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Androgen Receptor Expression in Epithelial and Stromal Cells of Prostatic Carcinoma and Benign Prostatic Hyperplasia

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Abstract

BACKGROUND: Prostatic carcinoma (PCa) derives from prostatic epithelial cells. However stromal microenvironment, associated with malignant epithelium, also plays a role in prostatic carcinogenesis. Alterations in prostatic stromal cells contribute to the loss of growth control in epithelial cells that lead to progression of PCa.

AIM: To analyse the differences between Androgen Receptor (AR) expression in both epithelial and stromal cells in PCa and the surrounding benign prostatic hyperplasia (BPH) and to compare the results with tumour grade.

MATERIAL AND METHODS: Samples from 70 cases of radical prostatectomy specimens were used. The expression and intensity of the signal for AR was analysed in the epithelial and stromal cells of PCa and BPH, and the data was quantified using histological score (H-score).

RESULTS: AR showed significantly lower expression in both epithelial and stromal cells of PCa compared to BPH. In PCa a significant positive correlation of AR expression was found between stromal and epithelial cells of PCa. AR expression showed a correlation between the stromal cells of PCa and tumour grade.

CONCLUSION: AR expression is reduced in epithelial and stromal cells of PCa. Expression of AR in stromal cells of PCa significantly correlates with tumour grade.

Introduction

Prostatic carcinoma (PCa) is the most frequently diagnosed malignancy and the second leading cause of cancer-related death in men in industrialized countries [1]. Androgens play a vital role in growth, differentiation and maintenance of prostate tissue via Androgen Receptor (AR). AR in stromal cells contributes to the development and growth of prostate during fetal stages as well as during prostate carcinogenesis and cancer progression [1]. Researchers have mainly focused on studying epithelial AR expression whereas there is limited data concerning stromal AR expression.

AR expression represents a potential prognostic marker for prostatic carcinoma, but more studies are needed in order to prove the usefulness of this factor in the future.

Materials and Methods

We reviewed 70 patients who underwent radical prostatectomy for prostatic carcinoma in the Pathology Laboratory in Clinical Hospital Acibadem – Sistina, Skopje, Macedonia between January 2010 and July 2015. All cases presented with localized disease without the lymph node metastatic involvement and all cases were assigned to acinar types of prostatic adenocarcinoma. Representative samples were chosen from the periphery of PCa that contained relatively equal amounts of tumour and surrounding benign prostatic hyperplasia (BPH).

The immunohistochemical analysis was performed using Androgen Receptor (AR) mouse antibody clone AR441 (DAKO) isotype IgG1 kappa dilution 1:50. For visualization of the antigen-antibody

complex DAKO REALTM En VisionTM Detection System. Peroxidase/DAB+, Rabbit/Mouse was used.

To achieve a relevant analysis of the immunostaining, we analysed the positive signal and the intensity of the signal. We found the positive nuclear signal in stromal and epithelial cells of prostatic carcinoma and normal prostate tissue. The signal was detected on small magnification (x 40) in order to find an area with the most intense signal in the first step. Then on large magnification (x 400), we counted at least 100 epithelial and stromal cells. The number of positive nuclei was shown as a percentage of the total number of counted nuclei. The intensity of the signal was graded on a scale from 0 to 3 where the 0=no signal, 1= weak signal, 2=moderate signal and 3=strong signal. Additionally a histological score (H-score) was constructed that measures the intensity and distribution of the signal using the formula: [3 (strong signal) x (percentage of cells with strong signal)] + [2 (moderate signal) x (percentage of cells with moderate signal)] + [1 (weak signal) x (percentage of cells with weak signal)]. The histological score ranges from 0 to 300 [2, 3]. The data were compared between stromal and epithelial cells in PCa and the surrounding BPH.

