein structure in humans, simians, porcine, bovine, and mice [37]. Despite having quite similar structures, they have distinct temporal and spatial expression patterns throughout development and in mature tissues. This suggests that the three isoforms have differing functions prenatally and that these functions are conserved between species [37-39]. The isoforms usually bind initially to the TGF-β type-2 receptor (TGFBR2), which then recruits the TGF-β type-1 receptor (TGFBR1). This complex then acts by phosphorylating SMAD2/3, which then recruits SMAD4 resulting in activation of gene transcription. The individual isoforms differ in how they bind to the type-2 receptor. While TGF-β1 and TGF-β3 bind directly to the receptor, TGF-β2 requires betaglycan (TGFBR3) to assist binding to TGFBR2 [40].

There are multiple ways that TGF-β signalling is regulated. A common method of regulation of TGF-β signal transduction is through inhibitory SMADs, I-SMADs, and other associated molecules

such as E3 ubiquitin-protein ligase, SMURF1 and SMURF2 proteins [41,42]. TGF-βs may bind to latent TGF-β binding proteins (LTBP) which can prolong the half-life of the molecule as well as provide recognition sites to promote the activation of the molecules. These various mechanisms of control allow tight regulation of the effects of TGF-β [43]. Tight regulation of TGF-βs' signalling is important because of their multiple roles in development, tissue maintenance and wound healing, which when unbalanced may lead to pathologies such as cancer. Other modulatory molecules that regulate TGF-β activity include micro ribonucleic acids (miRNA) [44-46]. These have been shown to modulate both the canonical and non-canonical signalling pathways and potentially provide another degree of control. As well as signalling through SMAD2/3, there is evidence that TGF-β also signals via SMAD1/5/8, through co-expression of the WNT pathways in chondrocytes. This co-expression of WNT signalling pathway and TGF-β was shown to increase TGF-βs' potential to phosphorylate SMAD1/5/8 and decrease the phosphorylation of SMAD2/3 [47]. A possible action of TGF-β phosphorylated SMAD1 molecules is to form complexes with SMAD3, this signalling molecule complex may bind to BMP-responsive promoters, thus inhibiting BMP SMAD1 signalling [48]. This type of cross-signalling has also been shown in breast cancer [49], but such a relationship has yet to be proven in the prostate.

### *2.1. Expression of TGF-β in the prostate and prostate cancer*

The three TGF-β isoforms are all expressed in the prostate [50]. Their expression is regulated by androgens, with increased androgen expression associated with decreased TGF-β production [51]. In humans, TGF-β1 is predominantly localised intracellularly in the stromal and epithelial cells of healthy prostate tissue. In prostate cancer tissues expression of the TGF-β1 protein in epithelial cells increases, with only a slight increase in TGF-β1 in the surrounding stroma in comparison to healthy tissue [50]. There is evidence that the prostatic epithelia of both rats and humans primarily produce TGF-β2, which is localised to the apical region of the epithelial cells. TGF-β2 protein expression is more diffuse in malignant tumour epithelia and expression is greater in stromal cells surrounding malignant epithelia than stromal cells surrounding healthy prostate epithelia [50,52]. TGF-β3 protein, like TGF-β2, is predominantly localised to the apical region of the epithelial cells of the prostate. In malignant epithelia TGF-β3 expression is more diffuse, but there is no change in expression in the stromal cells [50]. Another *ex vivo* study showed a decrease in TGF-β3 expression in prostate cancer tissue compared to the other isoforms of TGF-β and also when compared to the healthy prostate and benign prostate hyperplasia [53-55].

### *2.2. The role of TGF-β in prostate cancer*

The role of TGF-β in prostate cancer appears to depend on the stage of the disease. The three isoforms have tumour suppressive effects early in cancer development associated with cell cycle arrest and apoptosis. However, in later stages, their presence exacerbates the disease [56,57]. The effectiveness of each isoform in inducing aggressive cancer characteristics differs. TGF-β1 has been shown to be up-regulated in patients with prostate cancer, increasing angiogenesis and tumour growth [58-60]. TGF-β1's role in prostate cancer metastasis has recently been shown to be linked with both tumour necrosis factor receptor (TNFR)-associated factor 6 (TRAF6) cleaving the intracellular domain of the TGFBR1, and localisation of that cleaved fragment to the nucleus of

