HORM CANC (2017) 8:100–107
DOI 10.1007/s12672-017-0283-8

ORIGINAL PAPER

Over-Expression of Activin-β_C Is Associated with Murine and Human Prostate Disease.

Edward C. Ottley[1] · Karen L. Reader[1] · Kailun Lee[1] · Francesco E. Marino[1] · Helen D. Nicholson[1] · Gail P. Risbridger[2] · Elspeth Gold[1]

Received: 26 September 2016 / Accepted: 9 January 2017 / Published online: 23 January 2017

Abstract Activins are members of the TGF-β superfamily and have been linked to prostate cancer. There are four mammalian activin subunits (β_A, β_B, β_C, and β_E) that dimerize to form functional proteins. The role of activin-A (β_A-β_A) has been relatively well characterized and has been shown to generally inhibit growth in the prostate. In contrast, little is known about the biological function of the β_C and β_E subunits. Previous work indicated activin-C (β_C-β_C) to be an antagonist of activin-A. This is important because resistance to activin-A growth inhibition occurs during prostate cancer progression. This paradox is not currently well understood. Hence, we hypothesize that local expression of the activin-β_C subunit antagonizes activin-A-dependent growth inhibition and represents a key factor contributing to acquired insensitivity to activin-A observed in prostate cancer progression. To test our hypothesis, we characterized the ventral prostate lobes of 9-month-old transgenic mice over-expressing activin-β_C and examined the expression of activin-β_A, activin-β_C, and the activin intracellular signaling factor, Smad-2, in human prostate diseases. Prostate epithelial cell hyperplasia, low-grade prostatic intraepithelial neoplasia (PIN) lesions, alterations in cell proliferation, and reduced Smad-2 nuclear localization were evident in mice over-expressing activin-β_C. Increased activin-β_A and -β_C subunit immunoreactive scores and decreased Smad-2 nuclear localization were also evident in human prostate cancer. This study suggests that over-expression of activin-β_C is associated with murine and human prostate pathologies. We conclude that the activin-β_C subunit may have therapeutic and/or diagnostic implications in human prostate disease.

Introduction

In the USA, UK, Australia, and New Zealand, prostate cancer is one of the most commonly diagnosed male cancers and one of the leading causes of cancer deaths [1]. At the time of diagnosis, the majority of men have localized disease and treatment options include radiotherapy, radical prostatectomy, or active surveillance. About one third of these men will develop an aggressive form of the disease. Androgen deprivation therapy (ADT) is commonly used as standard care for the aggressive form because it induces a rapid atrophic response, reducing tumor size. However, as the tumor progresses, it becomes castration-resistant, evading the androgen deprivation therapy. This is mainly due to the ability of prostate cancer cells to adapt to low systemic androgens by activating androgen receptor mutations and utilizing other sources of androgens [2]. Tumor adaptation to ADT represents a major clinical challenge for prostate cancer treatment.

Both endocrine and paracrine factors are essential for the normal maintenance of tissue homeostasis within the prostate [3]. Activins, members of the TGF-β superfamily, are known to play a pivotal role in prostate homeostasis. Activins take the form of either homo (β_A-β_A, β_B-β_B)- or heterodimers (β_A-β_B) of the β_A or β_B subunits linked by a disulfide bridge. Activin-A (β_A-β_A) is a potent growth regulator that inhibits the proliferation of prostate epithelial cells [4]. Following

Dr. Elspeth Gold passed away on August 21, 2015.

Edward C Ottley and Karen L Reader are joint first authors.

✉ Karen L. Reader
karen.reader@otago.ac.nz

[1] Department of Anatomy, University of Otago, PO Box 913, Dunedin 9054, New Zealand

[2] Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia

binding of activins, or other TGF-β superfamily members (including growth differentiation factor-1 and nodal) [5, 6] to one of the two type 2 receptors (ACVR2A or ACVR2B), a type 1 receptor (ACVR1B) is recruited and phosphorylated [1]. This in turn phosphorylates the intracellular signaling molecules Smad-2 and Smad-3 which complex with Smad-4 and translocate to the nucleus. This results in the activation or repression of target genes.

After the initial discovery of activin-A, another subset of activin-β subunits (β_C, β_D, β_E) was identified, based on their homology to the β_A and β_B subunits. It has been shown that mice bearing a functional deletion of the activin-β_C and/or -β_E subunit genes do not show developmental defects and are phenotypically normal [7]. This evidence was used to conclude that activin-β_C was not biologically relevant. In 2009, our group proposed the hypothesis, that in the context of the null mouse, there may be functional redundancy with other transforming growth factor-β family members and that over-expression of activin-β_C, rather than under-expression, is more likely to have physiological consequences. Recent work has shown that activin-C antagonizes activin-A both in vivo and in vitro [8–10]. In these studies, we clarified the biological mechanism by which activin-C antagonizes activin-A, and we suggested that activin-C should be placed in the same domain as well-characterized regulators of activin-A, such as follistatin.

