COMMENTARY

Recent progress in our understanding of inhibin in the prostate gland

G P Risbridger

Inhibin was originally described as the active principle or substance in the testis which was able to prevent the appearance of castration cells in the male rat pituitary gland (McCullagh 1932). It was not until the early 1970s that interest in the identity of the putative feedback regulator of follicle-stimulating hormone (FSH) was renewed. Using castrate male rats, Franchimont (1982) showed that the subcutaneous injection of seminal plasma from normal men or patients with oligospermia significantly inhibited serum FSH; seminal plasma from men with azoospermia did not. The identity of the 'inhibin' in seminal plasma was a subject of considerable debate over the following years.

Seminal plasma was used as a source for the purification of inhibin by Seth and his colleagues (1984) who isolated 'prostatic inhibin peptide' (PIP), a 14 kDa, 98 amino acid non-glycosylated protein. Ramasharma *et al.* (1984) also identified an 'inhibin-like peptide' from seminal plasma which was a 31 amino acid peptide of 5 kDa which suppressed FSH *in vitro*. Neither of these proteins showed a consistent inhibition of FSH and they are unrelated to dimeric inhibin which was finally isolated from follicular fluid by Robertson *et al.* in 1986. In the testis, the Sertoli cells are the principal source of inhibin. Dimeric inhibin is composed of an α subunit and one of two β subunits linked by disulphide bonds to form inhibin A or inhibin B. The inhibin β subunits can also form homo- or heterodimers: activin A, activin B and activin AB (Burger 1988). In contrast to inhibins, activins stimulate rather than inhibit pituitary FSH (Mason *et al.* 1985).

Following these original discoveries, it has become clear that activins are growth and differentiation factors with roles in a number of cells and tissues, and are members of the transforming growth factor β (TGF β) superfamily. In terms of cell growth, activins regulate cell proliferation and apoptosis in a number of cells and tissues (Hedger *et al.* 1989, Mather *et al.* 1990, Nishihara *et al.* 1993, Kaipia *et al.* 1994). Prostate cell growth in normal and disease states is controlled by the balance between the regulation of cell death and proliferation, and this led us to re-examine the presence of inhibin-like proteins in the prostate gland.

In 1997, studies from this laboratory showed that the human prostate gland had the capacity to synthesise dimeric inhibins and activins. Using samples collected

from men with benign prostatic hyperplasia (BPH), Thomas *et al.* (1997) reported expression of mRNA for the inhibin α and β subunits; thus the capacity to produce all the known inhibins and activins resides in the human prostate. Interestingly, mRNA for the putative β C subunit which was originally cloned from human liver and believed to be specifically expressed in liver (Hotten *et al.* 1995), was also detected in the prostate. If the activin-like bioactivity of homo- or heterodimers of the β C subunit is confirmed in future studies, then it is possible that novel activins are present in the human prostate.

In addition to the α and β subunits, the human prostate expressed activin type II receptor which implies that activins have a local role in the prostate itself. The role of these proteins in the function of the non-malignant prostate is not known, but *in vitro* actions of activins on human prostate cancer cells were described. Three groups reported that activin A inhibited cell proliferation and/or induced apoptosis in the human prostate tumour cell line LNCaP (Dalkin *et al.* 1996, Wang *et al.* 1996, McPherson *et al.* 1997). This was rather surprising, because the growth inhibitory actions of activin A and B on the LNCaP tumour cells contrasts with the malignant characteristics of these cells; this, therefore, raised some questions. Are the actions of activins observed using LNCaP cells also recorded with other prostate tumour cell lines? Does the expression and localisation of the activin β , or the α inhibin, subunits differ in the non-malignant compared with malignant prostatic epithelium?

The growth inhibitory action of activin A on LNCaP cells is not reproduced in the androgen-independent PC3 cell line which is resistant to activin A; an intermediate response to activin A occurs with DU145 cells. Therefore, *in vitro*, there is a differential response to exogenously added activin A by the three cell lines LNCaP, DU145 and PC3 (McPherson *et al.* 1997). Interestingly, all these cell lines expressed activin β_A and β_B subunit mRNA (Batres *et al.* 1995, Furst *et al.* 1995, Ying *et al.* 1995), although no dimeric activin immunoreactivity was detected in the media from these tumour cells in culture, and it is not certain that prostate tumour cells produce detectable levels of endogenous activin ligands (McPherson *et al.* 1997). What was known about the

expression of the activin β subunits in poorly differentiated tumour tissue *in vivo*? Studies from this laboratory used specific antibodies to the activin β A and β B subunits and reported the presence of activin β subunit mRNA and protein in the malignant and non-malignant regions of tissue from men with high grade cancer (Thomas *et al.* 1997). The pattern of β subunit expression in epithelial cells was the same as that in tissue from men with BPH (Thomas *et al.* 1998). Collectively, these results did not show any significant change in the pattern of activin β subunit with progression to high grade cancer, which suggested that cells in poorly differentiated tumour tissues and cell lines, such as PC3 cells, were resistant to the growth inhibitory actions of activins. The possible mechanisms for conferring resistance were subsequently investigated.