prostate cancer cells [61]. Conversely, the up-regulation of TGF-β2 has been shown to arrest growth in PC3 cells [62,63]. TGF-β3 is the most potent in inducing invasive capabilities in PC3 cells, acting via SMAD3 signalling. TGF-β3 is expressed at higher levels in PC3 cells compared to TGF-β1 and TGF-β2, but has no effect on proliferation of the PC3 cell line. It instead causes loss of cell adhesion and increased cell motility. TGF-β3 does, however, inhibit the proliferation of the prostate cancer cell line DU145 [64-66].

Disruption of TGF-β signalling seems to be a major component in the progression of TGF-β related prostate cancer. Loss of TGFBR2 was found by Williams et al. (1996) in all 22 patients with prostate cancer studied when compared to healthy prostate controls and BPH tissue [67]. SMAD4 signalling, a target of TGF-β signalling, has been shown to be protective against the development of various cancers, with mutations being associated with cancer progression [68,69]. Therefore inactivation of TGF-β signalling, either through the loss of receptors, or inactivation of the canonical signalling pathway have been found to be associated with the rapid development of invasive metastatic tumours [70]. The anti-tumour effects of TGF-β include their ability to induce apoptosis through multiple targets such as: phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1 (SHIP) proteins, death-associated protein 1 (DAP)-kinases, Kruppel-like factor 10 (TIEG) and cJUN-N-terminal kinase (JNK) activation [71] and mitogen-activated kinase kinase 3 [72]. Knocking out or inhibiting TGF-β signalling has been shown to induce malignant phenotypes [17]. Activation of the canonical signalling of TGF-β leads to recruitment of SMAD4 which is associated with increased apoptotic bodies in prostate tumour tissue [12,73].

The tumour promoting effects of TGF-β are based around increasing the ability of the cells to proliferate, invade, and evade the immune response. For example, TGF-β can induce cells to differentiate into myofibroblasts, which as secretory cells, may secrete factors that promote tumour development [74]. TGF-βs have been shown to stimulate the collective migration of cells through the non-canonical ERK pathway [17]. Exposure of naive T cells to TGF-β3 inhibits their development into the tumour targeting immune cells CD4+ helper T cells or CD8+ cytotoxic T cells [18,75]. There are a multitude of variables that change how TGF-β signalling may be controlled. These may included multiple non-SMAD signalling molecules which are able to direct signalling to induce metastasis in prostate cancer [76]. With the structural similarity between TGF-β molecules and other TGF-β family members, alteration in ligand expression levels may result in promotion of prostate cancer. Further studies are needed to better understand the effect of each isoform in relation to the prostate and prostate cancer.

## 3. Activins and inhibins

Other members of the TGF-β family include the activins and inhibins. These proteins are composed of dimers of the inhibin-α and -β subunits; inhibin consisting of an inhibin-α and an inhibin-β subunit and activin formed by inhibin-β dimers. Activin is made up of two inhibin-β subunits that exist as 4 isoforms A, B, C, and E and may form either heterodimeric or homodimeric forms. These activin dimers bind to type-2 activin receptors A or B (ACRV2A/ACVR2B) to initiate signalling with the recruitment of the type-1 activin receptor (ACVR1). Activin E is expressed solely in the liver, while activin A, B and C are expressed in the prostate.

