ORIGINAL ARTICLE

Comparison of annexin II, p63 and α -methylacyl-CoA racemase immunoreactivity in prostatic tissue: a tissue microarray study

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J Clin Pathol 2007;**60**:773–780. doi: 10.1136/jcp.2006.040808

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Accepted 31 July 2006
Published Online First
17 August 2006

Background: Current ancillary markers for diagnosis in prostate biopsies include p63 and α -methylacyl-CoA racemase (AMACR). Annexin II (ANXII), a calcium and phospholipid binding protein, is lost in prostate cancer.

Aims: To investigate ANXII expression in order to assess its utility as a novel diagnostic marker in comparison to p63 and AMACR.

Methods: Using immunohistochemistry on six tissue microarrays, ANXII, p63, and AMACR expression was analysed from 210 radical prostatectomy cases. Staining was evaluated in benign and atrophic glands, high-grade prostatic intraepithelial neoplasia (HGPIN), and prostatic adenocarcinoma. Separate scores were given for ANXII, AMACR and p63 expression.

Results: Diffuse cytoplasmic expression of ANXII correlated with p63 reactivity in basal cells. Benign glands were positive for ANXII in 286/292 cores (98%) and negative for AMACR in all 292 cores. HGPIN showed heterogeneous expression of AMACR and ANXII. A significantly larger proportion of HGPIN glands were correctly identified as ANXII negative than as positive for AMACR. ANXII loss in prostate cancer was found in 282/320 cores (88%) and correlated with positive AMACR expression (272/320 cores, 85%), which was not statistically significant. There was no statistically significant correlation between ANXII scores and the clinical parameters examined.

Conclusions: Immunohistochemical staining for ANXII is a consistent and reliable marker of prostatic neoplasia. The findings of this study suggest the potential utility of ANXII as a diagnostic aid in prostate cancer histopathology.

The use of serum prostate specific antigen as a screening tool for detection of prostate cancer has greatly increased the number of biopsies performed. A diagnosis of prostate cancer is made by morphological assessment of prostatic tissue. However, the differential diagnosis of prostate cancer includes many benign mimickers.¹ Therefore, immunohistochemical markers are commonly used to assist in diagnosis. Currently applied markers include p63 and α -methylacyl-CoA racemase (AMACR, P504S). p63 is a homologue of the p53 tumour suppressor gene.² In the prostate, p63 is a nuclear basal cell marker, which is absent in prostate cancer.³ p63 is thought to be involved in the development and maintenance of the prostatic secretory epithelial cell layer.⁴ AMACR, an enzyme involved in peroxisomal β -oxidation of fatty acids,⁵ has been identified as a cytoplasmic marker of prostate cancer.^{6,7} Although AMACR is useful in assisting in the diagnosis of prostate cancer, wide variations in AMACR sensitivity and intensity have been observed. Recent studies have shown that 68–95% of prostate cancer stains positively for AMACR; however the intensity of immunoreactivity is often heterogeneous.^{7–14} High-grade prostatic intraepithelial neoplasia (HGPIN) is the putative premalignant lesion of prostate cancer.¹⁵ Positive reactivity with AMACR is reported in 41–90% of HGPIN glands and 8–29% of benign glands.³ Therefore, AMACR and p63 must be used in conjunction as diagnostic tools.³ Recent work has shown that antibody cocktails of AMACR and p63 are as effective as using the two markers separately; however they do not allow separate optimisation of the antibodies.¹⁶

Annexins are a family of calcium binding proteins with the ability to bind phospholipids in the plasma membrane.^{17,18}

Annexin II (ANXII, p36, lipocortin II, calpactin I) is implicated in migration inhibition by regulation of F-actin and intermediate filament organisation,¹⁹ extracellular matrix alteration through its effects on plasmin generation²⁰ and regulation of inflammation via inhibition of cytoplasmic phospholipase A2.²¹ ANXII has been reported to be over-expressed in the malignancies of numerous tissues, including brain, breast, colon, liver, and pancreas.^{22–26} Conversely, various studies have revealed loss of ANXII expression in prostate cancer.^{27–29} These studies also showed strong immunoreactivity of basal cells, and strong membranous expression in benign epithelium. The characterisation of ANXII expression in HGPIN has not been defined.

While AMACR and p63 are useful at present, the discovery of new reliable, consistent, and robust markers would be of value. In addition, novel markers may allow the refinement of the selection criteria for re-biopsy after a diagnosis of HGPIN and may allow better prognostication.