In many cells and tissues activins and inhibins have dual opposing roles (Hsueh *et al.* 1987), and it was possible that benign and malignant prostate cell growth was balanced and regulated by interaction between these ligands. This was supported by the failure to detect inhibin α subunit expression in prostate tumour cell lines (Batres *et al.* 1995, Furst *et al.* 1995, Ying *et al.* 1995). This hypothesis implies that inhibins have growth promoting, rather than growth inhibiting, actions on human prostate tumour cell lines. However, no effect of inhibin A on LNCaP, DU145 or PC3 cells (McPherson *et al.* 1997) was observed. *In vivo*, we reported that inhibin α gene expression and protein localisation was down regulated in tissues from men with high grade cancer (S L Mellor, M G Richards, J S Pedersen, D M Robertson & G P Risbridger, unpublished observations). Together with the demonstration that functional deletion of the inhibin α gene resulted in the development of gonadal tumours, these results supported the hypothesis that the inhibin α gene was tumour suppressive (Matzuk *et al.* 1992). However, the data contrasted directly with the observations that inhibin was elevated in women with ovarian cancer. The inhibin α subunit is considered a valuable marker for ovarian cancer because serum inhibin is elevated in postmenopausal women with granulosa and mucinous cell ovarian tumours (Healy *et al.* 1993, Jobling *et al.* 1994, Cooke *et al.* 1995, Gurusinghe *et al.* 1995, Phocas *et al.* 1996, Lambert-Messerlian *et al.* 1997). The role of the inhibin α gene in the progression of prostate cancer requires further evaluation.

The absence of inhibin α gene expression and the detection of activin β subunits in cells from poorly differentiated tumours or human prostate tumour cell lines implies that these cells continue to synthesise activins. If these ligands are growth inhibitory, then other mechanisms of conferring resistance may be evoked. By analogy with TGF β it is possible that there are inactivating mutations of the activin receptors, but this is unlikely given that the tumour cells are able to respond to low levels of activin A in the pg/ml to ng/ml range

(McPherson *et al.* 1997). An alternative means of regulating the bioactivity of activins may involve binding proteins (e.g. follistatins) which have specific and high affinity for the activin ligands (Sugino *et al.* 1993). Follistatins have been shown to neutralise some of the actions of activins (including apoptosis), by forming an inactive complex with the activin β subunits. In the absence of inhibin formation, the interplay between activins and follistatins may be important in the regulation of the growth responses to activins by human prostate tumours. Several observations support this hypothesis. As discussed above, there were differential responses to activins by three human prostate tumour cell lines. The only difference that we observed between the tumour cell lines was the expression of follistatin 288 mRNA by the activin-resistant PC3 cells but not by LNCaP or DU145 cells where the predominant mRNA species for follistatin was follistatin 315 (McPherson *et al.* 1997). The relative levels and types of follistatin proteins produced by the three prostate tumour cell lines remain to be shown but it was reported that follistatin 288 had a higher neutralising activity than follistatin 315 (Sugino *et al.* 1993). Therefore, it is possible that the presence of 288 is important in conferring activin resistance. *In vivo*, the localisation of follistatin mRNA and protein was confusing. Using *in situ* hybridisation, expression of follistatin mRNA was localised to the basal epithelial cells and the fibroblastic stroma in the non-malignant regions of tissue from men with high grade cancer (Thomas *et al.* 1997). However, the localisation of the follistatin proteins using two different antisera demonstrates that there is a difference between the follistatin isoforms expressed in basal cells and those expressed in the stroma. Using an antibody to follistatin 288 (OxB 288), protein was predominantly localised to the basal cells, but using another follistatin antibody (Monash #202), follistatin protein was localised to the stromal tissue and not to the basal cells. These results suggest that the follistatin proteins produced *in vivo* in the basal epithelial cells and in the stromal cells are different. In the malignant regions of the tissue sections, the tumour cells showed the expression of mRNA for follistatin and immunoreactivity was detected using antisera. These data suggested that the expression of follistatin mRNA in malignant and non-malignant tissues from men with high grade cancer results in the production of different follistatin isoforms in the specific cell types and that there is a change in the pattern of localisation of follistatin isoforms in the progression to malignancy. The development of new, specific and sensitive assays for follistatins is required to determine which follistatin isoforms are produced in the prostate and bound or unbound to activin ligands.

