Prostate Cancer

Overexpression of ELAV-like Protein HuR is Associated with Increased COX-2 Expression in Atrophy, High-grade Prostatic Intraepithelial Neoplasia, and Incidental Prostate Cancer in Cystoprostatectomies

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Abstract

\textbf{Background:} The human ELAV-like protein HuR regulates the stability of several mRNA targets, including that of cyclooxygenase-2 (COX-2). Their expression in prostatic carcinogenesis is uncertain.

\textbf{Objective:} To analyze HuR and COX-2 expression in cystoprostatectomies (CyPs) with incidental prostate cancer and compare their expression with those in radical prostatectomies (RPs) with clinically detected cancer.

\textbf{Design, setting, and participants:} HuR and COX-2 were immunohistochemically evaluated in normal-looking epithelium (NEp), atrophy, high-grade prostatic intraepithelial neoplasia (HGPIN), and prostate carcinoma (PCa) in 20 CyPs and 20 RPs, both types of specimens with pT2a Gleason score 6 PCa.

\textbf{Measurements:} At least 1000 cells were counted in contiguous 400X microscopic fields in each case, separately for NEp, atrophy, HGPIN, and PCa.

\textbf{Results and limitations:} There was an increase in the percentage of secretory cells with cytoplasmic HuR staining from NEp to atrophy, HGPIN, and PCa. The mean percentages in NEp, atrophy, and HGPIN adjacent to PCa were greater than away from cancer, both in the CyP and RPs. There was a trend towards a reduced nuclear HuR expression in atrophy, HGPIN, and PCa, compared to NEp. COX-2 staining was seen in the cytoplasm of the basal and secretory cells. There was a reduction in the mean proportion of positive basal cells and progressive increase in the percentage of positive secretory cells from atrophy to HGPIN and PCa, compared to NEp. Cytoplasmic HuR overexpression was correlated with COX-2 expression. There was no difference in HuR and COX-2 expression between cancers with tumour volume <0.5 ccm or >0.5 ccm.

The limitations of this study were the small number of cases investigated and lack of a control group without cancer.

\textbf{Conclusions:} The secretory cells showed shift in HuR staining from nuclear in NEp to cytoplasmic in PCa. This is associated with a parallel shift in COX-2 expression from basal to secretory cells.
1. Introduction

Prostate cancer is identified in three different settings: (1) clinically diagnosed by physical examination, laboratory tests, and/or symptoms; (2) discovered incidentally when the prostate is removed (eg, during cystoprostatectomy for bladder cancer), and (3) discovered at autopsy without ever having caused symptoms during the person’s lifetime (latent cancer) [1]. In general, PCs in CyPs and in autopsy prostates shows lower stage and lower Gleason score than in RPs [2].

Previous studies [1,3–5] have shown differences in marker expression between incidental and clinical cancers, pointing out that incidentally detected cancers have less aggressive features than clinically detected cancers and might represent indolent rather than aggressive cancers [1,5]. Such studies have shown that tissue characterization of the incidental and latent cancers is potentially important to identify differences between significant or aggressive and insignificant or indolent cancers. A very recent investigation has demonstrated overexpression of HuR and of COX-2 in clinically detected PCA [6]. There are no previous studies on HuR and COX-2 in CyPs with incidental PCA.

HuR has recently gained attention in cancer research because it regulates the mRNA stability of many growth-promoting genes [7]. HuR (HuA) is a member of the Hu family of RNA-binding proteins and is ubiquitously expressed in many cell types, in contrast to HuB, HuC, and HuD, which are almost exclusively found in neuronal cells. HuR recognizes and binds mRNAs containing adenosine and uracil-rich elements (ARE). These elements are frequently found in the 3’ untranslated regions of several unstable transcripts encoding cytokines, cell-cycle regulators, or protooncogenes [7]. mRNA binding by HuR stabilizes the mRNA and prevents its rapid degradation by exonucleases [7]. The mechanism by which HuR stabilizes RNAs is not completely understood. Several studies suggest that its cytoplasmic localization might be relevant for its mRNA stabilizing function [8,9]. In resting cells, HuR is predominantly localized in the nucleus and translocates to the cytoplasm upon stimulation. As increased cytoplasmic HuR expression has been detected in human malignant tumours, it has been suggested that a deregulated HuR pathway is implicated in cancer biology by promoting an abnormal expression of several proteins [10–12].