The present study evaluates the effectiveness of ANXII as a diagnostic marker of prostate cancer and HGPIN compared to AMACR and p63 using tissue microarrays.

MATERIALS AND METHODS

Tissue specimens

A total of 210 cases of radical prostatectomy from 2002 to 2004, with a diagnosis of prostate cancer were selected from the University Health Network (UHN) surgical pathology files, with UHN research ethics board approval. None of the patients had

Abbreviations: AMACR, α -methylacyl-CoA racemase; ANXII, annexin II; HGPIN, high-grade prostatic intraepithelial neoplasia

Table 1 Patient characteristics

	No patients		
	6(3+3)	PIN	n
Prostate weight (g)			
<30	14	11	25
30–39	32	31	63
40–49	31	28	59
50–59	10	17	27
60–79	13	12	25
>80	5	6	11
Mean	48.0	45.2	46.6
Age (years) (at RP)			
40–49	7	4	11
50–59	42	26	68
60–69	47	65	112
70–79	9	10	19
Mean	60.5	62.1	61.2
Pathological stage			
pT2	9	9	18
pT2a	3	2	5
pT2b	3	1	4
pT2c	74	57	131
pT3	1	0	1
PT3a	15	29	44
PT3b	0	7	7

PIN, prostatic intraepithelial neoplasia; RP, radical prostatectomy; 6(3+3), Gleason score 6(3+3) adenocarcinoma.

undergone hormonal therapy prior to prostatectomy. Pathological and patient data were obtained from patient records. All tissue was fixed in 10% neutral buffered formalin and embedded in paraffin.

Tissue microarray construction

Foci of HGPIN were selected from 105 cases with a diagnosis of Gleason score 7(3+4) adenocarcinoma for the construction of three HGPIN tissue microarrays, as previously described.³⁰ An additional 105 cases with a diagnosis of Gleason score 6(3+3) adenocarcinoma were used in the construction of three prostate cancer tissue microarrays. Each case was represented by three 1 mm diameter cores. The tissue microarrays contained three benign prostate cores as a control. Four arrays contained normal kidney or normal liver, which served as positive and/or negative controls.^{31–32} A total of ten cores from the HGPIN arrays and seven cores from the prostate cancer arrays were lost in processing, leaving 305 and 308 cores, respectively. The missing cores were all from different patients; these patients were still included in the final analysis.

Immunohistochemistry

Four consecutive 4 µm histological sections were cut from the six paraffin array blocks and mounted on positively charged slides. One section of each array was stained with H&E and serial levels were stained for ANXII (mouse IgG1; 1/1000; 610069, BD Transduction Laboratories, San Jose, California, USA), AMACR (monoclonal P504S; 1/100; Zeta Corporation, Sierra Madre, California, USA) and p63 (monoclonal 7JUL; 1/50; Vector Laboratories, Burlingame, California, USA) using standard immunohistochemical procedures. Briefly, sections were dewaxed, rehydrated and blocked for endogenous peroxidase activity with 3% aqueous hydrogen peroxide for 15 minutes. Antigen retrieval was performed in a pressure cooker in 10 mM sodium citrate buffer, pH 6.0, for 20 minutes within a microwave oven, followed by treatment with protein blocker (Signet Laboratories, Inc., Dedham, Massachusetts, USA) for 10 minutes. The primary antibodies were diluted to the above concentrations with antibody diluting buffer (DAKO,

Mississauga, Ontario, Canada). The slides were incubated with ANXII antibody overnight at room temperature in a moist chamber. AMACR and p63 staining was performed on an automated immunohistochemistry stainer (Ventana Medical Systems, Inc., Tucson, Arizona, USA). Immunoreactivity was subsequently detected by washing and incubating with Signet USA Level 2 biotinylated linking antibody (diluted 1:4) for 30 minutes at room temperature, followed by washing and another 30 minutes' incubation with Signet USA Level 2 labelling reagent (Ultra Streptavidin–Horseradish Peroxidase Complex, diluted 1:4). Colour development involved application of NovaRed (Vector Labs, Burlingame, California, USA; Cat#SK-4800) for 5–10 minutes. Sections were then counterstained lightly with Mayer's haematoxylin.