In summary, the results from more recent studies of prostatic inhibin confirmed that the human prostate gland is itself a site of synthesis and production of inhibins and the related proteins activins, together with activin binding proteins such as follistatins. Future studies may identify

Figure 1 Diagrammatic summary of the changes in expression of inhibin-related proteins in tissues from men with BPH or high grade prostate cancer. Progression to malignancy is characterised by a loss of inhibin α subunit expression which may result in the production of activins. These ligands have growth inhibitory actions in some human prostate tumour cell lines, but it is presumed that any such action on tumour tissue *in vivo* is neutralised by follistatin (FS). The expression of follistatin proteins is different in malignant and non-malignant tissues, and it is concluded that in malignant tumour tissue the interplay between follistatins and activins is more important than that between inhibin and activins.

novel activin or inhibin ligands involving the putative β C subunit. There are significant changes in the pattern of expression of the inhibin α subunit and follistatins in non-malignant and malignant prostate tissues from men with high grade cancer (see Figure 1). Paracrine actions between stromal and epithelial cell types in the non-malignant epithelium may involve inhibins, activins and follistatins. It is likely that these proteins regulate cell proliferation and it is noteworthy that immunoreactivity for these proteins was localised to the basal as well as the secretory cells of the prostatic epithelium because ~70% of the proliferating cells in the prostatic epithelium are of the basal cell phenotype (Bonkoff *et al.* 1994). In malignancy, the autocrine regulation of epithelial tumour cell growth may involve a different interplay between activins and follistatins. The *in vivo* localisation of follistatin immunoreactivity and activin immunoreactivity to tumour cells implies that resistance to the growth inhibitory actions of endogenously produced activin may be conferred by follistatins. Future studies will identify the nature and the role of secreted molecules, like follistatins, in the regulation of ligand access to receptors. The activins are members of the TGF β superfamily and it is known that secreted molecules such as *noggin* regulate the access of BMP4 to its receptor. Therefore the study of follistatin and activin in prostate tumour cells may identify processes of

regulating cell growth that are similar to those present in other cells and tissues involving members of the TGF β superfamily during development or tumorigenesis.

References

- Batres Y, Zhang A & Ying S-Y 1995 Expression of activins and activin receptor messenger RNAs in LNCaP cells, a human prostatic adenocarcinoma cell line. *International Journal of Oncology* **6** 1185–1188.
- Bonkoff H, Stein U & Remberger K 1994 The proliferative function of basal cells in the normal and hyperplastic human prostate. *Prostate* **24** 114–118.
- Burger HG 1988 Inhibin: definition and nomenclature including related substances. *Journal of Endocrinology* **117** 159–160.
- Cooke I, O'Brien M, Charnock FM, Groome N & Ganesan TS 1995 Inhibin as a marker for ovarian cancer. *British Journal of Cancer* **71** 1046–1050.
- 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** 5230–5235.
- Franchimont P 1982 Human gonadotropin secretion. *Journal of the Royal College of Physicians of London* **6** 283–298.
- Furst BA, Zhang Z & Ying S-Y 1995 Expression of activin and activin receptors in human prostatic carcinoma cell line DU145. *International Journal of Oncology* **7** 239–243.
- Gurusinghe CJ, Healy DL, Jobling T, Mamers P & Burger HG 1995 Inhibin and activin are demonstrable by immunohistochemistry in ovarian tumor tissue. *Gynecologic Oncology* **57** 27–32.