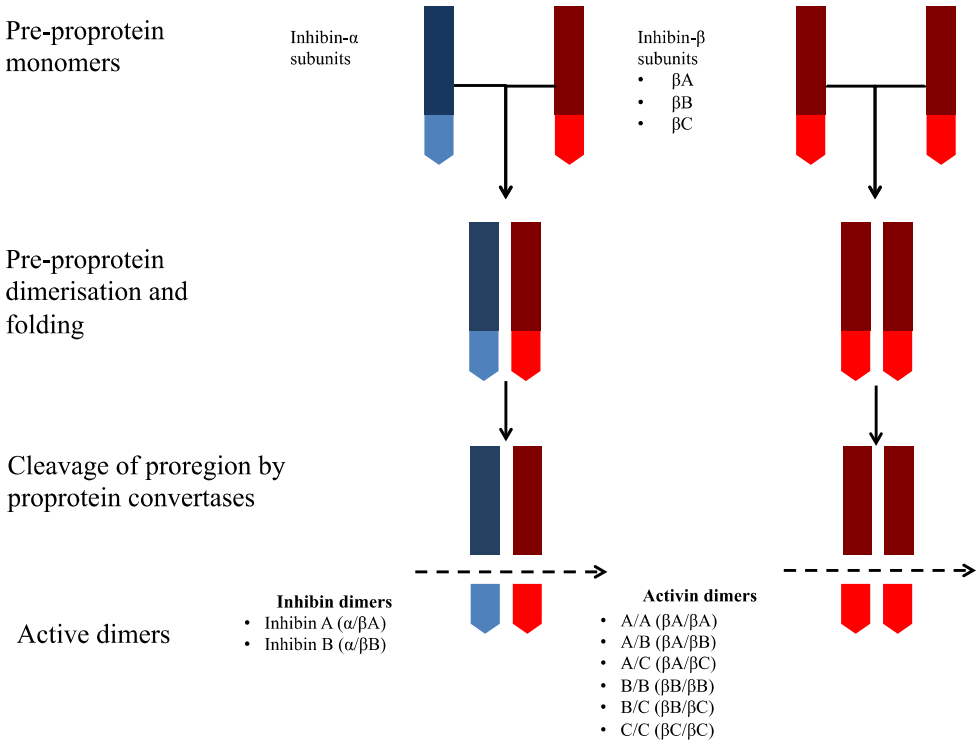


**Figure 1.** Diagram of activin and inhibin formation from proprotein monomers to active homo- and hetero-dimers. Inhibin-α subunits in blue and inhibin/activin-β subunits in red.

Activins have been shown to have the greatest capacity of the TGF-β family to interact with each other and other family members. Activin was named for its ability to stimulate secretion of follicle stimulating hormone (FSH). Conversely inhibin, a molecule that inhibits activin signalling, was named for its ability to oppose FSH signalling. Inhibin antagonism of activin signalling is derived from its competition for the type-2 receptor, which is aided by betaglycan which acts as a co-receptor, preventing the subsequent dimerization and phosphorylation of the type-1 receptor [10]. The most studied isoforms of activin are the subunits inhibin-βA and inhibin-βB which share 63% sequence homology. These form the homodimers activin A and activin B, and the heterodimer activin AB. Though these subunits are similar in structure their expression patterns are distinct during development, and knockout of the genes has specific effects [77]. Activins are structurally similar to the TGF-βs hinting at a possible redundancy in signalling. The differing roles that TGF-β and activin have are hard to elicit postnatally, due to activation of SMAD2 and SMAD3 by both families, as well as non-canonical signalling [35,78,79]. Activin A has been shown to have many inhibitors. Inhibin and follistatin are the most well-described inhibitors, while activin C has also been shown to be antagonistic towards activin A and B signalling, competing for the type-2 activin receptor [23,80,81]. The inhibin-βC subunit also has the ability to dimerise with the inhibin-βA and -βB subunits forming activin AC and activin BC heterodimers. These heterodimers have altered binding and signalling capabilities, with the AC heterodimer having less affinity for the receptor and less bioactivity than activin A [82]. The formation of these heterodimers decreases the formation of activin A [83].

Activins and inhibins are secreted as large monomeric subunits consisting of the proregion bound noncovalently to the mature region. These proregion bound monomers then dimerise and fold

to form the pre-mature protein. The formed dimers are aided in their binding to the receptor by their proregion which is cleaved by proprotein convertases prior to receptor binding (Figure 1) [10,84,85]. The proregion fragments have the potential to alter the signalling and binding of inhibin and activin. The inhibin-α subunit proregion fragment prevents the binding of inhibin A to betaglycan, which inhibits binding of inhibin to the type-2 receptor. Alterations to the cleavage site of the proregion also greatly affect synthesis of inhibin [9]. The presence of the proregion in inhibin-β subunits, however, increases the half-life of activin *in vivo* when compared to its mature protein counterpart, and as such the role of the prodomain has been studied as a possible therapeutic modulator of activin [10,86].