Additionally, the data were compared with tumour grade using the Gleason score retrieved from the medical records. Gleason score was grouped in Gleason prognostic grade groups as follows: Gleason prognostic grade group 1 – Gleason sum 1-6; Gleason prognostic grade group 2 – Gleason score 3+4; Gleason prognostic grade group 3 – Gleason score 4+3; Gleason prognostic grade group 4 – Gleason score 4+4 and Gleason prognostic grade group 5 – Gleason sum 9-10.

For statistical analyses of the data the following methods were used: descriptive methods (average, median), methods of testing significance of differences among analyzed parameters (Chi-square test, Student t test, Wilcoxon matched test, Analysis of Variance, Kruskal-Wallis ANOVA) and methods of determining correlation among designated parameters (Spearman coefficient of rang correlation and Pearson coefficient of correlation). Statistically significant values were determined to be $p < 0.05$ and highly statistically significant values were determined to be $p < 0.01$.

Results

AR immunoreactivity was exclusively nuclear and was detected in tumour epithelial cells, epithelial cells of benign glands, peritumoral stromal cells and intraglandular stroma in BPH (Fig.1A and Fig. 1B).

Tables 1 shows the mean values, average values as well as minimal and maximal values of AR expression in epithelial and stromal cells of prostatic carcinoma and the surrounding BPH.