The identification of activin-C as a new antagonist of activin-A has important implications because deregulation of the activin-A signaling pathway is associated with prostate disease development [11]. Many tumor cells, including those of prostate cancer, escape the growth inhibitory effects of TGF-β by acquiring mutations in the TGF-β receptors and intracellular signaling molecules. Similarly, some tumor cells develop a mechanism to evade the growth inhibitory effects of activin-A. Resistance to activin-A-induced growth inhibition becomes evident during prostate tumor progression [12] and represents an acquired capability of cancer cells [1, 13]. Activin-A has been shown to inhibit proliferation in the human prostate cancer cell lines LNCaP (low-grade, androgen-dependent) and DU145 (medium-grade, androgen-independent) but not in high-grade, androgen-independent PC3 cells [4, 14, 15]. The addition of PC3 conditioned media to LNCaP cells blocks activin-A-mediated growth inhibition of these cells indicating PC3 cells secrete an inhibitor of activin-A. Circulating levels of activin-A have been shown to significantly increase in high-grade metastatic prostate cancer [16, 17]. While the majority of studies have shown activin-A to be growth inhibitory in prostate cancer, some groups have shown that activin-A has the ability to promote growth [18, 19]. The mechanisms by which high-grade metastatic prostate tumors evade the growth inhibitory effects of activin-A are not well understood but are likely to involve activin-A antagonists such as follistatin and/or the novel antagonist activin-C. Further clarification of activin-A function in prostate and prostate cancer is needed.

The current study aimed to test our hypothesis that local expression of the activin-β_C subunit antagonizes activin-A-dependent growth inhibition and is a factor mediating insensitivity to the growth inhibitory effects of activin-A during prostate cancer progression. We have examined the effect of activin-β_C over-expression on the ventral prostate of aged transgenic mice measuring changes in proliferation, apoptosis, and morphology. Levels of activin-β_A, activin-β_C, and the activin signaling molecule Smad2 were compared between normal and diseased human prostate biopsy sections. The results demonstrate a role for activin-β_C in prostate cancer progression.

Materials and Methods

Experimental Animals

All experiments were approved by the Animal Ethics Committee of the University of Otago and conducted in accordance with the New Zealand code of practice in adherence with the NIH Guide for the care and use of laboratory animals. All animals were housed under a 12:12 h light-dark cycle, with food and water available ad libitum. Human-activin-β_C over-expressing mice (ActβC++) were obtained from Monash University, Australia, as previously described [8] and bred at the University of Otago, New Zealand. In our previous study [8], three independent lines were established and crossed with wild-type (WT) C57BL/6 mice and heterozygous littermates to obtain single-heterozygous (SH) haploid and double-heterozygous (DH) diploid transgenes. Southern blot and semi-quantitative polymerase chain reaction (PCR) were used to determine the transgene copy number. SH1 had 2–5 copies of the transgene, SH2 5–10 copies, and SH3 20–30 copies. The double-heterozygous (DH2) line was used for the current study. In the same study [8], we also demonstrated that the transgene mRNA expression showed the highest expression in the testis and the ventral prostate of the independent line 2 (SH2), whereas the independent line 3 showed the highest expression in the liver. We also determined that over-expression of human activin-βC did not alter endogenous mouse activin-βC expression levels.

Tissue Collection

Animals were anesthetized and euthanized by cervical dislocation. A tail biopsy was obtained and snap-frozen to confirm genotype using a competitive genomic PCR screening strategy with specific primers [8]. Wild-type (WT) and transgenic (ActβC++) mice aged 9 months were used for the study with 4–7 mice included in each group. The ventral prostate (VP)

lobes were removed under a dissecting microscope, weighed, and immersion-fixed in Bouin's solution for 4–6 h. Tissues were washed in 70% ethanol, embedded in paraffin, and sectioned at 4 μm onto Superfrost microscope slides (Menzel-Glaser) to obtain a total of 100 sections for each animal.

Pathological Assessment

Hematoxylin and eosin-stained sections of the ventral prostates from WT and ActβC++ mice were evaluated for pathology using the Bar Harbor classification system of mouse prostate pathology [20].

Human Tissue Microarrays

Human tissue microarrays, which included normal control and prostate disease biopsies, were purchased from US BIOMAX (PR805, PR807, and T194). Samples were classified in relation to the histopathological features of the tumor as follows: benign prostatic hyperplasia (BPH), $n = 41$; organ-confined prostate cancer (T1-2N0M0), $n = 38$; and extracapsular prostate cancer (T3-4N1M1), $n = 35$. Normal controls, $n = 14$.