### *3.1. Expression of inhibins and activins in the prostate and prostate cancer*

Inhibin is primarily produced in the testis, however the prostate has a method of concentrating inhibin resulting in inhibin concentrations being much higher than those in the testis [87]. In the prostate, inhibin expression is mostly localised to the epithelial cells and is more concentrated in the central zone of the prostate [88]. In the non-malignant prostate, inhibin-βA subunit mRNA and protein are localised in the basal and secretory epithelial cells. The inhibin-βB subunit protein is predominantly located in the basal epithelial cells of non-malignant prostate tissue rather than the secretory epithelial cells [89].

### *3.2. The role of activins and inhibins in prostate cancer*

The role of inhibin in prostate cancer is complex. It was initially thought to be an inhibitor of prostate cancer development because it is not detected in prostate cancer cell lines [90] and expression is reduced in some prostate cancer patients [91]. Inhibin expression has, however, been found to be increased in metastatic prostate cancers *in vivo* [92]. Further roles in cancer for activins and inhibins are suggested by the evidence that inhibin knockout mice develop sex cord stromal tumours with activin being found in supraphysiological levels in these mice [80,93,94].

Activin is usually associated with growth inhibitory effects in the normal prostate and in low grade prostate cancer cell lines, where it has apoptotic and cell cycle arrest inducing roles [95-97]. Like TGF-β, activin has been shown to have dual roles in cancer. The role of activin A and B in cancer follows a similar pattern to TGF-β, where they are growth inhibitory early on in cancer development, but have no effect or promote tumour development in advanced forms of the disease [95]. Activin A has been shown to be up-regulated in breast and prostate cancer, with the up-regulation in prostate cancer correlating with an increased Gleason score [98]. Increased activin A is also associated with metastasis to bone, a common site of metastasis in prostate cancer [21,99]. The activin C knockout mouse has no evidence of prostate abnormality. Up-regulation of activin C, however, has been shown to significantly decrease activin A signalling and induce prostate epithelial cell hyperplasia (personal communication). Activin C antagonises activin A growth inhibition in the activin-sensitive prostate cancer cell line LNCaP [23,100] and slows down the progression of cancer where activin A signalling is an underlying factor [80]. Further research is needed to understand how the complex associations between activins and inhibins are involved in the progression of prostate cancer.

## 4. Bone morphogenetic protein (BMP)

The BMP family is comprised of 10 ligands [101,102]. Like other members of the TGF-β family,

the members share conserved cysteine residues and exist as dimers. The BMP family shares approximately 35% homology with the other TGF-β family members and within the BMP family there exists between 60–80% sequence homology. The BMPs normally bind to the BMP type-2 receptor (BMPR2) and recruit the type-1 BMP receptor (BMPR1). Canonical signalling utilizes SMAD1, 5, and 8, though like other members of the TGF-β family they are able to signal via non-canonical pathways. BMPs were first implicated in regulation of bone formation via osteoblast differentiation [103,104]. They are also important in dorsal ventral axis formation and have other developmental functions [105].

Inhibitors of BMP function include BMP3, BMP binding endothelial regulator (BMPER), noggin, and BMP and activin membrane-bound inhibitor homolog (BAMBI) [106-108]. BMP3 is a unique member of the BMP family in that it is the only inhibitory BMP, fulfilling a role similar to that of activin C in the inhibition of activin A and B. BMP3 inhibits the signalling of other BMP isoforms through receptor competition. Knockdown of BMP3 results in increased bone density, while its up-regulation causes the development of fracture-prone bones [109]. BMP3 not only competes for BMP receptor binding, preventing signalling from occurring, but may also inhibit activin A signalling [110]. BMP3 is able to promote adipogenesis via activin A pathways, providing an example of how the different SMAD pathways may interfere with each other, as well as the structural and functional similarities between family branches [111]. This interference between the BMPs and activins is further evidenced by BMP3 inhibition of activin A signalling and activin inhibition of BMP6 and BMP9 signalling due to competition for binding of the type-2 receptors ACVR2A and ACVR2B [110,112]. Similar to the activin family, heterodimers of the differing BMP subunits can be formed. These BMP heterodimers appear to have altered signalling ability compared to their homodimer counterparts. An example of this is BMP2/7 which differentially targets TGF-β signalling when compared to BMP2 and BMP7 homodimers. The heterodimers have been shown to be more potent in inhibiting breast cancer and TGF-β directed SMAD signalling and less susceptible to noggin inhibition than BMP2 and BMP7 alone [34].