HuR has been shown to stabilize the mRNA of COX-2 [13,14], COX-2 protein is the inducible key-enzyme in the synthesis of prostaglandins [15]. Epidemiologic studies have shown that long-term intake of nonselective COX-2 inhibitors reduces the risk of PCa [16]. Functional studies have demonstrated the implication of COX-2 in PCA cell proliferation and invasiveness [17] and have underlined the role of COX-2 in the development and progression of PCA. COX-2 overexpression has been observed in clinically detected PCa [6,18–21], and has been associated with an unfavorable disease outcome in some studies [22,23].

The aim of the study was to analyze HuR and COX-2 expression in CyPs with incidental prostate cancer and compare their expression with those in RPs with clinically detected cancer.

2. Materials and methods

Forty prostatectomy specimens were obtained from the five pathology services associated with the Ancona United Hospitals. The material was included in a previous study [5]. Patient characteristics are shown in Table 1.

In group 1, there were 20 CyPs with incidental acinar PCa and HGPIN and no evidence of metastasis (latent cancer) (UC) in the prostate. These cases were from men with UC of the bladder and no history or clinical evidence of PCa.

In group 2, there were 20 RPs with hormonally untreated acinar PCa. HGPIN was present in all 20. These cases were from men with clinically detected PCa.

The whole-mount technique with complete sampling was used to process the specimens. Each prostate was covered with India ink and fixed for 24 h in 4% neutral buffered formalin; in the CyPs, the prostate was first severed from the bladder. After fixation, the prostate specimens were step-sectioned at 0.3 cm intervals perpendicular to the long axis (apical-basal) of the gland. The apex, base, and seminal vesicles were removed from each specimen and submitted in total for histologic examination. The cut specimens were post-fixed for 24 h and then dehydrated in graded alcohols, cleared in xylene, embedded in paraffin, and examined as 5 μm-thick whole-mount hematoxylin and eosin (H&E) stained sections.

Representative blocks containing compartments of NEp, atrophy, HGPIN, and cancer were selected. The samples selected for the study were all from the peripheral zone of the prostate to avoid having the results of HuR and COX-2 expression influenced by zonal distribution.

Table 1 – Patient characteristics

<table>
<thead>
<tr>
<th>Group type</th>
<th>Mean age (yr) and range</th>
<th>Total serum PSA (ng/ml) before surgery</th>
<th>Type of specimens</th>
<th>No of cases</th>
<th>No of cases with PCA</th>
<th>Cancer features*</th>
<th>No of cases with HGPIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidental</td>
<td>70 (56–84)</td>
<td>2.1</td>
<td>Cyp</td>
<td>20</td>
<td>20</td>
<td>pT2a, GS 6 (3+3), FZ, TTV, cc: 0.29 (0.03–2.23)</td>
<td>20</td>
</tr>
<tr>
<td>Clinical</td>
<td>66 (44–74)</td>
<td>0.4–3.9</td>
<td>Rp</td>
<td>20</td>
<td>20</td>
<td>pT2a, GS 6 (3+3), FZ, TTV, cc: 1.21 (0.40–3.51)</td>
<td>20</td>
</tr>
</tbody>
</table>

PSA = Prostate specific antigen; median and range; differences between incidental and clinical cancer groups not statistically significant.

Cyp = cytoprostatectomy; Rp = radical prostatectomy.

* The reason for prostate biopsy was an abnormal digital rectal examination and transrectal ultrasound in most of the cases. PSA velocity was the reason if few cases (data not shown).

** Pathologic stage according to the 2002 TNM revision (all the patients were N0 and there was no evidence of metastasis related to prostate neoplasia); GS = Gleason score; FZ = cancer origin in the peripheral zone; TTV = total tumour volume; cc = cubic centimeters; median and range; differences between incidental and clinical cancer groups statistically significant (p = 0.03).
The PCa of these two groups was pT2a and Gleason score 6 (3+3), because we planned to study a homogenous population, avoiding the influence of stage and grade.

Tissue representing all four compartments was mapped out on H&E-stained slides using marker pens. Different colors were used to mark out NEp, atrophy, and HGPIN areas that were 1 mm and 5 mm away from tumours. Glands that were in close proximity and within 1 mm distance of the tumours were considered to be near or adjacent, and glands that were at least 5 mm distance were considered to be away or remote [5]. Five-micron thick sections were cut from archival, formalin-fixed, paraffin-embedded specimens, and mounted on silane-coated slides. The procedure for this research conforms to the provisions of the Declaration of Helsinki.