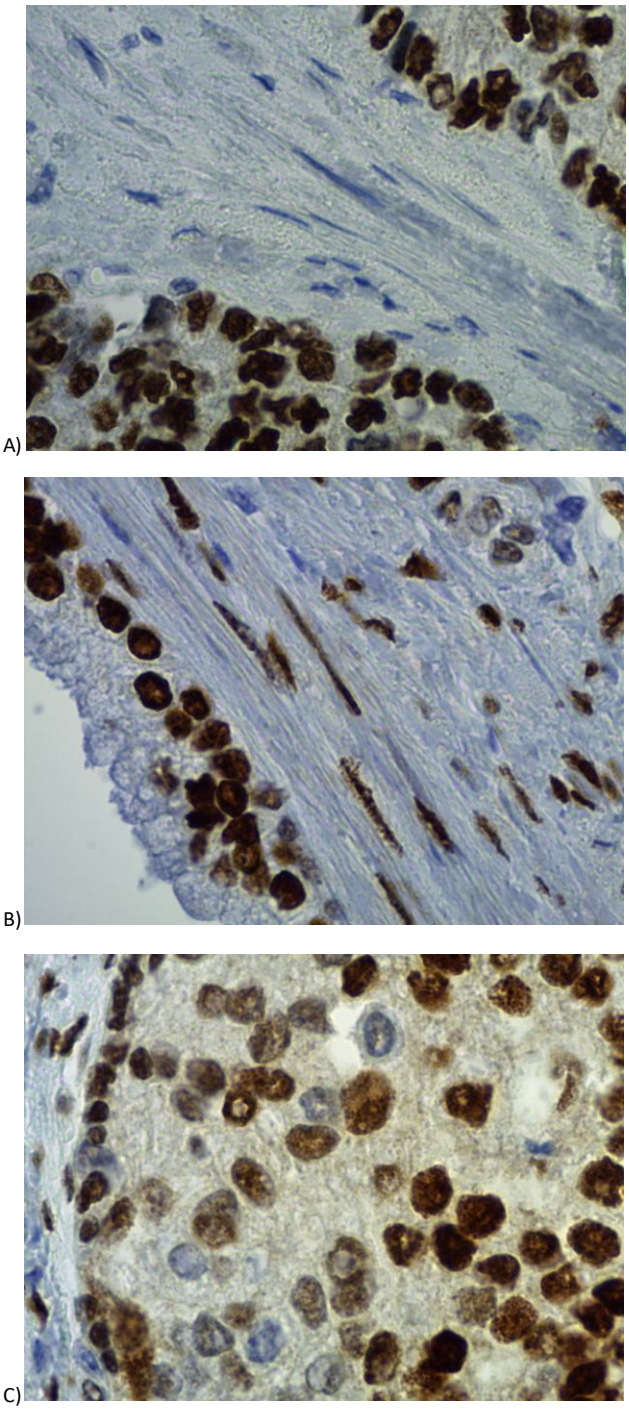

Figure 1: A) Expression of Androgen Receptor in epithelial and stromal cells in prostatic carcinoma (Androgen Receptor x 400); B) Expression of Androgen Receptor in epithelial and stromal cells in benign prostatic tissue (Androgen Receptor x 400); C) Heterogenous signal in epithelial cells of prostatic carcinoma in poorly differentiated tumors (Androgen Receptor x 600)

The expression of AR in the epithelial cells of PCa is significantly (Wilcoxon Matched Pairs $Z = 3.1$ $p = 0.002$) lower compared to the expression of AR in BPH (med 257.5 vs. 267). We registered significantly lower ($t = 9.5$ $p < 0.001$) average values of AR

expression in PCa compared to BPH (114.5 vs. 161) in the stromal cells. The average value of AR expression in the epithelial cells of PCa is 253.73 ± 22.9 and is significantly higher than the average value of AR expression in stromal cells of PCa with a score of 114.2 ± 37.3 ($t = 34.7$, $p < 0.01$). The value of AR expression in the epithelial cells of BPH is significantly (Wilcoxon Matched Pairs $Z = 7.3$ $p < 0.01$) higher than the expression of AR in stromal cells of BPH (med 267 vs. 161).

Table 1: Androgen Receptor (AR) expression using histological score (H-SCORE) in the epithelial and stromal cells in prostatic carcinoma and benign prostatic tissue

	N	Descriptive statistics – AR (H-SCORE)		
		mean ± SD	median	min - max
Carcinoma – epithelial cells	70	253.73 ± 22.9	257.5 (246 – 272)	153 - 285
Carcinoma – stromal cells	70	114.2 ± 37.3	114.5 (90 – 142)	37 – 208
Benign prostatic tissue – epithelial cells	70	264.14 ± 16.4	267 (260 – 275)	185 – 286
Benign prostatic tissue – stromal cells	70	161.61 ± 22.1	161 (148 – 177)	110 – 212

These data show that there was a drop in AR expression in both stromal and epithelial cells in prostatic carcinoma compartment but the drop of AR expression was more pronounced in the stromal compartment of prostatic carcinoma.

We studied the correlation of AR expression between the epithelial and stromal cells of PCa and BPH. The value of Pearson coefficient of linear correlation shows that expression of AR in the stromal cells of PCa significantly positively correlates with AR expression in the epithelial cells of PCa ($r = 0.046$ $p < 0.01$). The correlation between AR expression in the epithelial and stromal cells of BPH is positive but statistically not significant ($r = 0.22$ $p > 0.05$). A positive but not significant correlation exists between AR expression in epithelial cells of PCa and epithelial cells of BPH ($R = 0.056$, $p = 0.6$). Also, AR expression between stromal cells of PCa and BPH shows positive but not significant correlation ($R = 0.094$, $p = 0.44$).

The analyses of PCa in relation to tumor grade (Gleason score) according to the new Gleason score grouping showed that 12 cases belonged to Gleason prognostic grade group 1 (Gleason sum 2-6), 23 cases belonged to Gleason prognostic grade group 2 (Gleason score 3+4), 24 cases belonged to Gleason prognostic grade group 3 (Gleason score 4+3), 3 cases belonged to Gleason prognostic grade group 4 (Gleason score 4+4), and 8 cases belonged to Gleason prognostic grade group 5 (Gleason sum 9-10) (Table 2).

Table 2: Distribution of patients compared to Gleason prognostic grade group

Gleason prognostic grade group	N = 70 (%)
1	12 (17.14)
2	23 (32.86)
3	24 (34.29)
4	3 (4.29)
5	8 (11.43)

There was no significant statistical difference in the average values of AR expression and Gleason prognostic grade group ($F = 0.25$, $p = 0.9$) in the epithelial cells of PCa (Table 3).