### *4.1. The role of BMP in the prostate and prostate cancer*

Due to their role in development and their expression throughout the body BMPs have been studied for a potential role in cancer. Tissues from healthy prostate and prostate cancer have been shown to express members of the BMP family, namely BMP2, BMP3, BMP4, BMP6 [113] and BMP7 [114]. The potential role of BMPs in the prostate may be tied to development, for example BMP4 has been shown to restrict ductal formation and branching morphogenesis of the developing prostate [115]. The metastasis promoting contributions of BMPs are often associated with increased invasion into bone, whereas the cancer inhibiting roles of BMPs are associated with induction of apoptosis [116]. BMP2 and BMP6 have been shown to increase tumour invasiveness of both prostate cancer and other cancer types [117,118]. BMP2 has been reported to enhance the motility of the prostate cancer cell lines LNCaP, DU145 and PC3 [119] and both BMP2 and BMP4 have been shown to increase PC3 cell migration and invasion [120]. BMP6 secreted by prostate tumours may also have a role in aiding invasiveness by up-regulating vascular endothelial growth factor (VEGF), a protein involved in angiogenesis [121]. BMP7 and BMP4 have been shown to have an inhibitory effect on tumour invasiveness in glioblastomas [122] and BMP7 is down-regulated in prostate cancer [114,123]. BMP7 is a potent inhibitor of TGF-β induced EMT, counteracting TGF-β induced SMAD activation

as well as reducing metastasis to bone *in vivo.* BMP7 also has the ability to form heterodimers with BMP2 which can have inhibitory effects on certain cancers [34].

### *4.2. The role of GDFs in prostate cancer*

Often described as a sub group of the BMP family, the growth and differentiation factors (GDFs) are comprised of 10 members [101]. These ligands can also influence cancer development. GDF9 overexpression has been shown to increase proliferation in PC3 cells acting through TGFBR1 receptors [124,125]. GDF9 also has the ability to dimerise with BMP15, and is involved in regulating ovarian function [126,127]. BMP15 has not been identified in the prostate, but the ability of GDF9 to dimerise and its ability to induce EMT in PC3 cells warrants further investigation [125,128]. GDF15, also referred to as macrophage inhibiting cytokine, is another ligand that is up-regulated in prostate cancer. GDF15 is associated with stress, tissue damage and development. The alteration of both serum GDF15 and PSA levels seems to have a greater correlation with the high Gleason score of metastatic prostate cancer than prostate specific antigen (PSA) alone [129]. The expression of GDF15 is independent of PSA expression, which is important because PSA levels may be influenced by multiple conditions such as benign prostate hyperplasia and aging. Circulating GDF15 has potential as a diagnostic biomarker when it is used in conjunction with PSA, providing an increase in diagnostic accuracy in determining metastatic prostate cancer [130,131].

## 5. NODAL

NODAL is a TGF-β family ligand and is also an activin antagonist. As with many other members of the TGF-β family, NODAL has a multitude of functions in embryonic development and is essential in maintaining pluripotency and mesoderm patterning [132]. NODAL homodimers, aided by Cripto, bind to ACVR2B receptors and then recruit ACVR1B and ACVR1C type-1 receptors. They compete with activin for receptor binding, preferentially activating SMAD2 [133-135]. Like other TGF-β members, NODAL signalling has an additional layer of complexity with Cripto-independent mechanisms of signalling. Heterodimers consisting of BMP7 or BMP3 and NODAL are also possible. The affinity of NODAL for dimersiation with BMP7 and BMP3 is as strong as the affinity for NODAL homodimerisation. *In vitro* these heterodimers do not activate either SMAD1 or SMAD2 and act as a method of inhibiting BMP signalling [135]. NODAL signalling can be inhibited by Lefty and Cerberus proteins. Lefty inhibition of NODAL signalling occurs via two possible mechanisms that prevent NODAL from forming NODAL-receptor complexes; firstly by binding to Cripto, and secondly by binding directly to NODAL itself [136]. Likewise, Cerberus binds directly to NODAL preventing receptor-binding and signalling [137].

