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RESEARCH ARTICLE

CORRELATION OF ANDROGEN RECEPTOR EXPRESSION IN EPITHELIAL AND STROMAL CELLS OF PROSTATIC CARCNOMA WITH TUMOR GRADE

***,[1]Vanja Filipovski, [2]Vesna Janevska [1] Katerina Kubelka-Sabit and [1]Dzengis Jasar**

[1]Clinical Hospital ACIBADEM/SISTINA, Skopje, Skupi street No. 5a, Republic of Macedonia
[2]Institute of Pathology, Medical Faculty Skopje, University "Ss. Cyril and Methodius", Republic of Macedonia

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ABSTRACT

Introduction: Androgens are vital to prostate growth, development, and prostatic carcinogenesis. Studies have been conducted to evaluate androgen receptor expression in cancer cells and most of the studies have concentrated on cancer epithelial cells and not cancer stromal cells. Development of the prostate gland as well as development of prostatic cancer is closely associated with stromal-epithelial interactions and in the centre of these interactions stands the Androgen Receptor in both stromal and epithelial cells.

Material and Methods: A retrospective study was conducted using 70 cases of diagnosed prostatic cancer from radical prostatectomy samples. The immunohistichemical analysis was conducted using Androgen Receptor antibody. Quantification of the signal was analyzed using the histological score (HSCORE) that encompasses the percentage of stained cells and the intensity of the signal. Than histological score was correlated with tumor grade using the Gleason scoring system were the cancers were divided into well differentiated prostatic carcinoma (G1) (Gleason score 2-6), moderately differentiated carcinoma (G2) (Gleason score 7) and poorly differentiated carcinoma (G3) (Gleason score 8-10).

Results: In the epithelial cells Androgen Receptor expression declined as Gleason grade increased but this correlation was statistically not significant. In the stromal cells a decline in Androgen Receptor expression was also noted but this decline was more pronounced and statistically significant correlation of Androgen Receptor expression was noted between well differentiated carcinomas and moderately and poorly differentiated prostatic carcinomas.

Conclusion: Androgen Receptor expression in stromal cells of prostatic carcinoma shows more pronounced decrease as the tumor dedifferentiates compared to Androgen Receptor expression in epithelial cells of prostatic carcinoma.

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INTRODUCTION

Androgen control over normal growth of the prostate is transmitted via Androgen receptor (AR) that is expressed by the nuclei of both stromal and epithelial cells (Sar *et al*., 1990). The mechanisms responsible for initiation and propagation of prostatic carcinogenesis and the development of hormone resistance Prostatic Carcinoma (PCa) are unknown, but abnormalities in the activity of AR are thought to play an important part in these changes in the prostatic cell phenotype. Evaluation of AR expression in normal and abnormal prostatic tissues has been a major focus to unravel the mechanism responsible for malignant transformation and hormone-independent growth of the organ (Olapade *et al*., 1999). First reports suggest that AR expression is inversely proportional to histologic grade (Chodak *et al*., 1992; Masai *et al*., 1990) and than other reports showed high expression of AR and increased heterogeneity of the receptor in advanced cancers (Magi-Galluzzi *et al*., 1997; Ruizeveld de Winter *et al*., 1994). AR transmission of hormonal control of prostatic growth occurs via the regulation of the expression of the peptide growth factors and receptors involved in stromal-epithelial interactions that mediate the influence of androgens on the gland (Hiramatsu *et al*., 1988; Brass *et al*., 1995). Proliferation and differentiation of prostatic epithelium is initiated by binding of epithelial cell

****Corresponding author: Vanja Filipovski, Katerina,***
Clinical Hospital ACIBADEM/SISTINA, Skopje, Skupi street No. 5a, Republic of Macedonia.

membrane receptors by growth factor expressed in stromal cells (Shima *et al*., 1995). This intercellular relationship indicates that prostatic stroma plays an important intermediary role in the transmission of androgen-induced stimuli to the adjacent epithelial cells. Malignant transformation of prostatic epithelial cells may therefore involve abnormalities of a pathway that leads from the stromal nucleus through receptors on the epithelial cell membrane to the epithelial cell nucleus. AR expression of stromal cells in prostatic carcinoma is being only recently investigated as opposed to AR expression of epithelial cells. The transformation of prostatic epithelial cells could be associated with changes in AR expression in the nuclei of surrounding stroma.

