Original Article

Intraductal prostate cancer: An aggressive subset of prostate cancers? Immunophenotypic evaluation

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Abstract

Introduction: The presence of intraductal prostate cancer in a sample is often associated with large tumor volume, an advanced stage of the disease, a high Gleason score and an increased risk of recurrence, and resistance to androgen suppression and chemotherapy, which are also correlated with reduced progression-free survival and with postoperative, biochemical relapse.

Methods: The aim of our study was to investigate whether carbonic anhydrase IX (CA IX) is upregulated in prostate cancer and to investigate ERG and EZH2 as potential markers for cancer aggression in aggressive acinar disease with intraductal component prostate cancer. The series consisted of 79 cases of prostate cancer. Immunohistochemical staining was performed for EZH2 ERG and CA IX.

Results: The results of this study underline the fact that EZH2 protein expression is a powerful predictor of PSA relapse in prostate cancer and that this effect is stronger in ERG-positive cancers than in ERG-negative cancers. Evident EZH2 nuclear expression was found in prostatic tumor, proposing increased EZH2 expression important for the spread of prostate cancer.

Conclusions: The relationship to tumor phenotype and prognosis was more considerable in ERG-positive tumors than in ERG-negative tumors. EZH2 has gained great interest as a target for epigenetic cancer therapy. Although prostate cancer is a hypoxic tumor, it does not express CA IX and cannot be used as an endogenous marker for hypoxia.

Keywords: Carbonic anhydrase IX, ERG, EZH2, intraductal prostate cancer, prostate cancer

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INTRODUCTION

Intraductal prostate cancer (IDC-P) is a malignant lesion characterized by an expansive growth of malignant epithelial cells in the prostate ducts that present significant architectural and cytological atypia.[1] The presence of IDC-P in a sample is often associated with a large volume of cancer, an advanced stage of the disease, a high Gleason score and an increased risk of recurrence, and resistance to androgen suppression and chemotherapy.[2-5]

Well, IDC-P is a term that specifically refers to prostate adenocarcinomas that grow and spread in the prostate ducts, as described by Kovi *et al.*[6] The definition of IDC-P is based on a series of morphological criteria

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which have been evaluated by various authors.[7-9] It can have different growth models: solid, micropapillary, and rarely, flat architecture. The cells can be cubic or columnar with a considerable increase in the size of the nucleus.[10] Numerous studies report that the presence of IDC-P in radical prostatectomy (RP) is related to other adverse pathological features: higher Gleason score, higher tumor volume, and greater probability of extraprostatic infiltration, vesicular invasion, and metastasis from the pelvic lymph nodes (LNs). A correlation with reduced progression-free survival and postoperative biochemical recurrence was also highlighted.[11-13] Cohen *et al*.[14] after examining a small series of RP specimens concluded that reporting the presence of IDC-P in preoperative prostate biopsies could be useful in predicting the pathological stage before RP; also because the presence of IDC-P in the biopsy was strongly correlated to the biochemical failure. Recurrent ERG fusions represent the most common genetic modification in prostate cancer, TMPRSS2-ERG occurs in about 40%–50% of tumors.[15,16] The presence of this gene fusion is an early clonal event in the progression of prostate cancer, especially for IDC-P.

Basal layer cells are absent in prostate adenocarcinoma; immunohistochemical staining for p63, a high molecular-weight cytokeratin, is important for identifying the presence of basal cells.[17-22] This is especially true of the small areas of cancer that are common in needle biopsies. From a biological point of view, it has been hypothesized that the basal cells represent the compartment of the reserve cells within the prostate epithelium;[23-26] therefore, the loss represents an important step in the development of invasive carcinoma and intraepithelial prostate neoplasia of IDC-P.[27] Thus, the presence of clearly identifiable basal cells in a gland or duct excludes the diagnosis of carcinoma.Varambally *et al*.[28] showed a positive relationship between EZH2 protein expression levels and the increased aggressiveness of prostate cancer. Surprisingly, overexpression of EZH2, after RP, is not only associated with metastasis but also associated with a higher risk of recurrence in the prostate lodge. Therefore, EZH2 is considered a potential diagnostic and prognostic biomarker in prostate cancer; therefore, immunohistochemical evaluation of EZH2 could be useful in the presence of IDC-P in prostate biopsy. Finally, we know that hypoxia is a feature of a wide range of solid tumors important in promoting tumor progression[29] and treatment resistance[30] by reducing apoptosis. Carbonic anhydrase IX (CA IX) has been used as an endogenous marker for the assessment of hypoxia in numerous solid tumors, including bladder, kidney, lung, and head-and-neck cancers.[31-33] Usually, the expression of CA IX is associated with a more aggressive tumor phenotype and greater resistance to treatment.