Immunohistochemistry

Immunohistochemistry was performed on the mouse VP sections for proliferating cell nuclear antigen (PCNA) and Smad-2, and human tissue microarrays for activin-β_A, activin-β_C, and Smad-2. Sections were subjected to microwave antigen retrieval (1000 W for 12 min) in buffers as follows: PCNA, in 0.01 M citrate buffer (pH 6.0); activin-A and Smad-2 in 0.01 M sodium citrate buffer (pH 6.0); and activin-C in 0.01 M glycine buffer (pH 4.5). After cooling, slides were washed three times in PBS, and endogenous peroxidase activity was quenched using peroxidase-blocking solution (DAKO Real S2023). The sections were treated with CAS-blocking reagent (Invitrogen 00-8120) and primary antibodies incubated overnight at 4 °C. The primary antibodies used were the following: activin-β_A (1:70, abcam 56057); activin-β_C (1:250, abcam 73904); Smad-2 (1:200, cell signaling 3122); and PCNA (1:500, DAKO M0879). The secondary antibodies used were polyclonal goat anti-rabbit or anti-mouse immunoglobulins/biotinylated (DAKO E0432 and E0433). Negative controls included secondary antibodies only or immnunoglobulin matched to the primary antibody. The specificity of all the antibodies used for the immunohistochemical evaluation was tested by Western blot (data not shown) and has been previously published [10]. Antibodies were detected with avidin-biotin complex for 15 min at room temperature, and then color reacted with 3,3′-diaminobenzidine tetrahydrochloride for 5 min. Reactions were stopped in water, and sections counterstained with Gill's hematoxylin, dehydrated, and mounted.

Apoptosis was evaluated in mouse VP tissue sections by DNA fragmentation with the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay [21] using the ApopTag® peroxidase in situ apoptosis detection kit according to the manufacturer's instructions (Millipore S7100).

Immunoreactive Score of Human Tissue Microarrays

The intensity and distribution patterns of staining were evaluated for each tissue core by two blinded, independent, experienced observers, using the semi-quantitative immunoreactive score (IRS) [22]. The IRS was calculated by multiplication of the optical staining intensity (graded as follows: 0 = none, 1 = weak, 2 = moderate, and 3 = strong staining) by the percentage area of positively stained cells (0 = 0%, 1 = <10%, 2 = 11–50%, 3 = 51–80%, and 4 = >81%).

Stereological Assessment of the Human and Mouse Prostate Tissue

The mouse VP lobe sections stained for PCNA, ApopTag, or Smad-2 and human prostate cancer tissue microarrays (TMAs) stained for Smad-2 were assessed stereologically. Sections were examined at ×40 magnification on an Olympus BX-51 microscope from the beginning, middle, and end of the serially sectioned prostate block to acquire a representative sample through the entire prostate [8, 23]. Random systematic sampling was used to obtain images of each prostate section (Prior OptiScan, Spot Advanced software, and camera) with an average of 30 images taken per animal/patient. ImageJ software was used to count positive and negative epithelial nuclei staining via random placement of a 10 × 10 cm counting grid. The content of a single grid was counted per photograph, with an average of 1000 nuclei counted per animal.

For volumetric analysis, a point counting grid was overlaid randomly on six mouse VP sections from each of five WT and seven ActβC++ mice. Points were counted on epithelial cells, stromal cells, or luminal space and expressed as a percentage of total points counted to estimate the volume density of each partition. An average of 440 total points was counted per animal.

Statistical Analysis

For the mouse, the data for ventral lobe weight, volume density, proliferation (PCNA), apoptosis (ApopTag®), and Smad-2 nuclear staining were analyzed with Student's t test (GraphPad Prism version 5). For the human prostate cancer tissue microarrays, the IRS for each tissue was treated as ranked data and analyzed with the Mann-Whitney U test for two independent groups using IBM SPSS 20 statistic software. Significant differences were assumed at $p < 0.05$.