Analysis

ANXII, AMACR and p63 stained slides were digitally scanned using the T3 ScanScope by Aperio Technologies (Vista, California, USA) with a resolution of 0.5 µm/pixel. The H&E stained sections of each tissue microarray were histologically examined under bright field microscopy and the glandular percentage of benign, atrophic, HGPIN and prostate cancer lesions was scored for each core. This study refers to benign prostatic glands as those glands which do not show the morphological features of HGPIN, atrophy or carcinoma. The staining patterns for ANXII, p63 and AMACR on serial sections were visually scored and compared on adjacent computer screens. p63 images were used to confirm the glandular diagnosis on each core and to compare to ANXII staining in the basal cells. Separate scores for ANXII and AMACR immunoreactivity were assigned for each histological component on each core. Annexin II was scored based on the percentage of cells with positive membranous staining. Glands were classified as negative (0%), weak (1–20%), moderate (21–79%) or strong (80–100%). HGPIN glands were classified differently since they presented with diffusely positive cytoplasmic staining, focal loss of ANXII immunoreactivity and luminal staining patterns. ANXII reactivity in HGPIN was thus classified as negative, positive (diffusely cytoplasmic or membranous), focal (>5% cells negative in a gland), or luminal (strong luminal, but weak cytoplasmic or membranous). AMACR was scored based on the percentage of positive cells and the intensity of staining (0 = negative, 1 = weak, 2 = moderate, 3 = strong), as previously described.⁸ For the evaluation of AMACR, a combined weighted score was calculated from the sum of the percentage of positive cells multiplied by the intensity of staining, giving a total score with a maximum of 300.

Multiple and single variant linear regression analysis, as well as single-variable ANOVA (analysis of variance) were performed to assess if any correlation was present between the ANXII scores and AMACR scores or with the clinical variables examined. In order to perform such analysis a combined weighted score, encompassing the four staining patterns of ANXII in HGPIN, was calculated, such that those glands that were negative were weighted as 0 and those that were positive as follows: luminal as 1, focal as 2, and positive as 3. Clinical variables investigated included: pathological stage, Gleason score, age at radical prostatectomy, and prostate weight. Fisher's exact test, odds ratio analysis, and 95% CI calculations by the approximation of Woolf (GraphPad Prism 4) were used to examine the efficacy of ANXII versus AMACR staining in assisting correct diagnosis.

RESULTS

Patient characteristics and routine histology

Information on patients who had undergone radical prostatectomy was obtained from patient records (table 1). Due to the heterogeneity of prostatic tissue, benign, atrophic, HGPIN and

Figure 1 Annexin II (ANXII) reactivity diffusely in the cytoplasm of the basal cells. ANXII reactivity in the basal cell layer (A) correlates with positive nuclear staining for p63 (B). Original magnification $\times 20$.

prostate cancer glands were often available for scoring on an individual core, regardless of whether the core was present on the HGPIN or the Gleason 6(3+3) prostate cancer tissue microarrays. A total of 320 cores contained prostate cancer (14 of which were on the HGPIN arrays, and were, in fact, Gleason score 7(3+4), pattern 3). A total of 402 cores contained HGPIN, including 116 cores from the prostate cancer array. In addition to the three benign control cores on each array, 292 cores contained benign glands and 55 cores contained atrophic lesions.

Immunohistochemistry

Figure 1 shows the immunoreactivity of ANXII diffusely in the cytoplasm of the basal cell layer, corresponding to cells reactive

for p63. Such staining was not observed in prostate cancer glands and an interrupted basal cell staining was observed in the HGPIN glands.

Moderate to strong membranous staining for ANXII was observed in 286 of 292 benign tissues (98%) and 53 of 55 (96%) atrophic tissues (table 2, fig 2). Conversely, AMACR reactivity was negative in 210 (72%) samples with benign glands. This difference of staining patterns in benign glands was not statistically significant ($p = 1.00$). No significant difference was observed between the staining of ANXII and AMACR in atrophic glands ($p = 1.0$). Negative expression of ANXII was able to confirm a diagnosis of malignancy in 241 (75%) of Gleason 6(3+3) prostate cancer containing cores ($n = 320$), compared to the 272 (85%) that stained moderately to strongly for AMACR (fig 3). Positive ANXII expression in prostate cancer glands was typically luminal and rarely encompassing the whole membrane. There was no significant difference ($p = 0.29$) in the diagnostic value of ANXII compared to that of AMACR on prostate cancer glands.

