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EXPRESSION OF LMO2 IN PROSTATE CARCINOMA AND ADJACENT PROSTATIC PARENCHYMA

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SUMMARY – LMO2 *(LIM domain only)* is a member of transcription factor family of proteins characterized by their cysteine-rich, zinc-binding LIM domains. Its expression in prostate cancer cells, as well as in adjacent stroma, is described in a study in a cohort of 83 patients treated with radical prostatectomy for clinically localized prostate adenocarcinoma. Authors found that LMO2 overexpression in prostate cancer was strongly associated with features indicative of worse prognosis (higher preoperative PSA, higher Gleason score, positive surgical margins, and extraprostatic extension of disease). Expression of LMO2 was also associated with biochemical disease progression. We analysed immunohistochemical expression of LMO2 in prostate cancer epithelial and stromal cells, as well as in adjacent parenchyma. Significant negative correlation between glandular expression of LMO2 in carcinoma and stromal expression in BPH (ρ = -0.238, P = 0.033) was found, but also between stromal expression in carcinomas and glandular expression in BPH (ρ = -0.255, P = 0.021). Positive correlation was found between stromal expression in BPH and stromal expression in carcinomas (ρ = 0.306, P = 0.005). Study results support the potential role of LMO2 in prostatic carcinogenesis and cancer progression.

Key words: *LMO2; Prostate cancer; Stromal reaction*

Introduction

Prostate carcinoma (PCa) is the most common cancer worldwide[1]. It accounts for approximately 18% of all newly diagnosed malignant tumors in males in Croatia. It is responsible for up to 10% of all deaths in males in 2014[1,2]. There are well established features indicative of worse prognosis, but for some groups of PCa we still cannot precisely predict which tumors will behave aggressively. Today, it is well-known that the interaction between stroma and carcinomatous epithelial cells plays an important role in carcinogenesis, cancer progression and metastases[3]. Prostate cancer stroma is composed of (myo)fibroblasts, endothelial cells and immune cells. Extracellular matrix is also one of the keys for creating a suitable microenvironment for cancer progression[3].

LMO2 is a member of transcription factor family of proteins characterized by their cysteine-rich, zinc-binding LIM domains. Its expression is required early in hematopoiesis[4] and it was first identified in T-cell acute lymphoblastic leukaemia (T-ALL)[4,5]. LMO2

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proteins are important regulators in controlling cell growth and differentiation[5,6]. Its expression is a strong predictor of superior outcome in patients with diffuse large B-cell lymphoma[5,6]. Recent studies also suggest that LMO2 over-expression may be associated with several other malignant tumors, including gastric carcinoma[7], pancreatic carcinoma[8] and glioma[9].

There are several studies dealing with LMO2 in prostate cancer, showing the highest expression of LMO2 in prostate carcinoma epithelial cells and stromal cells of the peripheral zone, using immunohistochemistry and RT-PCR methods[4,10].

The aim of this study was to analyse the expression of LMO2 in prostate cancer epithelial cells, normal epithelial cells of adjacent prostatic parenchyma, as well as stromal cells within tumor tissue and surrounding non-tumor area.

Patients and methods

The study included 83 patients, median age 66.0 years (range 62-68 years), treated with radical retropubic prostatectomy for clinically localized prostate adenocarcinoma at the Department of Urology in Sestre milosrdnice University Hospital Centre in Zagreb, Croatia. All patient identifiers were removed and replaced by unique study numbers, linked to the original identifiers by a single file kept under high security. The retrieval of archival tissue block was conducted under institutional review board approval. None of the patients were treated with hormone or radiation therapy before or after radical prostatectomy, and none had secondary cancer.

Specimens were fixed in 10% buffered formalin, embedded in paraffin, cut at 5-µm thickness, and routinely stained with haematoxylin and eosin. The diagnosis of adenocarcinoma was histologically confirmed in all cases.