Our study aims to investigate whether CA IX is upregulated in prostate cancer and to evaluate the expression of ERG and EZH2 as potential markers of aggressiveness of prostate cancer with an intraductal component.

CASES AND CLINICAL INFORMATION

The series included 79 cases of prostate cancer retrieved from the Department of Surgical, Medical, Molecular Pathology, and Critical Areas at the University of Pisa. For each case, a representative section of the tumor was selected for immunohistochemistry and 4 μ thick sections were obtained from each selected formalin-fixed, paraffin-embedded tissue block. All patients who underwent a RP in this series were operated on by a single surgeon in urological surgery between 2004 and 2015 and received adjuvant or neoadjuvant hormone therapy or adjuvant radiation therapy.

HISTOLOGICAL EVALUATION

Hematoxylin and eosin-stained (HE) slides made from RP specimens were re-evaluated by a pathologist specializing in urological pathology. The following pathological parameters were analyzed for each patient: pathological staging (Tumor node metastasis), Gleason score, surgical margin (SM), presence of IDC-P, extraprostatic infiltration, seminal vesicular invasion, and LN metastasis. The Biopsy Gleason Score was also re-evaluated according to the rating system of the International Society for Urological Pathology 2016.

INTRADUCTAL CARCINOMA

IDC-P was defined according to McNeal criteria: Which are well-defined lesions with basal cells present but consisting of a population of clearly malignant epithelial cells, usually present around the invasive carcinoma [Figure 1].

IMMUNOHISTOCHEMISTRY

Four tissue sections were used for immunohistochemical analysis and stained with anti-p63 antibody (4A4, Ventana), anti-TMPRSS2-ERG antibody (EPR3864-anti-ERG Ventana), anti-EZH2 antibody (SP129), and anti-CA IX antibody (VENTANA EP161 Rabbit Monoclonal Primary Antibody). The colors for EZH2, ERG, and CA IX were performed with Benchmark Ultra, Ventana Medical Systems (Tucson, AZ). All IHC staining slides were assessed with a light microscope.

The nuclear staining for p63 [Figure 2] was complete positive or focal or negative if completely absent. EZH2 [Figure 3] and ERG [Figure 4] were evaluated positive or negative based on nuclear expression, tissue samples with a percentage of positive cells >10% were considered positiv, while for CA IX, it was assessed positive or negative based on cytoplasmic staining.

EZH2 and ERG immunohistochemistry

PCa specimens were assessed for ERG, EZH2, and CA IX protein expression by immunohistochemistry. Thirty-four samples showed ERG expression (34/79, 43.04%), while EZH2 expression was found in 36/79 cases (45.57%) [Figures 3 and 4]. Chi-square test displayed a strong correlation between ERG and EZH2 expression ($P < 0.0001$) [Figure 5]. All the samples were negative for CA IX, with a kidney sample used as a positive control in immunostaining [Figure 6]. ERG positivity was statistically associated with high Gleason ($P < 0.0001$) [Table 1 and Figure 7]; *t*-test showed that elevated ERG expression was significantly decreased with the Gleason score ($P < 0.0001$); mean ERG value was 0.5 in Gleason 9 samples, 0.46 in score 8, 0.44 in score 7 (4 + 3), and 0.11 in score 7 (3 + 4) [Figure 5]. Figure 8 and Table 1 show a similar association between EZH2 positivity and Gleason score (Chi-square test; $P = 0.0002$), with EZH2 expression levels lowering as Gleason score decreasing (*t*-test; $P < 0.0001$).

DISCUSSION

IDC-P is usually associated with invasive and biologically aggressive prostate cancer. Recent reports have highlighted the importance of recognizing IDC-P in RP to predict biochemical recurrence-free survival.[34] Epstein, from a morphological point of view, has proposed specific criteria to identify intraductal carcinoma of the prostate:[10] Malignant epithelial cells fill large acini and prostate ducts, with preservation of basal cells that form solid or dense