2.1. Immunohistochemistry

To evaluate HuR and COX-2, standard indirect biotin–avidin immunohistochemical analysis was performed. Antigen retrieval was carried out with EDTA buffer, 100 °C, in a pressure steamer for 90 min. The slides were stained on an automated immunostainer (DakoCytomation, Denmark), using the following antibodies: monoclonal anti-human HuR antibody (Santa Cruz Biotechnology, Santa Cruz, CA; 1:750 dilution), and monoclonal antibody directed against COX-2 (NeoMarkers, Fremont, CA; 1:100 dilution). Bound antibodies were detected with the Dako Envision System. Slides were counter-stained with a light haematoxylin. All staining was performed within 3 d of slide preparation to prevent antigen degradation. All the slides were prepared by one laboratory technician.

The maps made on the H&E-stained slides were traced out on the corresponding immunostained slides (see above). Immunohistochemistry was evaluated as follows:

- HuR. Secretary cell staining was evaluated separately for the nucleus and cytoplasm.
- COX-2. Cytoplasmic staining was evaluated separately for the basal and secretory cells.

For both HuR and COX-2, staining intensity was classified as negative, weak, moderate, or strong. In each case, the percentage of positive cells and that of cells with strong immunoreactivity were calculated and reported in this study.

At least 1000 cells were counted in contiguous 400X microscopic fields, in each case separately for NEp, atrophy, HGPIN, and PCa.

2.2. Statistics

Statistics were analyzed with the Statistical Package for Social Science software (SPSS Inc, Chicago, Illinois, USA). For each group, the mean and standard deviation were evaluated. The Mann-Whitney test was used. Results were considered significant at $p < 0.05$

3. Results

3.1. HuR

There was a sharp increase in the mean percentage of secretary cells with cytoplasmic staining from NEp to atrophy, HGPIN, and PCa. Even though there were differences between CyPs and RPs, they did not reach statistical significance.

Fig. 1A shows normal-looking epithelium. The mean values of cells with cytoplasmic staining in glands remote from cancer were lower than those adjacent to it (Fig. 2) (CyP, away: mean 10%, standard deviation [SD] 10.13%; adjacent: mean 12.63%, SD 12.4%; RP, away: mean 8%, SD 11.52%; adjacent: mean 10.9%, SD 10.7%). The differences were not statistically significant (Tables 2 and 3).

Fig. 1B shows atrophy. When atrophy was remote from cancer, the mean proportions of cells with cytoplasmic staining were 48.38% (SD 21.39%) in the CyPs and 39.50% (SD 18.49%) in the RPs, the values being lower than in the adjacent location (see Fig. 2 for additional information). The difference between the remote and adjacent locations did not reach statistical significance for either the CyPs or RPs (Tables 2 and 3). The mean proportions were higher than in NEp; the differences were statistically significant, either away from or adjacent to PCa, for both the CyPs and RPs (Tables 2 and 3).

Fig. 1C shows HGPIN. The mean proportions of cells with cytoplasmic staining in the CyPs were higher than in the RPs.
(CyP, remote: mean 71.97%, SD 19.28%; adjacent: mean 77.12%, SD 20.06%; RP, remote: mean 69.33%, SD 22.41%) (Fig. 2). The differences between the remote and adjacent locations did not reach statistical significance, either for the CyPs or RPs (Tables 2 and 3). The mean proportions were greater than those seen in NEp and atrophy; the differences were statistically significant (Tables 2 and 3).

Fig. 1D shows prostate cancer. The mean proportions of cells showing cytoplasmic staining in PCa were greater that those in the adjacent HGPIN (Fig. 2); the differences were not statistically significant (Tables 2 and 3). The mean values in the CyPs were slightly higher than in the RPs (CyP: 83.63% ± 17.65%; RP: 79% ± 21.68%).

Fig. 3 shows graphically the mean proportion of secretory cells with strong staining intensity of the cytoplasm in all groups, both in the CyPs and RPs. The values increased progressively from atrophy away from PCa towards PCa. Normal looking epithelium (NEp) shows cells with strong intensity neither remote from nor adjacent to PCa. (The columns represent the mean; the standard error is also shown.)