MATERIALS AND METHODS

The material for this study was obtained from 70 cases of diagnosed prostatic carcinoma from radical prostatectomy specimens in the histopathological laboratory of Acibadem / Sistina clinical hospital from May 2010 to February 2015. The archive slides were reviewed and selected samples were chosen from the peripheral areas of prostatic carcinoma with a relatively equal amount of Prostatic Carcinoma (PCa) and surrounding Benign Prostatic Hyperplasia (BPH). For determination of immunohistochemical expression of Androgen Receptor (AR) the antibody Androgen Receptor, clone AR441 (DAKO) isotype IgG1 kappa was used with 1:50 dilution. The samples were prepared using instruction guidelines. Taking into account the heterogeneity of the signal the samples were observed on low magnification (x40) to find a spot with the most intensive signal. Than the signal was analyzed on high magnification (x400) were at least 100 epithelial and stromal cells were counted. The signal had a nuclear localization. The number of positive nuclei was shown as a percentage of total number of counted nuclei. Considering the heterogeneity of the signal "histological score" was constructed (HSCORE) that measures intensity and distribution of the signal using the formula Pi(i+1). Intensity of the signal (i) was subjectively graded from scale of 0-3 where 0 = no signal, 1 = weak signal, 2 = moderate signal, and 3 = strong signal, while Pi represents the percentage of stained cells for each intensity signal. This semi quantitative method has been proven as a method with high interobserver and intraobserver reproducibility (Qui *et al*., 2008). These results were then correlated with tumor differentiation (histologic grade) using the Gleason grading system. The prostatic carcinoma cases were stratified in 3 groups: well differentiated prostatic carcinomas (G1) Gleason score 2-6, moderately differentiated prostatic carcinoma (G2) Gleason score 7, and poorly differentiated prostatic carcinomas (G3) Gleason score (8-10).

RESULTS

The mean value of AR in the epithelial cells of PCa is highest in the group of well differentiated PCa (G1)(85,06± 12,4), lower in the group of moderately differentiated PCa 84,79 ± 5,8 and lowest in the group of poorly differentiated PCa 83,12 ± 8,7. The statistical analysis showed insignificant differences in the mean value of AR of epithelial cells of PCa with in various tumor grades. The mean values of AR are insignificantly higher in epithelial cells of well differentiated PCa compared to moderately or poorly differentiated PCa (F=0, 24 p=0,79) (Table 1).

Table 1. Correlation between AR expression in PCa epithelial cells and G score

G score	N	Descriptive Statistics – AR Ca epithelium	
		mean ± SD	minimum - maximun
G1	12	85,06 ± 12,4	51,0 – 95,0
G2	47	84,79 ± 5,8	63,7 – 94,0
G3	11	83,12 ± 8,7	70,7 – 93,3
Tested differences		Analysis of Variance F = 0,24 p = 0,79 *NS	

*NS – not significant

However the mean value of AR in stromal cells of PCa shows statistically significant differences with tumor grade (F=8,16 p=0,0007). In G1 PCa (well differentiated PCa) the mean value of AR is highest (48,0 ± 13,9), in moderately differentiated PCa (G2) is lower 37,64 ± 10,8, and in poorly differentiated PCa (G3) is lowest 29,06 ± 10,2. Post hos analysis showed that in the group of well differentiated PCa, the mean value of AR in stromal cells is significantly higher compared to the group of moderately differentiated (p=0,017) and the group of poorly differentiated PCa (p=0,0005). The difference in the mean value of AR in the stromal cells of moderately and poorly differentiated PCa was insignificant (p=0,07) (Table 2).