- Healy DL, Burger HG, Mamers P, Jobling T, Bangah M, Quinn M, Grant P, Day AJ, Rome R & Campbell JJ 1993 Elevated serum inhibin concentrations in postmenopausal women with ovarian tumors. *New England Journal of Medicine* **329** 1539–1541.
- Hedger MP, Drummond AE, Robertson DM, Risbridger GP & de Kretser DM 1989 Divergent actions of inhibin activin and transforming growth factor β on [^3H]thymidine incorporation by rat thymocytes and 3T3 fibroblasts *in vitro*. *Molecular and Cellular Endocrinology* **61** 133–138.
- Hotten G, Neidhardt H, Schneider C & Pohl J 1995 Cloning of a new member of the TGF- β family: a putative new activin β_c chain. *Biochemical and Biophysical Research Communications* **206** 608–613.
- Hsueh JW, Dahl RD, Vaughan J, Tucker E, Rivier J, Bardin CW & Vale W 1987 Heterodimers and homodimers of inhibin subunits have different paracrine actions in the modulation of luteinizing hormone stimulated androgen biosynthesis. *Proceedings of the National Academy of Sciences of the USA* **84** 5082–5086.
- Jobling T, Mamers P, Healy DL, MacLachlan V, Burger HG, Quinn M, Rome R & Day J 1994 A prospective study of inhibin in granulosa cell tumors of the ovary. *Gynecologic Oncology* **55** 285–289.
- Kaipia A, Toppari J, Huhtaniemi I & Panken J 1994 Sex difference in the action of the activin-A on cell proliferation of differentiating rat gonad. *Endocrinology* **134** 2165–2170.
- Lambert-Messerlian GM, Steinhoff M, Zheng W, Canick JA, Gajewski WH, Seifer DB & Schneyer AL 1997 Multiple immunoreactive inhibin proteins in serum from postmenopausal women with epithelial ovarian cancer. *Gynecologic Oncology* **65** 512–516.
- McCullagh DR 1932 Dual endocrine activity of the testis. *Science* **76** 19–20.
- McPherson SJ, Thomas TZ, Wang H, Gurusinge CJ & Risbridger GP 1997 Growth inhibitory response to activin A and B by human prostate tumour cell lines, LNCaP and DU145. *Journal of Endocrinology* **154** 535–545.
- Mason AJ, Hayflick JS, Ling N, Esch F, Ueno N, Ying S-Y, Guilleman R, Niall H & Seeburg P 1985 Complementary DNA sequences of ovarian follicular fluid inhibin show precursor structure and homology with transforming growth factor- β . *Nature* **318** 659–663.
- Mather JP, Attie KM, Woodruff T, Rice GC & Phillips DM 1990 Activin stimulates spermatogonial proliferation in germ-sertoli cell co-cultures from immature rat. *Endocrinology* **127** 3206–3214.
- Matzuk MM, Finegold MJ, Su JG, Hsueh AJ & Bradley A 1992 Alpha inhibin is a tumor suppressive gene with gonadal specificity in mice. *Nature* **360** 313–319.
- Nishihara T, Okahashi N & Ueda N 1993 Activin A induces apoptotic cell death. *Biochemical and Biophysical Research Communications* **192** 985–991.
- Phocas I, Sarandakou A, Sikiotis K, Rizo D, Kalambokis D & Zourlas PA 1996 A comparative study of serum alpha-beta immunoreactive inhibin and tumor-associated antigens CA125 and CEA in ovarian cancer. *Anticancer Research* **16** 3827–3831.
- Ramasharma K, Sairam MR, Seidah NG, Chretien M, Manjunath P & Schiller PW 1984 Isolation, structure, and synthesis of a human seminal plasma peptide with inhibin-like activity. *Science* **23** 1199–1201.
- Robertson DM, Foulds LM, Leversha L, Morgan FJ, Hearn MTW, Burger HG, Wettenhall REH & de Kretser DM 1986 Isolation of inhibin from bovine follicular fluid. *Biochemical and Biophysical Research Communications* **126** 220–226.
- Seth AR, Arabatti N, Carlquist M & Jornvall H 1984 Characterization of a polypeptide from human seminal plasma with inhibin (inhibition of FSH secretion)-like activity. *FEBS Letters* **1** 11–15.
- Sugino K, Kurosawa N, Nakamura T, Takio K, Shimasaki S, Ling N, Titoni K & Sugino H 1993 Molecular heterogeneity of follistatin, an activin binding protein. *Journal of Biological Chemistry* **268** 15579–15587.
- Thomas TZ, Chapman SM, Wang H, Gurusinge CJ, Mellor SL, Fletcher R, Pedersen J & Risbridger GP 1998 Inhibins, activins and follistatins: expression of mRNAs and cellular localization in tissues from men with benign prostatic hyperplasia. *The Prostate* **34** 340–43.
- Thomas TZ, Wang H, Niclasen P, O'Bryan MK, Evans LW, Groome NP, Pedersen J & Risbridger GP 1997 Expression and localisation of activin subunits and follistatins in tissues from men with high grade prostate cancer. *Journal of Clinical Endocrinology and Metabolism* **82** 3851–3858.
- Wang QF, Tilly KI, Tilly JL, Preffer F, Schneyer AL, Crowley WF Jr & Scuss PM 1996 Activin inhibits basal and androgen-stimulated proliferation and induces apoptosis in the human prostate cancer cell line, LNCaP. *Endocrinology* **137** 5476–5483.
- Ying S-Y, Zhang Z & Xing W 1995 Expression of activin and activin receptors in PC3 human prostatic tumour cell lines. *International Journal of Oncology* **6** 602–606.

Institute of Reproduction and Development,
Monash Medical Centre,
Level 3, Block E,
246 Clayton Road,
Clayton,
Victoria 3168,
Australia