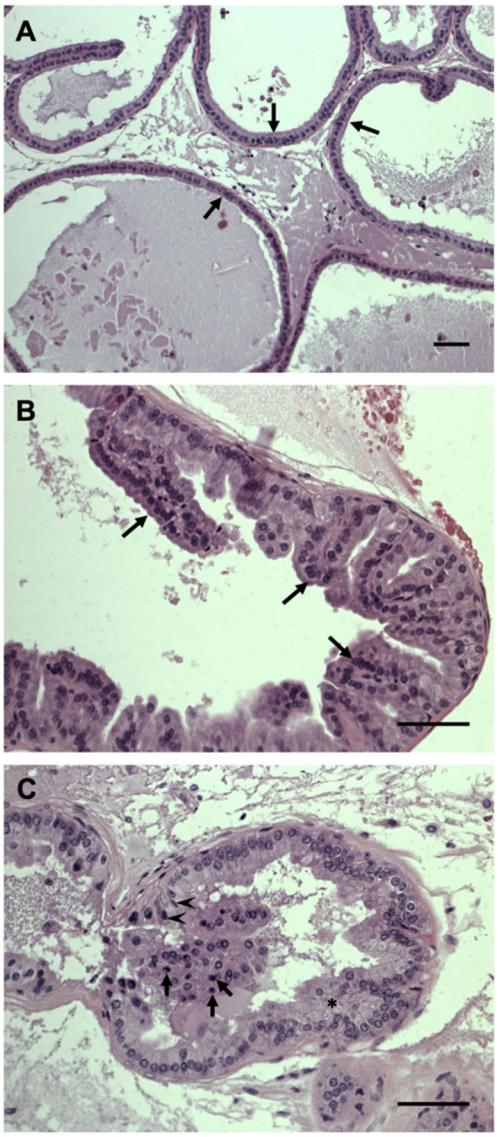

Fig. 1 Examples of ventral prostate histology in WT and Actβ_C++ mice. **a** In WT mice, a single layer of epithelial cells lining the lumen is evident (*arrows*). However, in the Actβ_C++ mouse **b**, evidence of epithelial hyperplasia was common (*arrows*). **c** Cells indicative of prostatic intraepithelial neoplasia (PIN) were evident in the VP lobes from Actβ_C++ mice including cribiform pattern (*), nuclear elongation (*arrowheads*), and hyperchromatic nuclei (*arrows*) [20]. *Scale bars* =50 μm

Results

Over-Expression of Activin-β_C Increased Lobe Weight and Epithelial Cell Volume Density in the Mouse Prostate

The VP lobes of WT animals exhibited acini with normal lumina lined with a single layer of epithelial cells. In contrast, transgenic mice over-expressing activin-β_C showed evidence of luminal epithelial cell hyperplasia, characterized by increased stratification in the form of tufting, papillary in-folding, and an increase in hyper-chromatically stained nuclei (Fig. 1a, b). Mouse pathological analysis revealed no evidence of prostate cancer in mice aged 9 months, but prostatic intraepithelial neoplasia (PIN) lesions were observed (Fig. 1c). The hyperplasia was reflected in an increase in mouse VP lobe weight ($p < 0.01$) in the ActβC++ mice compared to that of the WT controls as shown in Fig. 2a. An independent validation of this was evident in Fig. 2b, where the epithelial, stromal, and luminal volume densities were evaluated by point counting in the mouse VP lobe. Epithelial cell density increased ($p < 0.001$), luminal density decreased ($p < 0.001$), and no difference was observed for stromal cell density in ActβC++ compared to WT animals.

Over-Expression of Activin-β_C Increased Proliferation and Reduced Apoptosis in the Mouse Prostate

To assess cell proliferation and apoptosis, the percentage of PCNA and ApopTag® positive epithelial nuclei were estimated. There was a significant increase in PCNA-positive epithelial nuclei ($p < 0.05$) (Fig. 3a) and a significant decrease in ApopTag®-positive epithelial nuclei in over-expressing activin-β_C compared to the WT mice ($p < 0.05$) (Fig. 3b). Smad-2 levels were assessed to examine possible changes to activin-A signaling when activin-β_C was over-expressed. The percentage of Smad-2-positive nuclei decreased in mice over-

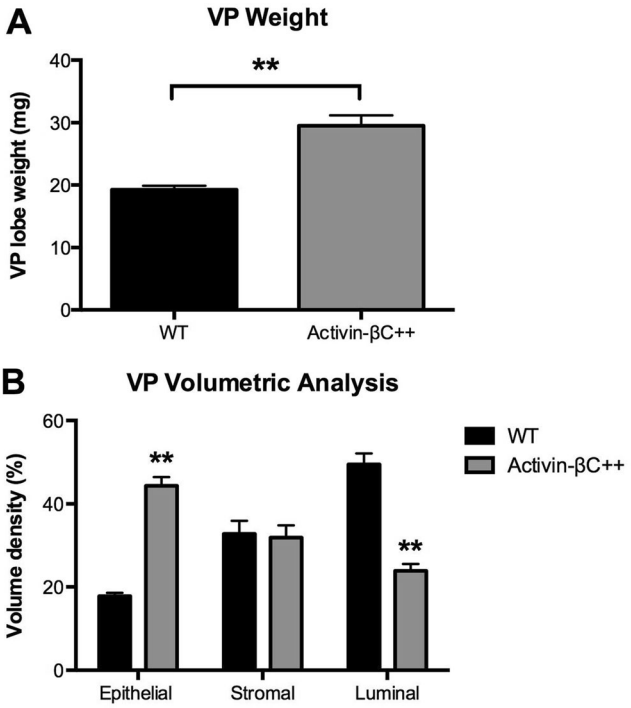

Fig. 2 Mouse prostate weights and volume densities. **a** VP lobe weights (mg) were obtained from WT ($n = 4$) and Actβ_C++ ($n = 6$) mice. **b** Volume densities of epithelial cells, stromal cells, and luminal spaces for WT ($n = 5$) and Actβ_C++ ($n = 7$) mice. Values are mean ± SEM, ** $p < 0.01$ with a Student's *t* test