Table 2. Correlation between AR expression in PCa stromal cells and G score

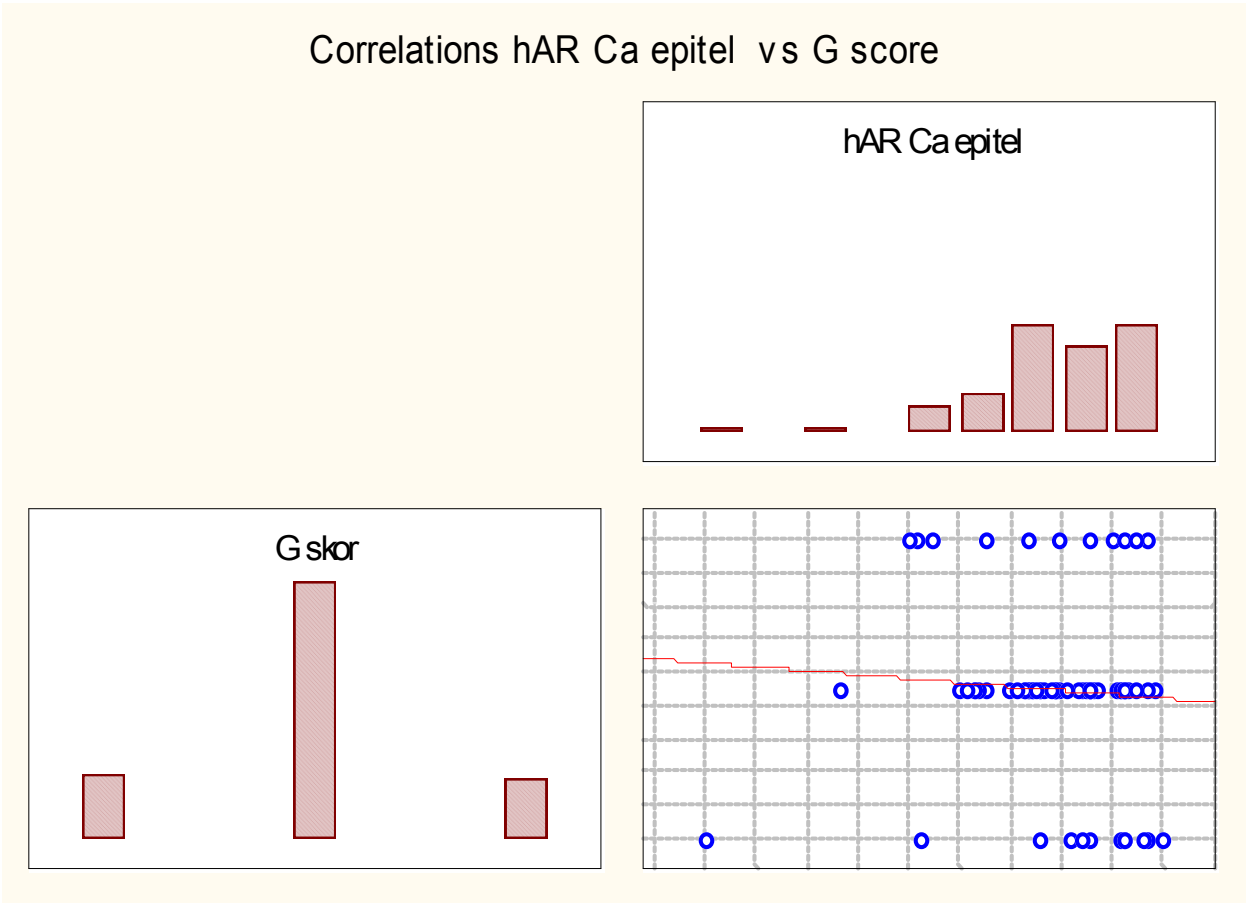
G score	N	Descriptive Statistics – hAR Ca stroma	
		mean ± SD	minimum - maximun
G1	12	48,0 ± 13,9	14,0 – 69,3
G2	47	37,64 ± 10,8	16,0 – 59,3
G3	11	29,06 ± 10,2	12,3 – 45,7
Tested differences		Analysis of Variance F = 8,16 p = 0,0007** p<0,01 Post hoc Tukey test G1 vs G2 p=0,017 G1 vs G3 p=0,0005 G2 vs G3 *NS	