Table 3: Expression of Androgen Receptor (AR) in epithelial cells of prostatic carcinoma compared to Gleason prognostic grade group

Gleason grade group	N	Descriptive statistics – AR expression in epithelial cells of prostatic carcinoma	
		mean ± SD	minimum - maximum
1	12	255.17 ± 37.1	153 – 285
2	23	251.87 ± 21.2	191 – 282
3	24	256.79 ± 12.9	228 – 277
4	3	251.33 ± 20.5	234 – 274
5	8	248.62 ± 29.2	212 – 280
Tested differences		Analysis of Variance $F = 0.25$, $p = 0.9$	

The average value of AR expression in the stroma of PCa showed a statistically significant difference compared to Gleason grade group ($F = 4.33$, $p = 0.0036$). In the Gleason prognostic grade group 1 the average value was 144.0 ± 41 that was significantly higher compared to Gleason prognostic grade groups 3, 4 and 5 ($p = 0.033$; $p = 0.0034$; $p = 0.0085$ consecutively) (Table 4).

Table 4: Expression of Androgen Receptor (AR) in stromal cells of prostatic carcinoma compared to Gleason prognostic grade group

Gleason grade group	N	Descriptive statistics – AR expression in stromal cells of prostatic carcinoma	
		mean ± SD	minimum - maximum
1	12	144.0 ± 41.9	42 – 208
2	23	117.87 ± 34.4	50 – 167
3	24	108.17 ± 30.3	48 – 178
4	3	79.0 ± 36.5	37 – 103
5	8	90.25 ± 30.4	51 – 137
Tested differences		Analysis of Variance F=4.33 p=0.0036**; Post hoc Turkey test; Gleason grade group 1 vs. Gleason grade group 3 p=0.033*; Gleason grade group 1 vs. Gleason grade group 4 p=0.0034**; Gleason grade group 1 vs. Gleason grade group 3 p=0.0085**	

*p < 0.05; **p<0.01.

We also analysed the correlation between Gleason grade group and AR in both the epithelial and stromal compartments of PCa. Tumor differentiation expressed through Gleason score did not show significant correlation with AR expression in the epithelial cells of PCa ($R = -0.15$, $p = 0.02$). The correlation between Gleason score and AR expression in the stromal cells of PCa was negative and statistically significant ($R = -0.44$. $p < 0.01$) which means that as a tumour showed less differentiation AR expression in the stromal cells of prostatic carcinoma decreased significantly and vice versa.

Discussion

The role of stroma in prostate development, prostate function and the maintenance of tissue differentiation is well established [1]. Androgen Receptor (AR) plays a critical role in prostatic

development through regulation of androgen effects on epithelial cells. AR expression is mainly localized in the mesenchymal tissue in the fetal period while AR is mainly localized in epithelial compartment in the post-natal period [1]. The mesenchymal AR initiates and controls proliferation and differentiation of epithelial cells while epithelial AR plays a role in functioning and differentiation of the prostatic gland hence stromal-epithelial interactions are reciprocal in the development of mature prostatic tissue [1].

AR in epithelial cells of prostatic carcinoma was studied by several authors [3-18]. Some authors found greater expression of AR in epithelial cells in well-differentiated tumours compared to moderately and poorly differentiated tumours [4-8]. In our study also, there was a slightly greater expression of AR in epithelial cells in better-differentiated tumours but the difference was statistically not significant ($F = 0.25$, $p = 0.9$). In some prospective studies, authors presented higher expression of AR to be associated with better prognosis [4, 5, 9] while other authors suggested higher AR expression in the epithelial cell to be associated with worse prognosis [10-13]. However, most authors agree that a phenomenon known as AR expression heterogeneity was a consistent finding in poorly differentiated tumours [4, 8, 9, 14, 15]. This phenomenon was also observed in our study in poorly differentiated carcinomas (Fig. 1C).