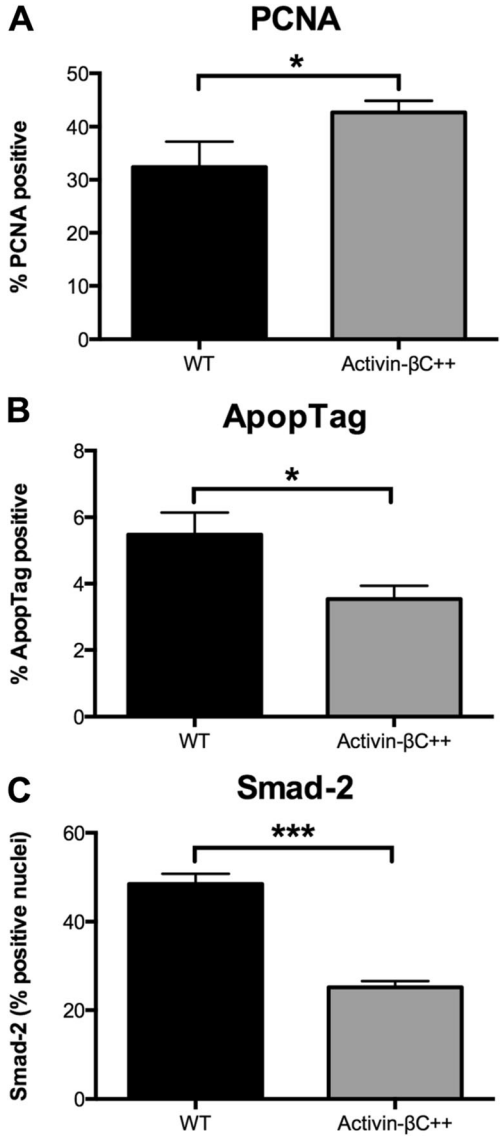

Fig. 3 Stereological assessment of PCNA, ApopTag®, and nuclear Smad-2 in WT and Actβ_C++ mice. Positive nuclear localization of staining was calculated as a percentage of total epithelial nuclei. The percentage of positive nuclei is presented for PCNA **(a)**, ApopTag® **(b)**, and Smad-2 **(c)**. Values are mean ± SEM * $p < 0.05$, *** $p < 0.001$, with a Student's t test, $n = 4$–6 animals with a mean of 1000 cells counted per animal

expressing activin-β_C compared to that of the WT counterparts ($p < 0.001$) (Fig. 3c).

In Human Prostate Cancer, Increased Activin-A, Activin-C, and Alterations in Smad-2 Were Evident

Activin-A staining was significantly increased in all prostate diseases compared to normal controls ($p < 0.001$) (Fig. 4a). No statistically significant changes were noted for activin-C in patients with benign prostatic hyperplasia (BPH) and organ-confined prostate cancer. However, activin-C staining was significantly increased in patients with prostate cancer presenting with extracapsular spread ($p < 0.01$) compared to normal controls (Fig. 4b).

The percentage of Smad-2-positive nuclei was significantly up-regulated in tissues from patients with BPH and organ-confined prostate cancer ($p < 0.01$). Interestingly, in tissues from patients with prostate cancer presenting extracapsular spread, no changes were noted compared to those of normal controls (Fig. 4c).

Discussion

Previous work has highlighted a novel and previously unappreciated function of activin-C as an antagonist of activin-A bioactivity both in vivo and in vitro [8–10]. Additionally, over-expression was associated with the development of a range of pathologies in 3-month-old mice, which included hyperplasia in the ventral prostate. Hence, the aims of the present study were twofold; firstly, to assess whether increased exposure to elevated levels of activin-C in mice was sufficient to cause malignancy, and secondly, to investigate the expression and role of activin-C in human prostate disease.

Activin-A generally acts as a negative growth regulator—promoting apoptosis and inhibiting cancer progression [24]—favorable characteristics for impeding prostate cancer progression. However, if cells become insensitive to activin-A-induced growth inhibition, cancer progression can occur. Three plausible mechanisms could underlie this loss of activin-A growth inhibition. Firstly, inactivating mutations of activin signaling molecules, including the activin receptor and Smad signaling proteins, have been identified in colon, pancreas, and prostate cancer [25–27]. Secondly, alterations to the isoform of follistatin and follistatin-related gene have been demonstrated in liver and breast cancer [28, 29]. Thirdly, other local factors that block the activity of activin-A may be present or over-expressed [1, 11, 30].

Popular candidates for local activin-A inhibitors include follistatin, inhibin, and activin-C, but no prostate phenotype is exhibited when follistatin or inhibin is over-expressed in mice [31, 32]. Prostate hyperplasia is observed, however, when activin-C is over-expressed in mice [8].