*NS – not significant

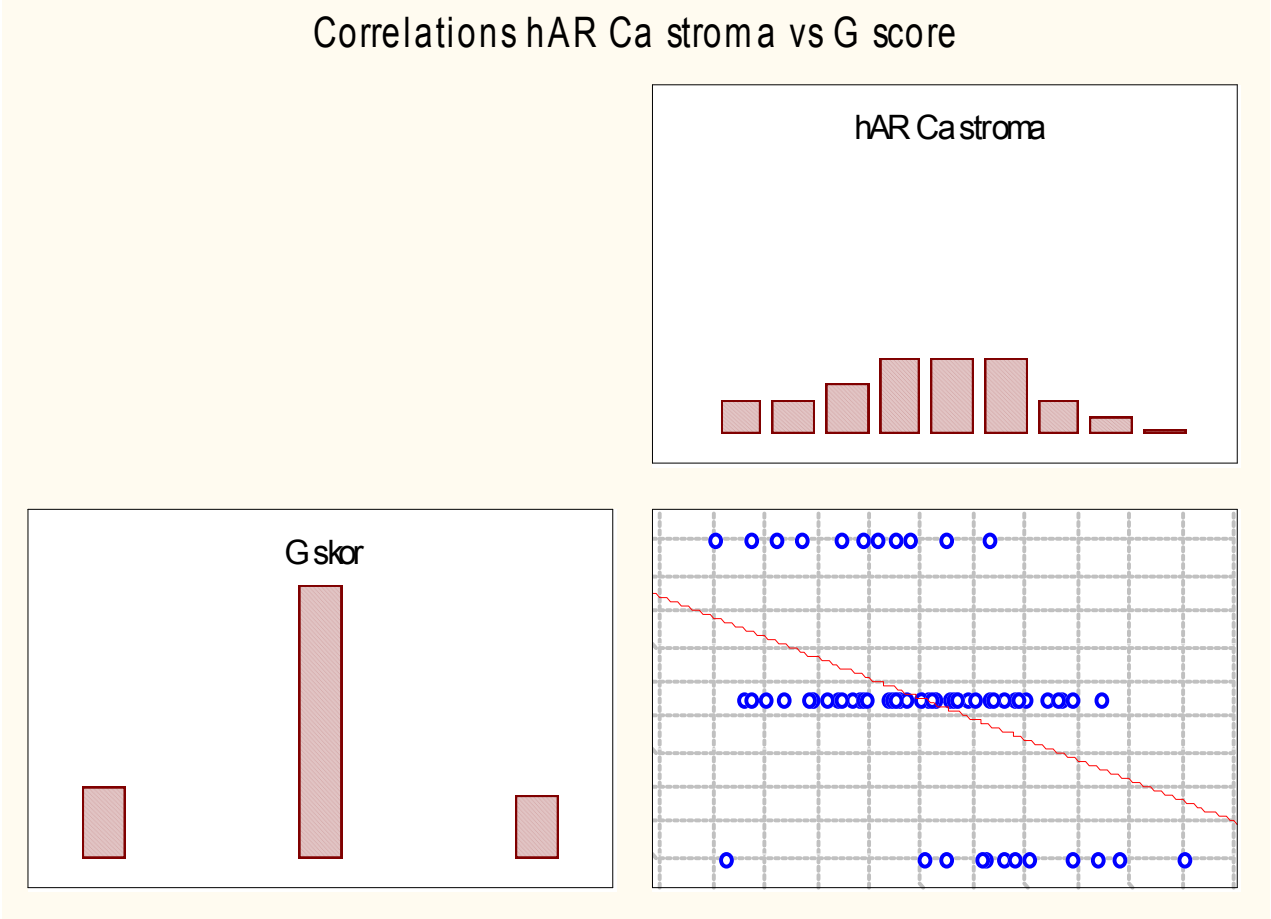
Tumor differentiation expressed through G score and expression of AR in the epithelial cells of PCa show insignificant correlation (R = - 0,15 p>0,05). Correlation between G score and expression of AR in stroma is inverse, but statistically significant (R = - 0,44 p<0,05) meaning that as G score increases (tumor dedifferentiation) the expression of AR in stromal cells decreases.

DISCUSSION

Most of the studies conducted so far concerning the expression of AR in prostate cancer cells have been concentrated on the expression in the nuclei of epithelial cells. Association of AR expression and tumor differentiation (Gleason grade) has been correlated and there are generally conflicting reports. Some of the other studies showed presence of association between AR and Gleason score but this correlation was not statistically significant (Qui *et al*, 2008; Olapade-Olaopa *et al*., 1999; Sweat *et al*., 1999; Noordzij *et al*., 1997; Gaston *et al*., 2003; Ford *et al*., 2003). However this correlation was sometimes positive (Qui *et al*., 2008; Magi-Galluzzi *et al*, 1997, Ruizeveld de Winter *et al*., 1994) and sometimes negative (Chodak *et al*., 1992; Masai *et al*, 1990) but not statistically significant.