Heterogeneous staining patterns were observed in HGPIN containing cores ($n = 402$) with both ANXII and AMACR. The reactivity of AMACR in HGPIN glands ranged from negative to very strong, and occasionally both strong and weak staining were observed in adjacent HGPIN glands in a single core (fig 4A,B). On the contrary, four inter-related staining patterns were observed for ANXII in HGPIN (figs 4C,F, 5). No expression of ANXII with the exception of the basal cells was observed in 177 cores (44%) which were considered to be negative. A subset of glands emerged in 141 HGPIN cores (35%) which showed strong and diffuse cytoplasmic staining throughout the gland, scored positive. In 60 cores (15%) containing HGPIN glands, a portion of the gland did not express ANXII. Interestingly, such negative foci consistently showed more obvious morphological features of HGPIN, such as more prominent nucleoli and an increased nuclear to cytoplasmic ratio (fig 6). In glands staining focally for ANXII, the positively staining component showed a membranous staining pattern similar to the benign glands. Finally, a small proportion of glands (24 cores, 6%) showed no cytoplasmic or membranous reactivity with the exception of the luminal membrane of the secretory epithelial cells.

A significant proportion of HGPIN glands were negative for ANXII compared to glands that stained positively for AMACR ($p < 0.0001$). Odds ratio analysis showed that ANXII was 1.827 times more likely than AMACR to accurately aid in the diagnosis of HGPIN (95% CI 1.367 to 2.442). This analysis may be an underestimate of the actual ability of ANXII staining to correctly identify HGPIN glands, since positive, focal and luminally staining glands were grouped together as positive for ANXII in this comparison. It is noteworthy that focal and luminal staining were characteristic of HGPIN. Re-examination of the odds ratio indicated that a negative, focal, or luminal staining pattern with ANXII was 4.29 times more likely to correctly identify HGPIN glands compared to positive AMACR

Table 2 Staining patterns for annexin II (ANXII) and α -methylacyl-CoA racemase (AMACR)

	ANXII			AMACR			p-value†
	% Negative	% Weak	% Moderate-strong	% Negative	% Weak	% Moderate-strong	
Benign	0.3	1.7	98	72	28	0	1.00
Atrophy	4	0	96	75	24	2	1.00
HGPIN	44	0	56*	28	42	30	<0.0001
Prostate cancer	75	12	13	1	13	85	0.2969

HGPIN, high-grade prostatic intraepithelial neoplasia.

*The positive, focal and luminal staining patterns of HGPIN are grouped into this subset.

†The staining patterns for ANXII and AMACR were shown to be significantly different when comparing the moderate-strong and negative-weak staining patterns.

Figure 2 Benign gland reactivity with annexin II (ANXII), p63 and α -methylacyl-CoA racemase (AMACR). (A) H&E. (B) Benign glands show strong membranous expression of ANXII and diffuse cytoplasmic immunoreactivity in the basal cells. (C) Basal cell nuclei stain for p63. (D) Negative to weak expression of AMACR in benign glands. Original magnification $\times 20$.

staining (95% CI 3.198 to 5.778). The proportion of glands identified by ANXII compared to AMACR remained statistically significant ($p < 0.001$).

Weak reactivity was considered to be non-specific. ANOVA comparison of ANXII and AMACR for all tissue types investigated showed that ANXII had a significantly smaller proportion of non-specific staining than we observed with AMACR ($p = 0.013$).

Correlation with patient variables

Both linear and multiple regression analysis showed no correlation, in any of the histological tissue types examined, between ANXII staining patterns and age at radical prostatectomy, Gleason score, pathological stage, or prostate weight (prostate cancer, $F = 0.04013$; HGPIN, $F = 8.201 \times 10^{-6}$). In addition, no correlation was found between the immunoreactivity of AMACR and the staining patterns observed with ANXII ($R^2 < 0.05$, $F < 0.01$). Further regression analysis of the 125 cores that contained foci of both prostate cancer and HGPIN, revealed that there was no correlation between ANXII scores in prostate cancer and HGPIN in these patients ($R^2 = 0.02431$, $F = 0.0303$).

DISCUSSION

ANXII is expressed in the cytoplasm in the prostatic basal cells.²⁷ This study is the first to compare the immunohistochemical expression of ANXII and p63 and shows that ANXII is as reliable as p63 in the detection of prostatic basal cells. The robust and diffuse cytoplasmic staining of basal cells by ANXII provides a precise marker of the integrity of the basal cell layer. Thus ANXII may aid in the diagnosis of HGPIN in which a distinguishing morphological feature is a discontinuous basal cell layer.