Here, we present further evidence for the potential biological relevance of activin-C in mice and men. Over-expression of activin-C was sufficient to antagonize known effects of activin-A in mice aged 9 months. A reduction in the levels of nuclear, and thus activated Smad-2, was observed in the VP of mice over-expressing activin-C indicating a reduction in activin-A signaling. Correspondingly, staining for PCNA, a marker of proliferation, was increased and apoptosis was decreased in the VP of these mice when compared to that of WT mice. The evidence of decreased apoptosis noted within the current study differs from our previous study [8], where no significant effect on apoptosis was observed in 3-month-old

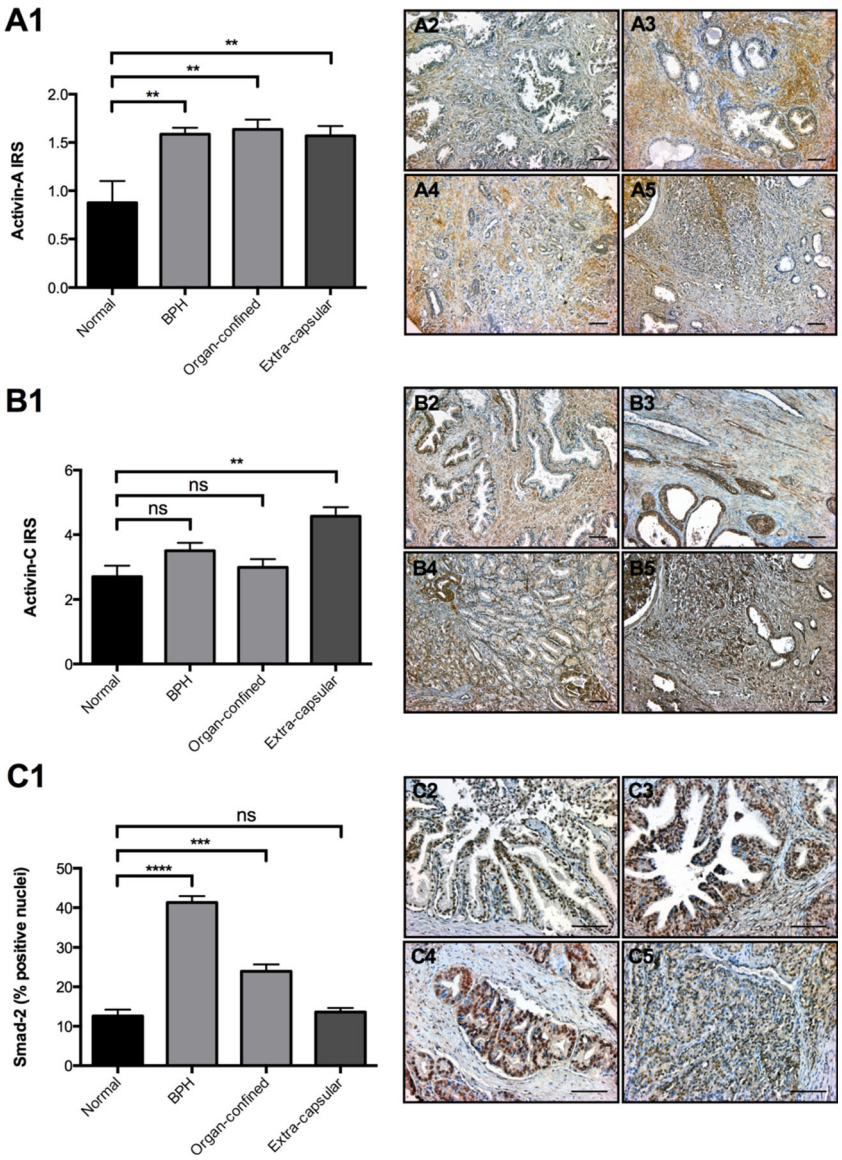

Fig. 4 Human tissue microarray assessment. Immunoreactive score (IRS) and representative images for activin-A **(a),** activin-C **(b)** and Smad-2-positive nuclei **(c)** staining in human biopsy cores from patients are the following: normal controls, $n = 14$ (*A-C2*); benign prostatic hyperplasia (*BPH*), $n = 41$ (*A-C3*); organ-confined prostate cancer (*T1-2N0M0*), $n = 38$ (*A-C4*); and extracapsular prostate cancer (*T3-4N1M1*), $n = 35$ (*A-C5*). Values are mean ± SEM. ** $p < 0.01$, *** $p < 0.001$. Mann-Whitney *U* test was used for the activin-A/C IRS data and a Student's *t* test for the Smad-2 data. *Scale bars* =100 μm

mice over-expressing activin-C. This difference could be attributed to aging, especially when considering that the mouse VP is more sensitive to age-related differences in apoptosis compared to other prostate lobes and organs [33]. While the aforementioned results are consistent with the notion that activin-C is antagonizing activin-A-induced apoptosis, it must be noted that these effects may not be attributed to activin-A only. Indeed, it is possible that over-expression of activin-C could be antagonizing other members of the TGF-β superfamily that also utilize Smad-2 such as growth differentiation factor-1 (GDF-1), nodal, and TGF-β [5, 6].

Given that in our previous study, hyperplasia was observed in 3-month-old ActβC++ mice, we predicted that increased aging to 9 months would be sufficient to allow malignancy to develop. Pathological analysis in the experimental mice revealed no evidence of frank malignancy, however, low-grade PIN lesions were present throughout the VP lobe in transgenic mice indicating early development of prostate cancer (Fig. 1c). Hyperplasia was evident to the extent that mouse VP lobe weights were significantly increased in the transgenic mice compared to WT counterpart (Fig. 2a). Additionally, evidence of epithelial hyperplasia is also reflected in the volumetric analysis, where increased epithelial and decreased luminal volume densities were observed (Fig. 2b). It is likely that the increased epithelial volume density contributed to an increased VP lobe weight in the transgenic mice, despite a decrease in the luminal volume density, because the cells and tissue would weigh more than luminal fluid. It is also possible that there is an increase in the number of glands, but this could not be quantified in this study.