The discrepancies in these findings can be attributed to several factors. First of all, the lack of unified criteria about the method used in the analyses could be an important factor. Second the specimens can also attribute to these varied results since some authors performed their analyses on biopsy specimens [4, 5, 9, 14], some of transurethral resection specimens [5, 6, 16, 17] and most of them on radical prostatectomy specimens [3, 7, 8, 10-13,18]. At the end AR expression heterogeneity complicates the matter further since the selection of analysed area is compromised. In order to eliminate any possible miss assessments, we used only radical prostatectomy specimens and selected the area of analysis where the staining signal was strongest, and also we used the H-score to incorporate both the staining intensity and percentage of stained cells as it is specified in the section of Material and Methods. With this approach, we tried to address these issues concerning the inconsistencies in previous assessments of AR expression. Concerning the issue of the method used to assess AR expression, we used H-score as was recommended by some authors as a most valid method of AR expression analysis [3]. The second issue concerning material used to assess AR expression we only used radical prostatectomy specimens as they contain a lot of material to choose adequate samples for analysis. In the end, the problem of heterogeneity was address by choosing areas of highest staining intensity and performing the analyses in the chosen areas as was recommended by some authors [3].

Stromal expression of AR was much less studied than an epithelial expression of AR in PCa [1, 19]. Authors found a decline in stromal AR expression in the stromal cells surrounding prostatic cancer. Also, the drop of expression was higher in poorly differentiated carcinomas. Authors found a statistically significant decrease of stromal AR expression in carcinomas compared to stromal AR expression in the benign prostatic hyperplasia surrounding the prostatic carcinoma [1]. These findings are consistent with our data. In our study, the decrease in AR expression in the stromal cells in poorly differentiated carcinomas showed statistical significance. There was a negative statistically significant correlation of AR expression compared to Gleason grade group meaning that AR expression was significantly higher in well-differentiated carcinomas compared to poorly differentiated carcinomas.

AR expression belongs to the category of prognostic factors of prostatic carcinoma [20, 21]. There are three categories of prognostic factors. The first group encompasses well established histopathological factors of prostatic carcinoma like the pathologic stage of the disease, status of surgical margins in radical prostatectomy specimens and Gleason grade. The second category is comprised of factors that are presumed to be established in the near future like histological types of prostatic carcinoma, tumour volume, and DNA ploidy. The third category belongs to factors that do not possess enough data that they may represent prognostic factors shortly. These factors are genetic markers, neuroendocrine markers, proliferative markers, perineurial, vascular or lymphatic invasion, small vessel density, nuclear morphometry and AR expression of epithelial cells [20, 21]. For this third category of prognostic factors, authors are welcomed to perform studies in order to prove their clinical usefulness [20, 21].

AR expression in epithelial cells of prostatic carcinoma has been studied more than AR expression in stromal cells. Our study shows the significance of AR expression assessment in both epithelial and stromal cells because both epithelial and stromal cells may contribute to initiation and progression of prostatic carcinoma.

Further studies are needed in order to prove the clinical usefulness of this potential prognostic factor and correlations are needed with the already well established prognostic factors like the Gleason grade.

In conclusion, our study shows a significant drop of AR expression in both epithelial and stomal cells of prostatic carcinoma compared to benign prostatic tissue that indicates that there is a quantitative change in AR expression in the malignant prostatic tissue. The drop in AR expression is more pronounced in the stromal cells. Also, this drop of AR expression continues as prostatic carcinomas evolve

from well differentiated to poorly differentiated carcinomas. The drop of AR expression in the epithelial cells, as they progress to poorly differentiated carcinomas, is not statistically significant but the drop of AR expression in the stromal cells is statistically significant. This concludes that AR expression in stromal cells of prostatic carcinoma could represent one of the prognostic factors for prostatic carcinoma in the future.

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