### *Role of NODAL in prostate cancer*

Expression of NODAL is largely confined to embryonic development and stem cells, and is generally absent from normal adult tissue. Its presence in adult cancerous tissue makes it a promising target for cancer therapy, especially in prostate cancer [132,138]. NODAL and its receptors are up-regulated in the progression of various metastatic cancers, such as pancreatic and gastric cancer. NODAL signalling has been reported to be influential in other cancers with its up-regulation

increasing glioblastoma proliferation [139]. Up-regulation of Cerberus and Lefty has been able to inhibit the metastatic ability of NODAL expressing breast cancer cells [140]. The expression of NODAL *in vitro* is associated with the more aggressive prostate cancer cell lines DU145 and PC3, and when expression is induced in the less aggressive LNCaP cells, they exhibit increased invasive abilities [138,141]. *In vivo* there is a possible association between NODAL expression and prostate cancer progression, with increased NODAL staining in high grade prostate cancer biopsy tissue when compared to low grade prostate biopsy cancer biopsy tissue, and little to no expression of NODAL in benign prostate glands. The expression of the embryonic developmental gene NODAL in prostate cancer highlights a potential role for NODAL in the progression of prostate cancer [138].

## 6. Interactions of the TGF-β family

Many studies that explore the TGF-β family's involvement in cancer examine a few members in isolation [94,110,121,123]. Whilst this is useful for teasing out the function of a specific gene and associated protein, these studies ignore the fact that due to structural and functional similarities, there may be cross-talk, interactions or redundancy occurring between ligands and their signalling pathways. Consideration of these possible interactions are important when many ligands can interact with a single receptor (Figure 2), and other co-factors and inhibitory factors exist [78].