In the human tissue microarrays, a statistically significant up-regulation of activin-A staining in all prostatic diseases examined compared to normal controls was evident. Interestingly, statistically significant up-regulation of activin-

C was only observed in tissue cores from patients with prostate cancer with extracapsular spread. Accordingly, only in tissue cores from patients with extracapsular spread the percentage of Smad-2-positive nuclei was not statistically different compared to that of normal controls. This suggests that activin-C is an important modulator of the activin-A signaling pathway in human advanced prostate cancer.

Conclusions

In conclusion, this study further supports the hypothesis that local expression of activin-C antagonizes activin-A-mediated negative growth regulation during the development of prostate cancer. In the mouse, increased exposure to elevated levels of activin-C led to the development of low-grade PIN lesions, prostate epithelial cell hyperplasia, increased proliferation, decreased apoptosis, and decreased activation of Smad-2. Similarly in human prostatic tissues, increased activin-C corresponded to decreased Smad-2 signaling in the aggressive form of prostate cancer. The selective up-regulation of activin-C in aggressive prostate cancer only, suggests that activin-C may play a role in the advanced stages of the disease. Therefore, activin-C could represent a new diagnostic tool in advanced prostate cancer.

Acknowledgements The authors would like to thank Sylvia Zellhuber-McMillan for assistance with the ApopTag staining and immunoreactive scoring, Sara Ferreira for assistance with the immunohistochemical staining, and Maree Gould for assistance with the volumetric assessment method. This project was funded by the Health Research Council NZ and National Health and Medical Research Council Australia. KLR was funded by a Rutherford Foundation NZ Postdoctoral Fellowship.

Compliance with Ethical Standards All experiments were approved by the Animal Ethics Committee of the University of Otago and conducted in accordance with the New Zealand code of practice in adherence with the NIH Guide for the care and use of laboratory animals.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Gold E, Risbridger G (2012) Activins and activin antagonists in the prostate and prostate cancer. Mol Cell Endocrinol 359(1):107–112
2. Risbridger G, Taylor R (2013) To target or not to target the enemy within localized prostate cancer. Cell Cycle 12(21):3349–3350
3. Risbridger GP, Ellem SJ, McPherson SJ (2007) Estrogen action on the prostate gland: a critical mix of endocrine and paracrine signaling. J Mol Endocrinol 39(3):183–188
4. McPherson SJ, Thomas TZ, Wang H, Gurusinghe CJ, Risbridger GP (1997) Growth inhibitory response to activin A and B by human prostate tumour cell lines, LNCaP and DU145. J Endocrinol 154(3):535–545
5. Shi Y, Massagué J (2003) Mechanisms of TGF-β signaling from cell membrane to the nucleus. Cell 113(6):685–700
6. Loomans HA, Andl CD (2014) Intertwining of activin A and TGFβ signaling: dual roles in cancer progression and cancer cell invasion. Cancers (Basel) 7(1):70–91
7. Lau AL, Kumar TR, Nishimori K, Bonadio J, Matzuk MM (2000) Activin βC and βE genes are not essential for mouse liver growth, differentiation, and regeneration. Mol Cell Biol 20(16):6127–6137
8. Gold E, Jetly N, O'Bryan MK, Meachem S, Srinivasan D, Behuria S, Sanchez-Partida LG, Woodruff T, Hedwards S, Wang H, McDougall H, Casey V, Niranjan B, Patella S, Risbridger G (2009) Activin C antagonizes activin A in vitro and overexpression leads to pathologies in vivo. Am J Pathol 174(1):184–195
9. Gold E, Marino FE, Harrison C, Makanji Y, Risbridger G (2013) Activin-βc reduces reproductive tumour progression and abolishes cancer-associated cachexia in inhibin-deficient mice. J Pathol 229(4):599–607
10. Marino FE, Risbridger G, Gold E (2014) The inhibin/activin signalling pathway in human gonadal and adrenal cancers. Mol Hum Reprod 20(12):1223–1237
11. Risbridger GP, Schmitt JF, Robertson DM (2001) Activins and inhibins in endocrine and other tumors. Endocr Rev 22(6):836–858
12. McKenzie S, Kyprianou N (2006) Apoptosis evasion: the role of survival pathways in prostate cancer progression and therapeutic resistance. J Cell Biochem 97(1):18–32
13. Ottley E, Gold E (2012) Insensitivity to the growth inhibitory effects of activin A: an acquired capability in prostate cancer progression. Cytokine Growth Factor Rev 23(3):119–125
14. Furst B, Zhang Z, Ying S (1995) Expression of activin and activin receptors in human prostatic carcinoma cell line du145. Int J Oncol 7(2):239–243
15. Dalkin AC, Gilrain JT, Bradshaw D, Myers CE (1996) Activin inhibition of prostate cancer cell growth: selective actions on androgen-responsive LNCaP cells. Endocrinology 137(12):5230–5235
16. Incorvaia L, Badalamenti G, Rini G, Arcara C, Fricano S, Sferrazza C, Di Trapani D, Gebbia N, Leto G (2007) MMP-2, MMP-9 and activin A blood levels in patients with breast cancer or prostate cancer metastatic to the bone. Anticancer Res 27(3B):1519–1525
17. Leto G, Incorvaia L, Badalamenti G, Tumminello FM, Gebbia N, Flandina C, Crescimanno M, Rini G (2006) Activin A circulating levels in patients with bone metastasis from breast or prostate cancer. Clin Exp Metastasis 23(2):117–122
18. Hofland J, van Weerden WM, Steenbergen J, Dits NF, Jenster G, de Jong FH (2012) Activin A stimulates AKR1C3 expression and growth in human prostate cancer. Endocrinology 153(12):5726–5734
19. Kang HY, Huang HY, Hsieh CY, Li CF, Shyr CR, Tsai MY, Chang C, Chuang YC, Huang KE (2009) Activin A enhances prostate cancer cell migration through activation of androgen receptor and is overexpressed in metastatic prostate cancer. J Bone Miner Res 24(7):1180–1193
20. Shappell SB, Thomas GV, Roberts RL, Herbert R, Ittmann MM, Rubin MA, Humphrey PA, Sundberg JP, Rozengurt N, Barrios R (2004) Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the Bar Harbor meeting of the mouse models of human cancer consortium prostate pathology committee. Cancer Res 64(6):2270–2305
21. Gavrieli Y, Sherman Y, Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 119(3):493–501
22. Remmele W, Stegner H (1987) Vorschlag zur einheitlichen Definition eines Immunreaktiven Score (IRS) für den