Table 2 – HuR positive secretory cells in the cystoprostatectomy specimens; results of the statistical analysis (Mann-Whitney test) applied to pairs of compartments, ie, normal looking epithelium, NEp, (away from and adjacent to PCa), atrophy (away from and adjacent to PCa), HGPIN (away from and adjacent to PCa), and prostatic adenocarcinoma (PCa)

<table>
<thead>
<tr>
<th></th>
<th>NEp adjacent</th>
<th>Atrophy away</th>
<th>Atrophy adjacent</th>
<th>HGPIN away</th>
<th>HGPIN adjacent</th>
<th>PCa</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEp away</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NEp adjacent</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atrophy away</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atrophy adjacent</td>
<td></td>
<td>&lt;0.016</td>
<td>&lt;0.025</td>
<td>&lt;0.018</td>
<td>NS</td>
<td>0.013</td>
</tr>
<tr>
<td>HGPIN away</td>
<td>0.016</td>
<td>0.025</td>
<td>0.018</td>
<td>NS</td>
<td>NS</td>
<td>0.009</td>
</tr>
<tr>
<td>HGPIN adjacent</td>
<td>0.001</td>
<td>0.018</td>
<td>0.009</td>
<td>NS</td>
<td>NS</td>
<td>0.009</td>
</tr>
<tr>
<td>PCa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.013</td>
</tr>
</tbody>
</table>

NS = not significant.

Table 3 – HuR positive secretory cells in the radical prostatectomy specimens

<table>
<thead>
<tr>
<th></th>
<th>NEp adjacent</th>
<th>Atrophy away</th>
<th>Atrophy adjacent</th>
<th>HGPIN away</th>
<th>HGPIN adjacent</th>
<th>PCa</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEp away</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NEp adjacent</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atrophy away</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atrophy adjacent</td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HGPIN away</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>HGPIN adjacent</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>PCa</td>
<td></td>
<td></td>
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<td>NS</td>
</tr>
</tbody>
</table>

NS = not significant.
moderate intensity was seen in the nuclei, mostly in NEp away from and adjacent to PCa, and of weak intensity in the cytoplasm in atrophy and HGPIN. Nuclear staining was also identified in the ejaculatory duct epithelium, the stromal cells, and the endothelial cells, as well as the inflammatory infiltrates (data not shown).

3.2. COX-2

There was progressive reduction in the mean proportion of positive basal cells from NEp to atrophy and HGPIN (Fig. 1E–1H and Fig. 5) and an increase in the mean percentage of secretory cells with cytoplasmic staining from NEp to atrophy, HGPIN, and PCa (Fig. 6). Similarly to HuR, the mean values in atrophy and HGPIN remote from PCa were lower than when adjacent to it, both in CyPs and RPs. The results of statistics are reported in Tables 4 and 5 for the basal cells and in Tables 6 and 7 for the secretory cells. The differences for most of the comparisons were statistically significant. The differences between CyPs and RPs did not reach statistical significance. Fig. 7 shows graphically the proportion of secretory cells with strong staining intensity of the cytoplasm. The mean values increased from atrophy remote from PCa to PCa.

COX-2 staining of weak to moderate intensity was also seen in the cytoplasm of the stromal, endothelial, and inflammatory cells, and of strong intensity in the ejaculatory duct epithelium (data not shown). Cytoplasmic HuR overexpression was correlated with increased COX-2 expression in the secretory cells, both in the CyPs and RPs (CyPs: $R = 0.75$, $p < 0.001$; RPs: $R = 0.76$, $p < 0.001$). There was no difference in HuR and COX-2 expression between cancers with tumour volume $< 0.5$ ccm or $>0.5$ ccm.
4. Discussion

The study shows an increase in the percentage of secretory cells with cytoplasmic HuR staining from NEp to atrophy, HGPIN, and PCa. The values in NEp, atrophy, and HGPIN adjacent to PCa are greater than those away from it, both in the CyP and RPs, even though the differences did not reach statistical significance. There was a trend towards a reduced nuclear HuR expression from atrophy to HGPIN and PCa, compared to NEp.

Our findings have similarities with those reported by Niesporek et al [6], who compared HuR expression between prostate carcinomas and adjacent nonneoplastic glands. They found HuR expression to be shifted from nuclear in normal prostate to cytoplasmic in carcinoma. Their results, as well as ours, are in line with findings of a cytoplasmic overexpression of HuR in breast [12], ovarian [11,24], and colorectal cancer [10,13], and indicate that subcellular localization of HuR is deregulated in PCa.

Our observations that HuR expression is deregulated in atrophy and HGPIN, mainly when adjacent to PCa, give support to the presence of a field effect in prostatic carcinogenesis, both in CyPs and RPs [25–30], and confirm the findings of our recent study on AMACR, Ki67, and topoisomerase II alpha expression [5]. It was shown that AMACR expression and cell proliferation increased from NEp to atrophy and HGPIN, and were greater when they were evaluated adjacent to PCa. To the best of our knowledge, this is the first investigation to show HuR deregulation in prostatic carcinogenesis in incidental and clinical cancer.