This study shows positive ANXII immunoreactivity in the glandular epithelium of benign prostatic tissue. Previous studies have reported that 75–100% of benign prostatic glandular epithelium stained positively for ANXII,^{27–29 33}

consistent with the 98% reported in this study. No expression of AMACR was identified in benign glands in this study. No staining for ANXII was observed in less than 1% of cores that contained foci of benign glands. These negative benign glands may represent biologically transformed glands, which do not show morphological features of neoplastic transformation. The possibility of a false negative result cannot be excluded. AMACR is more reliable than ANXII in the identification of benign glands by statistical analysis. However, this may not indicate biological significance, since these stains were very similar in their ability to appropriately stain benign glands.

We confirm that ANXII is a negative marker of prostate cancer, since 75% of malignant glands showed no expression. This is consistent with the previously reported findings of ANXII loss in 68–100% of prostate cancer glands.^{27–29 33} Comparison of ANXII and AMACR staining patterns indicated no significant difference in the proportion of prostate cancer glands that were identified by either of these stains. ANXII is as useful as AMACR in its capacity to assist in the diagnosis of prostate cancer. We thus propose ANXII to be a dependable negative marker of prostate cancer. The use of ANXII as a negative marker in the diagnosis of prostate cancer is reliable since the majority of prostate biopsy cores contain benign glands that serve as an internal positive control for each specimen under investigation (fig 3E). Immunohistochemical staining for ANXII was performed manually with overnight incubation as part of a research protocol. However, we are currently developing methodology for the use of ANXII in the routine clinical laboratory.

Heterogeneous staining patterns of AMACR, specifically in HGPIN, are well recognised.^{7–14} For this reason, it is often recommended that AMACR be used in conjunction with basal cell markers, such as p63, for diagnostic clarification in equivocal cases. The staining pattern of ANXII in HGPIN appears to be heterogeneous, like AMACR. However, linear regression analysis indicates that AMACR and ANXII do not

Figure 3 Reactivity of Gleason 6(3+3) prostatic adenocarcinoma with annexin II (ANXII), p63 and α -methylacyl-CoA racemase (AMACR). (A) H&E. (B) Negative immunoreactivity for ANXII and the absence of a basal cell layer are identified with ANXII. (C) No p63 positive basal cell nuclei in prostate cancer glands. (D) Strong positive staining with AMACR. (E) ANXII negative prostate cancer with adjacent benign gland as an internal positive control. A–D, original magnification $\times 20$; E, original magnification $\times 10$.

always recognise the same subsets of glands. For instance, in HGPIN, the AMACR scores did not inversely correlate with the ANXII scores. This indicates that different subsets of HGPIN are being identified by ANXII and AMACR. Since the application of AMACR as a marker of prostate cancer, a number of studies have been unable to show a correlation of AMACR staining with clinical outcome.^{8–9} Such studies have not been performed for ANXII. Since ANXII staining overlaps and increases the detection of HGPIN compared to AMACR, it has the potential to further define the diagnosis of HGPIN with respect to clinical outcome. It is possible that the loss of ANXII expression in HGPIN may predict concomitant prostate cancer or may identify a subset of HGPIN glands with a greater propensity for malignant progression. These studies are currently under investigation.

Positive HGPIN glands showed a unique, diffusely cytoplasmic staining pattern for ANXII. This may be due to altered function and localisation of the protein during neoplastic progression. Liu *et al*³⁴ used immunofluorescence to show a reduced expression of ANXII in DU145 cells with residual protein expression exclusively in the cytosol. This coincided with increased migration of these cells compared to those that

expressed ANXII on the membrane. Thus our results may be indicative of increased migratory ability of these cells, a key alteration in neoplastic transformation. Alternatively, it may be hypothesised that the cytoplasmic expression represents an internalisation process removing ANXII from the plasma membrane since ANXII has been co-localised in lipid rafts and caveolae,^{35–37} and is thought to have a role in endocytosis.³⁸ The significance of the relocalisation of ANXII in HGPIN transformation remains undetermined.

We identified HGPIN glands that had foci of no reactivity, in which the remaining positively staining glandular epithelium was typically membranous, similar to benign glands. These foci often showed more prominent morphological features characteristic of HGPIN, with increased nuclear to cytoplasmic ratio and more prominent nucleoli. This staining pattern may be indicative of glands either undergoing transformation from benign to HGPIN or may represent focal transformation of a gland antecedent to invasion.