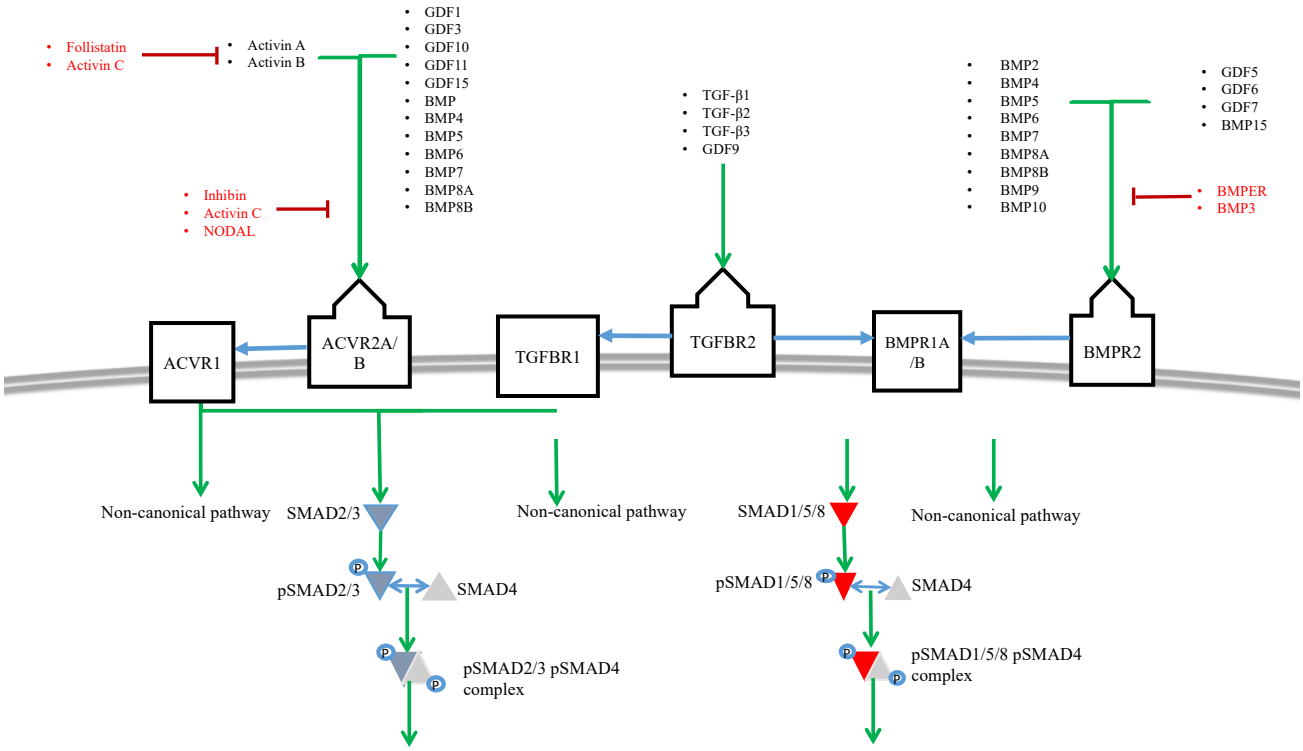


**Figure 2.** Simplified diagram of the signalling pathways that the main members of TGF-β family may employ. The diagram highlights the high number of ligands relative to the low number of receptors and signalling pathways. The ligands typically bind to type-2 receptors which then recruit type-1 receptors via phosphorylation. Inhibitory family members are shown in red.

Interactions between family members include competition for receptors and downstream signalling molecules, and heterodimer formation. These mechanisms have been demonstrated for specific members of the TGF-β family with members able to either prevent a ligand from binding to its receptor [110,112] or altering its signalling by the formation of heterodimers [34,83]. The receptors for TGF-β and activin are almost identical, and the TGF-β and activin signalling pathways share a total of 21 overlapping target genes [78]. This possible overlap in signalling could be achieved either through interactions between ligands, either directly or by competition for receptors, or due to shared recruitment of SMAD2, SMAD3 and SMAD4 signalling [35,79]. Such interactions and complexity may hinder the promise of TGF-β family associated treatments of cancer [23,80,142].

### *6.1. Heterodimerisation in the TGF-β family*

The dimeric structures of TGF-β family members allow for a wide variety of ligand formation both within and between family branches (Figure 3). Activins and inhibins are expressed in the prostate and have the capacity to form both homo and heterodimers and interact with each other *in vivo* [83]. The heterodimers of inhibin subunits are especially important to consider when there are dimers that oppose the signalling or interfere with the signalling of other ligands. This is apparent with inhibin, (inhibin-α and inhibin-β heterodimer), and activin C heterodimers (activin AC and activin BC), which are antagonistic to the signalling of activin A and activin B homodimers [83]. Likewise, BMP heterodimers may form and have been observed to have altered receptor-binding capabilities compared to their homodimer counterparts. BMP2 and BMP7 can form heterodimers which have different functions to their homodimer counterparts, potentiating the inhibition of breast cancer metastasis to bone and also reducing the size of the cancer stem cell population [34]. Other BMP heterodimers include BMP2/6, which has a higher affinity for ACVR2B and BMPR1A and exhibits increased signalling via SMAD1 when compared to either of the homodimers [143]. BMP4/7 produces increased SMAD1 activation in xenopus embryos when compared to BMP4 or BMP7 alone [144]. Heterodimers of BMP15 and GDF9 are far more potent in altering ovarian function than BMP15 and GDF9 alone [128]. NODAL may form a heterodimer with BMP7, with a similar kinetic affinity for heterodimerism as their homodimeric counterparts. This association between NODAL and BMP may be a mechanism for NODAL based inhibition of BMP signalling as the formed heterodimer does not activate phosphorylation of SMAD1 or SMAD2 [145]. Heterodimers also occur between GDF1 and NODAL which result in altered receptor-binding kinetics binding to the co-receptor Cryptic rather than Cripto. These heterodimers have a lower molecular weight with enhanced diffusion capabilities than NODAL homodimers which may aid signalling during embryonic development [146]. Aside from heterodimers of inhibin/activin subunits, heterodimers of other TGF-β family members have not been documented in the prostate or in prostate cancer. Potential heterodimers may contribute to the development of prostate cancer by altering receptor binding and signalling of TGF-β superfamily ligands. Such interactions between members of the TGF-β family adds further complexity to the role of these proteins in prostate cancer but may also provide potential targets for the development of new therapies.