An increased RNA-stabilizing function of HuR enhances the expression of proteins that are involved in angiogenesis, cell proliferation, and cell survival.
tumour-associated inflammation, and cellular growth. Several mRNA targets have been reported for HuR, for example, the mRNAs of the angiogenic factor vascular endothelial growth factor (VEGF), the inflammatory cytokines interleukin-8 (IL-8), interleukin-6 (IL-6), and tumour necrosis factor α [31], as well as cell cycle regulatory proteins such as cyclin A and cyclin B1 [32]. In addition are the mRNAs of the protooncogenes c-fos [9] and c-myc [33], urokinase plasminogen activator (uPA), and uPA receptor [34]. HuR has been shown to stabilize the mRNA of COX-2, an enzyme involved in PCa cell proliferation and invasiveness [18–21].

The current study also shows that there is reduction in the proportion of positive basal cells and increase in the percentage of secretory cells with cytoplasmic COX-2 positivity from NEp to atrophy, HGPIN, and PCa. Similarly to HuR, the changes in atrophy and HGPIN adjacent to PCa are greater than when away from it, both in CyPs and RPs.

Our findings on COX-2 expression in NEp have similarities with those reported by Cohen et al [22]. They investigated normal-benign glands away from clinically detected PCa and adjacent to it. They found that normal-benign glands away from PCa cells have low-grade staining, whereas the normal-benign glands that are adjacent to tumour cells showed high-grade staining if the specimen was from a patient who had recurred. This group did not find an increased degree of staining in those glands adjacent to cancer in patients who did not recur.

Concerning COX-2 in atrophy, we found overexpression in the secretory cells and a reduced expression in the basal cells. In particular, the increased staining in the secretory cells was related to the proximity of atrophy to PCa. Zha et al [35] investigated COX-2 expression in atrophic glands in the so-called proliferative inflammatory atrophy (PIA), a putative preneoplastic lesion [27]. They found COX-2 overexpressed in PIA, but not in HGPIN and cancer. In contrast to the study by Zha et al [35], we also found COX-2 overexpression in HGPIN and PCa.

COX-2 expression in HGPIN was evaluated in a small number of studies in specimens with clinically detected PCa [18–21,35]. They all found overexpression compared to NEp. Our data are similar to those published previously by others, the difference being that we also investigated HGPIN in relation to its proximity to PCa, both in CyPs and RPs. The previous studies, as well as ours, also dealt with PCa and all found that the highest level of overexpression was always in HGPIN, in our study when adjacent to PCa, and in PCa. The previous studies also dealt with the relationship with prognostic factors and prognosis. This was not done in our study, in which PCa cases were selected in terms of identical Gleason score and stage.

The association of HuR and COX-2 expression in clinically detected cancers has been reported by others in breast [12], ovarian [11,24], gastric [36], and colorectal cancer [10,13]. Niesporek et al [6], by combining descriptive observations in tumour tissue with data from cell culture, provided evidence that COX-2 overexpression in prostate carcinoma is due to deregulated HuR activity. Our study is the first to show that HuR overexpression is associated with increased COX-2 expression also in incidental cancer.

NEp, atrophy, and HGPIN near PCa showed higher levels of HuR and COX-2 compared to NEp, atrophy, and HGPIN away from cancer. Differences between the near and remote locations were recently observed by Santinelli et al [5] when racemase, Ki-67, and topoisomerase IIα were evaluated. Their interpretation was that of evidence for and differences in field effects in prostatic carcinogenesis.

In conclusion, the secretory cells showed the shift in HuR staining from nuclear in NEp to cytoplasmic in PCa. This is associated with a change in COX-2 expression from basal to secretory cells. Our results give support to the presence of a field effect in prostatic carcinogenesis in CyPs, as well as in RPs.

Author contributions: Rodolfo Montironi had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Montironi, Beltran.

Acquisition of data: Barbian, Mazzucchelli

Analysis and interpretation of data: Beltran.

Drafting of the manuscript: Scarpelli, Montironi.

Critical revision of the manuscript for important intellectual content: Cheng.

Statistical analysis: Santinelli.

Obtaining funding: Scarpelli, Montironi.

Administrative, technical, or material support: Mazzucchelli.

Supervision: Montorsi.

Other (specify): Santinelli.

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Funding/Support and role of the sponsor: Design of study, collection of data, management of data.

References