Spearman Rank Order correlation R = - 0,15 p>0,05



Spearman Rank Order correlation R = - 0,44 p<0,05

But some studies found decrease in AR expression in moderately differentiated carcinomas (G2) and increase in poorly differentiated carcinomas (Qui *et al*., 2008; Olapade-Olaopa *et al*., 1999). But there were studies that showed decrease in AR expression in poorly differentiated carcinomas as well as metastatic carcinomas (Takeda *et al*., 1996; Segawa *et al*., 2001; Miyamoto *et al*., 1993; Ruizeveld de Winter *et al*., 1990). In our study however there was a progressive linear decrease in AR expression in epithelial cells from well differentiated carcinomas to poorly differentiated carcinomas, but again this correlation was not statistically significant. Authors that conducted retrospective and prospective studies showed higher AR expression to be associated with better prognosis (Takeda *et al*., 1996; Segawa *et al*., 2001 Pertschuk *et al*., 1995) while other authors found the expression of AR to be associated with worse prognosis (Inoue *et al*., Li *et al*., 2004., Henshall *et al*., 2001 Sweat *et al*., 1999).

These conflicting reports were assigned to be to the most part a result of tumor heterogeneity that almost all authors encountered. We also observed this phenomenon of tumor heterogeneity but the method we used to quantify AR expression proved to minimize this phenomenon. Other discrepancies were attributed to different tissue samples like biopsy material or transurethral resection specimens that are small samples were the issue of thermal injury may play a role as AR are sensitive to thermal injury and may be damaged during this procedure. Also there is the issue of different evaluation methods. We used the preferred large radical prostatectomy samples and the preferred HSCORE method to obtain the most accurate results possible. Since androgen-regulated interactions between stromal and epithelial cells are mandatory for normal prostate development and prostatic carcinogenesis in this study we quantified also the expression of AR in stromal cells in PCa. So far several studies have reported AR expression decrease in stromal cells of PCa but only as an observation because those studies tested other hypotheses. One study concentrated on AR expression in stromal cells and found gradual decrease of AR expression as the tumor dedifferentiated but this correlation was statistically not significant. However in our study there was a statistically significant decrease of AR expression in stromal cells of PCa. Post hos analysis showed that in the group of well differentiated PCa the mean value of hAR in stromal cells is significantly higher compared to the group of moderately differentiated (p=0,017) and the group of poorly differentiated PCa (p=0,0005). The difference in the mean value of AR in the stromal cells of moderately and poorly differentiated PCa was insignificant (p=0,07). Rare studies from analyses of AR expression on stromal cells of PCa show uniformly decrease of AR expression as the tumor dedifferentiates (Olapade-Olaopa *et al*., 1999).

The decrease of AR expression in stromal cells is also not explained. One explanation is the presence of mutant receptors that are not recognized by the antibody used even though in general aberrant receptors are uncommon in primary prostatic tumors. The other possibility is the presence of abnormal interactions between stromal and epithelial cells of the malignant prostate gland. The gradual reduction of AR expression in the stroma in the vicinity of altered prostatic glands culminating in total absence of AR expression in fully malignant cells may be due to effects of as yet undetermined tumorogenic signals from transformed epithelial cells. Some in vivo studies support the existence of these tumorogenic signals and show that local extension of primary and metastatic cancers may rely, at least in part, on the mutagenic field effect of altered surrounding stroma (Chung *et al*., 1991; Gregoire *et al*., 1995; Radinsky *et al*, 1995).

Conclusion

According to the results of our study AR expression decreased in human prostatic epithelial and stromal cells in proportion to the degree of dedifferentiation of the tissues, and the decline of AR expression is more pronounced in the stroma. There is uniform evidence for the decrease of AR expression in stromal cells compared to decrease of AR expression in epithelial cells. Other studies are necessary to confirm these findings and we believe that in the future expression of AR receptor in stromal cells of PCa my present as a reliable marker for tumor dedifferentiation and tumor progression.

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