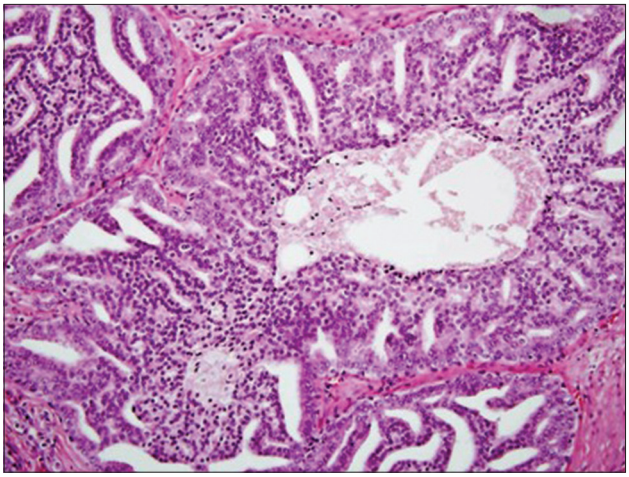

Figure 1: Histological features of intraductal cancer of the prostate. Large caliber smooth-contoured ducts surrounded by basal cells

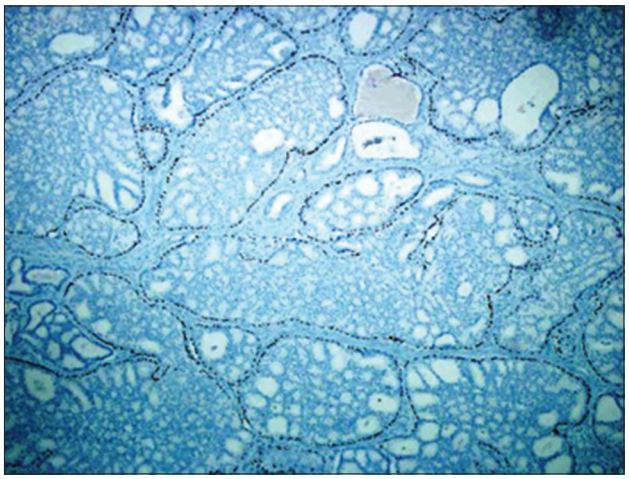

Figure 2: An example of complete positive P63

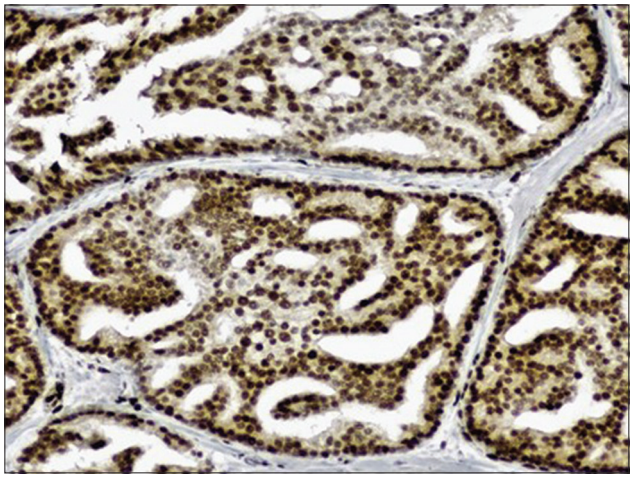

Figure 3: EZH2 nuclear expression

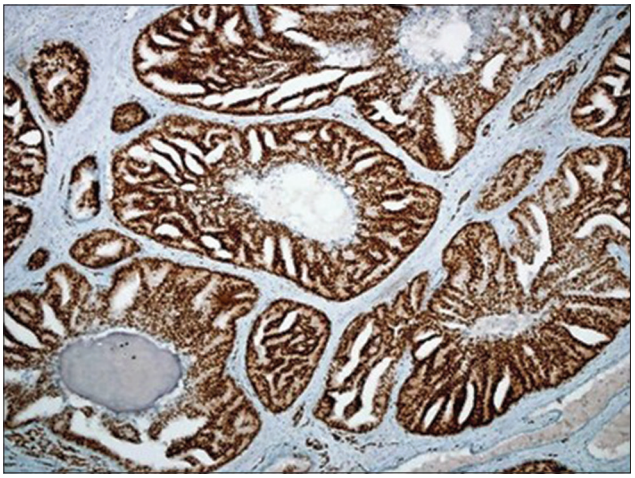

Figure 4: Strong expression nuclear ERG

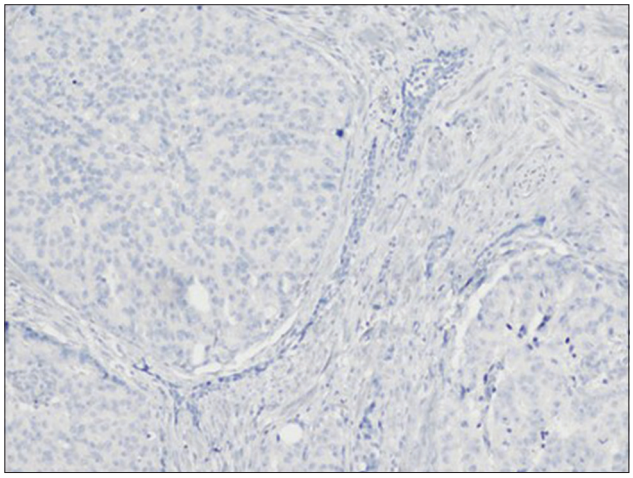

Figure 5: ERG/EZH2

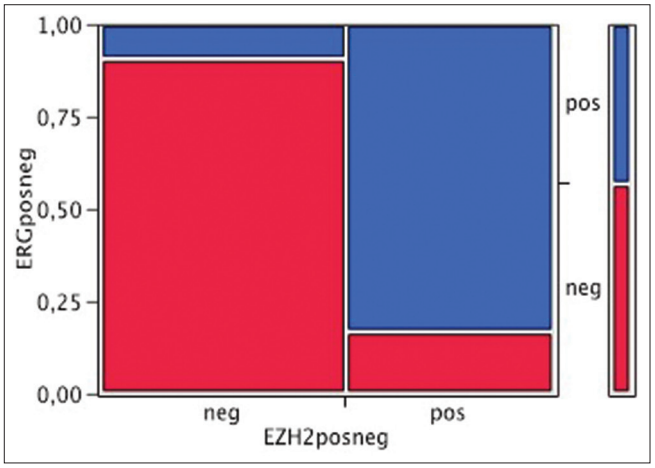

Figure 6: Absence of Carbonic anhydrase IX expression

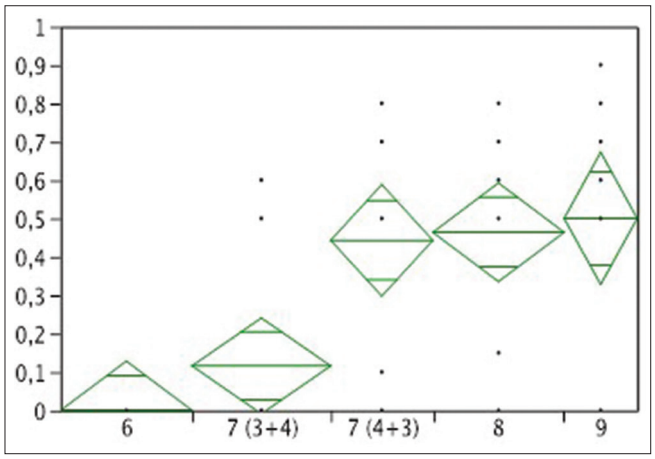

Figure 7: Correlation between ERG expression and Gleason score

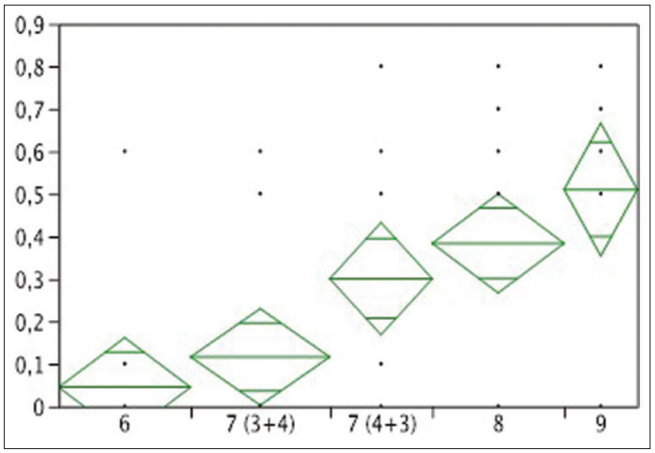

Figure 8: Correlation between EZH2 and Gleason Score

cribriform models or loose cribriform or micropapillary models with marked nuclear atypia (nuclei six times the normal or larger size) or comedonecrosis. In our study, in addition to the morphological aspect, we wanted to evaluate whether the expression of ERG and EZH2 was related to the aggressiveness of prostate cancer with an intraductal component. In recent years, the TMPRSS2/ERG (T/E) fusion gene has been shown to be present in approximately 50% of prostate cancers. Experiments on prostate cancer cells containing a T/E fusion[16] show that the promoter TMPRSS2, which contains promoter elements that respond to the androgen receptor, increases the expression of ERG in response to androgens. The T/E fusion gene has also been shown to act on the proliferation, invasion, and motility of prostate epithelial cells and it activates various proteins and pathways such as Ezh2, Wnts, TGFB, and Sox9.