Finally, we identified a population of HGPIN glands with luminal staining. In some instances this may represent loss of ANXII protein expression via exocytosis or shedding into the lumen. Alternatively, luminal staining may represent residual

Figure 4 High-grade prostatic intraepithelial neoplasia (HGPIN) staining patterns with α -methylacyl-CoA racemase (AMACR) and annexin II (ANXII). (A and B) Heterogeneous staining patterns for AMACR showing both weak and strong reactivity in adjacent HGPIN glands. Four ANXII staining patterns in HGPIN (C–E): (C) positive expression of ANXII remains in the basal cell layer, with no staining in the secretory epithelial cells; (D) diffuse positive cytoplasmic staining for ANXII; (E) focal staining for ANXII. (F) Luminal staining for ANXII. Original magnification $\times 20$.

ANXII positive benign cells sloughed into the lumen as they are replaced by transformed HGPIN cells. It has been proposed that the basal cell layer may harbour the cell of origin of prostatic neoplasia^{39–42}; however this hypothesis is controversial. We have observed what appears to be the extrusion of ANXII positive non-neoplastic cells into the lumen as they are replaced by ANXII negative transformed neoplastic cells. This may represent a possible mechanism for the progression of benign glands to HGPIN and supports the concept of the basal cell origin of prostatic neoplasia.

Our comparison of the efficacy of ANXII versus AMACR to identify HGPIN glands yielded significant results, which have not been previously described. We showed that a negative staining pattern with ANXII was able to identify approximately twice as many HGPIN glands than positive staining for AMACR. The ability of ANXII to assist in a diagnosis of HGPIN compared to AMACR was greatly increased to 4.3 times more likely when the focal and luminal staining patterns were included. The staining patterns observed in HGPIN with AMACR are variable, thus requiring the additional use of a concomitant basal cell marker for a definitive diagnosis.^{7–14} This

is unnecessary with ANXII because of its ability to positively stain basal cells. Therefore ANXII may be useful in the detection of HGPIN in prostate biopsies.

Our results indicate that the loss of ANXII is an early and frequent event in prostatic neoplastic progression, often occurring at the HGPIN stage. Unlike AMACR, ANXII has the ability to reliably distinguish between prostate cancer and HGPIN lesions due to its uniform staining of the basal cell epithelium and its distinctive staining patterns of the luminal epithelium. ANXII would be clinically applicable as a single immunohistochemical marker for the distinction of benign glands, HGPIN and prostate cancer, especially in prostate biopsies where the amount of tissue is restricted.

Limited clinical follow-up was available for our patients post-radical prostatectomy. Linear and multiple regression analysis revealed no correlation of ANXII reactivity in HGPIN or prostate cancer with age at radical prostatectomy, Gleason score, pathological stage, or prostate weight, in this cohort of patients. Although no significant correlations were found, more retrospective studies with longer clinical follow-up should be performed.

Figure 5 Distribution of annexin II staining patterns in high-grade prostatic intraepithelial neoplasia.

This study suggests that the loss of ANXII is an early and frequent event in prostate carcinogenesis. We have shown the potential utility of ANXII as a diagnostic marker in equivocal

Figure 6 Glands with focal negativity for annexin II (ANXII) compared to H&E stained sections. (A) High-grade prostatic intraepithelial neoplasia (HGPIN) glands showing a focal staining pattern with ANXII. The positively staining segments of such glands are more membranous, similar to benign glands. (B) These foci often show more typical morphological features associated with HGPIN on H&E stained sections. Original magnification $\times 20$.

Take-home messages

- Annexin II immunohistochemical staining positively identifies basal cells in prostatic epithelium.
- Annexin II is a consistent and reliable positive marker of benign secretory epithelium and a negative marker of invasive prostatic carcinoma.
- The expression of annexin II in HGPIN requires further investigation.
- Annexin II has potential utility as a diagnostic aid in prostate cancer histopathology.

cases of prostate cancer. To our knowledge this is the first body of work comparing the efficacy of ANXII as a novel diagnostic marker of prostate cancer with AMACR and p63, and the first to describe the staining patterns of ANXII in HGPIN. Since this marker consistently stains the cytoplasm of basal cells and negatively marks prostate cancer lesions, it could replace the use of AMACR and p63, or cocktails comprising both antibodies. This study indicates that ANXII is a consistent and reliable marker of prostatic neoplasia, although the prognostic significance remains to be determined.

ACKNOWLEDGEMENTS

The authors would like to thank Kelvin So for his expertise in immunohistochemistry.

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Funding for this project was provided by the Prostate Clinical Research Program at the Princess Margaret Hospital. Study sponsors had no involvement in the study design; the collection, analysis, or interpretation of data; the composition of the report; or the decision to submit the paper for publication.

Competing interests: None.

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