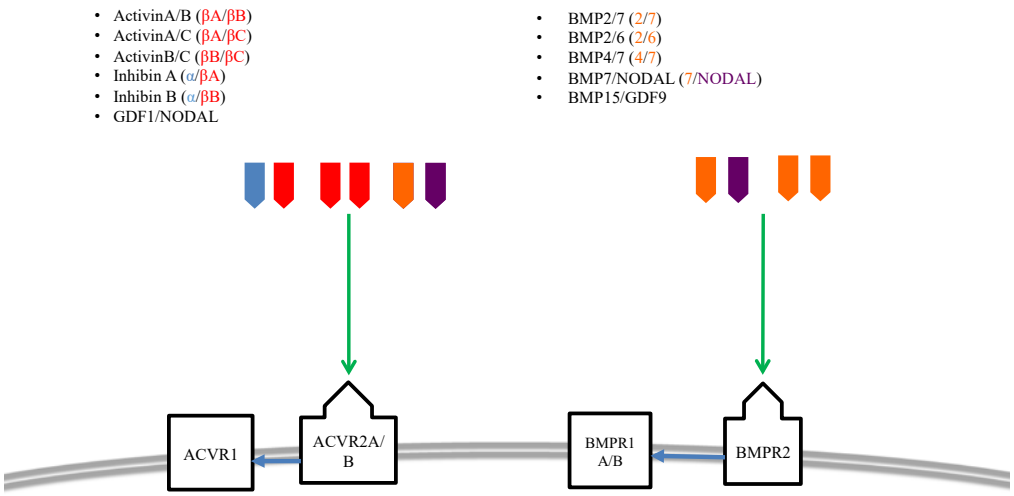


**Figure 3.** Known heterodimers of the TGF-β family that involve the inhibin-α (blue), inhibin-β (red), BMP (orange) and NODAL (purple) subunits and the receptors that they may signal through.

### *6.2. Receptor competition and inhibition*

The common structure between family members means that there are many shared modulators of the TGF-β family. For example, BMP and activin receptor binding can both be inhibited by BAMBI [134]. Activin A signalling is opposed directly by inhibin and activin C both of which compete for binding to the type-2 activin receptor. The inhibition of activin pathways in particular has been highlighted as an important area of research for possible therapeutics for prostate cancer [23,80,147]. BMP3, as well as inhibiting BMP signalling, has also been shown to inhibit activin A signalling. Again, this inhibition of activin A signalling occurs through competition for the activin receptor by BMP3, preventing activin signalling from occurring [111,112]. Cripto, the co-receptor of NODAL, helps NODAL form a stable NODAL-Cripto-receptor complex that recruits SMAD2, but Cripto has also been shown to bind activin and the ACVR2A and ACVR2B receptors. This interaction forms a non-signalling receptor complex with activin, preventing it from recruiting the type-1 receptor and thus inhibiting activin signalling [133]. Therefore the presence of Cripto aids NODAL signalling but inhibits activin signalling [135]. This prevention of activin signalling and promotion of NODAL related effects, may be a mechanism by which Cripto and NODAL expression can influence the progression of prostate cancer [138].

## 7. Conclusion

TGF-β family members have important roles in development [148]. Hijacking of these functions can lead to cancer promoting effects [17,19,66,79]. As such, there has been much study into the roles that this large group of proteins may play in various cancers, including prostate cancer [15,101]. As well as interactions between members of the same family, members of different families also interact with each other. This complexity of signalling could possibly alter the effectiveness of TGF-β

derived treatments [23,142]. The greatest hurdle to fully understanding TGF-β family signalling in relation to prostate cancer, is the sheer number of possible interactions that are not apparent when studying a protein or gene in isolation. A holistic view of the superfamily is therefore required. The availability of techniques, such as whole transcriptome sequencing, that allow the level of expression of multiple members of this family to be measured at the same time in tumours, will greatly benefit our overall understanding of the TGF-β family's role in prostate cancer. With improvements to genomic and proteomic techniques, the ability to study the co-expression and interactions between TGF-β family members is now easier, allowing for more holistic studies to be carried out. With a better understanding of the interactions between TGF-β family members and their downstream effects, diagnostic and therapeutic agents specific to tumour type may be developed.

**Acknowledgements**

Special thanks to Dr. Elspeth Gold, who was a great inspiration and driving force behind this review. You will be dearly missed and your passing is a loss to the scientific community and prostate cancer research.

**Conflict of interests**

All authors declare no conflict of interest in this paper.

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