[35] In 2002, it was discovered by a cDNA microarray study that EZH2 is associated with prostate cancer and it would be significantly upregulated in metastatic prostate cancer. The result indicated a positive relationship between the level of EZH2 protein and the aggressiveness of the disease.[28] In our study, a positive correlation was found between the expression of ERG and EZH2 in tumors with an intraductal component and a high Gleason score; we also highlighted significant correlations with the tumor stage and the presence of LN metastases.[36-39] The expression of the EZH2 protein was more evident in the positive ERG samples than in the negative ERG samples, confirming that both the proteins play a fundamental role in recognizing the more aggressive variants of prostate cancer such as IDC-P. We also wanted to verify whether the expression of ERG and EZH2 could be favored by the presence of a hypoxic environment by evaluating the presence, in our samples, of CA IX. CA IX usually stains heterogeneously. Stok *et al.* have shown that monocarboxylate transporters (MCTs) are involved in the outflow of lactic acid, pyruvate, acetate, and butyrate from the cell,[40] through the co-transport of H + with anions on monocarboxylates.[41] There are four known members (MCT1-4), MCT 4 is the most common in cells with a high glycolytic rate such as cancer cells[42] and is shown to accumulate on the migratory surface of cells.

Table 1: Comparison between erg and ezh2 expression and clinico-pathological data (Values are shown as n. [b]p-values are assessed by χ^2test)

	ERG expression		*P*	EZH2 expression		*P*
	Negative	Positive		Negative	Positive	
AGE						
≤69 years (TOT: 40 CASES)	50% (20/40)	50% (20/40)	0,2	48% (19/40)	52%(21/40)	0,210
>69 years (TOT: 39 CASES)	64% (25/39)	36% (14/39)		62% (24/39)	38%(15/39)	
PAHOLOGICAL STAGE T						
T2a	100% (2/2)	0% (0/2)		50%(1/2)	50%(1/2)	