immunhistochemischen Östrogenrezeptor-Nachweis (ER-ICA) im Mammakarzinomgewebe. Pathologe 8(3):138–140
23. Gold E, Zhang X, Wheatley A, Mellor S, Cranfield M, Risbridger G, Groome N, Fleming J (2005) betaA- and betaC-activin, follistatin, activin receptor mRNA and betaC-activin peptide expression during rat liver regeneration. J Mol Endocrinol 34(2): 505–515
24. Butler C, Gold E, Risbridger G (2005) Should activin C be more than a fading snapshot in the activin/TGF family album? Cytokine Growth Factor Rev 16(4–5):377–385
25. Jung B, Doctolero RT, Tajima A, Nguyen AK, Keku T, Sandler RS, Carethers JM (2004) Loss of activin receptor type 2 protein expression in microsatellite unstable colon cancers. Gastroenterology 126(3):654–659
26. Hempen PM, Zhang L, Bansal RK, Iacobuzio-Donahue CA, Murphy KM, Maitra A, Vogelstein B, Whitehead RH, Markowitz SD, Willson JK, Yeo CJ, Hruban RH, Kern SE (2003) Evidence of selection for clones having genetic inactivation of the activin A type II receptor (ACVR2) gene in gastrointestinal cancers. Cancer Res 63(5):994–999
27. Rossi MR, Ionov Y, Bakin AV, Cowell JK (2005) Truncating mutations in the ACVR2 gene attenuates activin signaling in prostate cancer cells. Cancer Genet Cytogenet 163(2):123–129
28. Grusch M, Drucker C, Peter-Vorosmarty B, Erlach N, Lackner A, Losert A, Macheiner D, Schneider WJ, Hermann M, Groome NP, Parzefall W, Berger W, Grasl-Kraupp B, Schulte-Hermann R (2006) Deregulation of the activin/follistatin system in hepatocarcinogenesis. J Hepatol 45(5):673–680
29. Razanajaona D, Joguet S, Ay AS, Treilleux I, Goddard-Leon S, Bartholin L, Rimokh R (2007) Silencing of FLRG, an antagonist of activin, inhibits human breast tumor cell growth. Cancer Res 67(15):7223–7229
30. Grusch M, Petz M, Metzner T, Ozturk D, Schneller D, Mikulits W (2010) The crosstalk of RAS with the TGF-beta family during carcinoma progression and its implications for targeted cancer therapy. Curr Cancer Drug Targets 10(8):849–857
31. Cho B, McMullen M, Pei L, Yates C, Mayo K (2001) Reproductive deficiencies in transgenic mice expressing the rat inhibin α-subunit gene. Endocrinology 142(11):4994
32. McMullen M, Cho B, Yates C, Mayo K (2001) Gonadal pathologies in transgenic mice expressing the rat inhibin α-subunit. Endocrinology 142(11):5005
33. Jara M, Carballada R, Esponda P (2004) Age-induced apoptosis in the male genital tract of the mouse. Reproduction 127(3):359–366