T2b	100% (4/4)	0% (0/4)	0,0002	100%(4/4)	0%(0/4)	0,0014
T2c	74%(28/38)	26%(10/38)		71%(27/38)	29%(11/38)	
T3a	39%(7/18)	61%(11/18)		39%(7/18)	61%(11/18)	
T3b	24%(4/17)	76%(13/17)		24%(4/17)	76%(13/17)	
PATHOLOGICAL STAGE N						
N0 (TOT: 26 cases)	38% (10/26)	62% (16/26)	<0,0001			0.0001
N1 (TOT: 17 cases)	24% (4/17)	76% (13/17)				
NX (TOT: 36 cases)	86% (31/36)	14% (5/36)				
GLEASON SCORE						
6 (3+3)	100% (18/18)	0% (0/18)	<0,0001	83% (15/18)	17% (3/18)	0,0002
7 (3+4)	79% (15/19)	21% (4/19)		79% (15/19)	21% (4/19)	
7 (4+3)	29% (4/14)	71% (10/14)		36% (5/14)	64% (9/14)	
8 (4+4)	28% (5/18)	72% (13/18)		33% (6/18)	67% (12/18)	
9 (4+5 or 5+4)	0% (0/7)	100% (7/7)		20% (2/10)	80% (8/10)	
MARGINS						
NEGATIVE (TOT: 34 cases)	85% (29/34)	15% (5/34)	<0,0001			
POSITIVE (TOT: 45 cases)	38% (17/45)	62% (28/45)				
RELAPSE						
ABSENT (TOT: 59 cases)	69% (41/59)	31% (18/59)	<0,0001			
PRESENT (TOT: 20 cases)	20% (4/20)	80% (16/20)				
IDC-P						
ABSENT (TOT: 45 cases)	100% (45/45)	0% (0/45)	<0,0001			
PRESENT (TOT: 34 cases)	0% (0/34)	100% (34/34)				

Values are shown as *n*. [b]*P*-values are assessed by test

It is known to be inducible to hypoxia in bladder cancer cells[43] and could play a role in pH acidification in prostate cancer. Although further studies are needed to elucidate the exact pH regulation mechanism involved in prostate cancer, we have clearly demonstrated that CA IX is not expressed in prostate cancer.

Although prostate cancer is a hypoxic tumor, it does not express CA IX and cannot be used as an endogenous marker of hypoxia which in the prostate may depend on alternative mechanisms for pH regulation.

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Conflicts of interest

There are no conflicts of interest.

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