Ninety prostate cancer specimens were used to construct a tissue microarray by manual tissue arrayer. Representative areas of each paraffin block were labelled by B.K. and M.U. and adequate specimens containing cancer from peripheral zone of the prostate were sampled. Haematoxylin and eosin stained sections from each block were analysed to ensure the collection of adequate tissue. Seven specimens were not included into the study due to lack of tumorous tissue in the recipient block.

Deparaffinization and immunohistochemical staining were performed following the Microwave Streptavidin ImmunoPeroxidase (MSIP) protocol on a DAKO Tech-MateTM Horizon automated immunostainer (DAKO, Copenhagen, Denmark). We used primary monoclonal antibodies to LMO2 (1F10/B8): sc-73516, dilution 1:100, Abcam Cruz Biotechnology, Inc., CA, USA). Breast cancer tissue served as positive control and the removal of primary antibody was used as negative control.

To evaluate the intensity of LMO2 expression in prostate cancer and adjacent prostatic parenchyma, the percentage of positively stained carcinoma cells was examined in the entire slide. We applied a system similar to that used in a previous study of LMO2 expression in prostate cancer, where at least moderate staining intensity was required in more than 25% of tumour and/or stromal cells to define LMO2 overexpression[6,10]. Staining was graded on a 0-3 scale and expressed as 0, up to 25% of positive carcinoma or stromal cells; low, 25%-50% of positive carcinoma epithelial/stromal cells; moderate, 50%-75% of positive carcinoma epithelial/stromal cells; and high, more than 75% of positive carcinoma epithelial/stromal cells. All samples were examined independently by two observers (M. U., and B. K.). Any disagreements were resolved by a joint review.

Statistical analysis was performed using Mann-Whitney U test, Kruskal-Wallis test, χ^2-test, Kaplan-Meier test and Cox proportional-hazards regression test. The levels of statistical significance were set at least at $p<0.05$.

Results

Median patient age was 66.0 (62.0 - 68.0) years. Gleason score was under 7 in 18 (21.7%) patients, and it was 7 in 50 (60.2%) patients (41 (49.4%) had 3+4 score, while 9 (10.8%) had 4+3 score). Gleason score >7 was recorded in 15 (18.1%) patients.

LMO2 expression in stromal and glandular tissue of carcinomas and benign prostate hyperplasia is presented in Table 1 and Figure 1. Overall, patients with BPH had lower expression of LMO2 in both stromal and glandular tissue ($P<0.001$). There was no correlation between age and Gleason score with LMO2 expression.

LMO2 was expressed in all 83 tissue array specimens of prostate cancer (Table 1 and Fig. 1). Seventy

Table 1. LMO2 expression in stromal and glandular tissue of carcinomas and adjacent uninvolved prostate tissue.

	BPH		Carcinoma		P value
Glands					
LMO2 expression n (%)					<0.001
1	1	(1.2)	0	(0)	
2	34	(41.0)	6	(7.2)	
3	48	(57.8)	77	(92.8)	
Stroma					
LMO2 expression n (%)					<0.001
0	38	(45.8)	1	(1.2)	
1	35	(42.2)	36	(43.4)	
2	5	(6.0)	39	(47.0)	
3	5	(6.0)	7	(8.4)	

seven cases (92.8%) showed strong and 7 cases (7.8%) showed moderate expression of LMO2 in tumor cells. Expression in the epithelium of benign gland in adjacent parenchyma was strong in 48 cases (57.8%), moderate in 34 (41.0%) and slight in 1 (1.2%) case. On the other hand, strong stromal reaction for LMO2 was found in the stromal component of 7 carcinomas, moderate in 39, slight staining in 36 cases and negative in 1 case only. Negative stromal reaction in adjacent benign prostatic tissue was recorded in 38 cases, slightly positive in 35, moderate in 5, while strong positivity was recorded in 5 cases. Immunohistochemical reaction was membranous and cytoplasmatic (Fig. 1).

We found a significant negative correlation between glandular expression of LMO2 in carcinomas and stromal expression in BPH ($\rho = -0.238$, $P = 0.033$), but also between stromal expression in carcinomas and

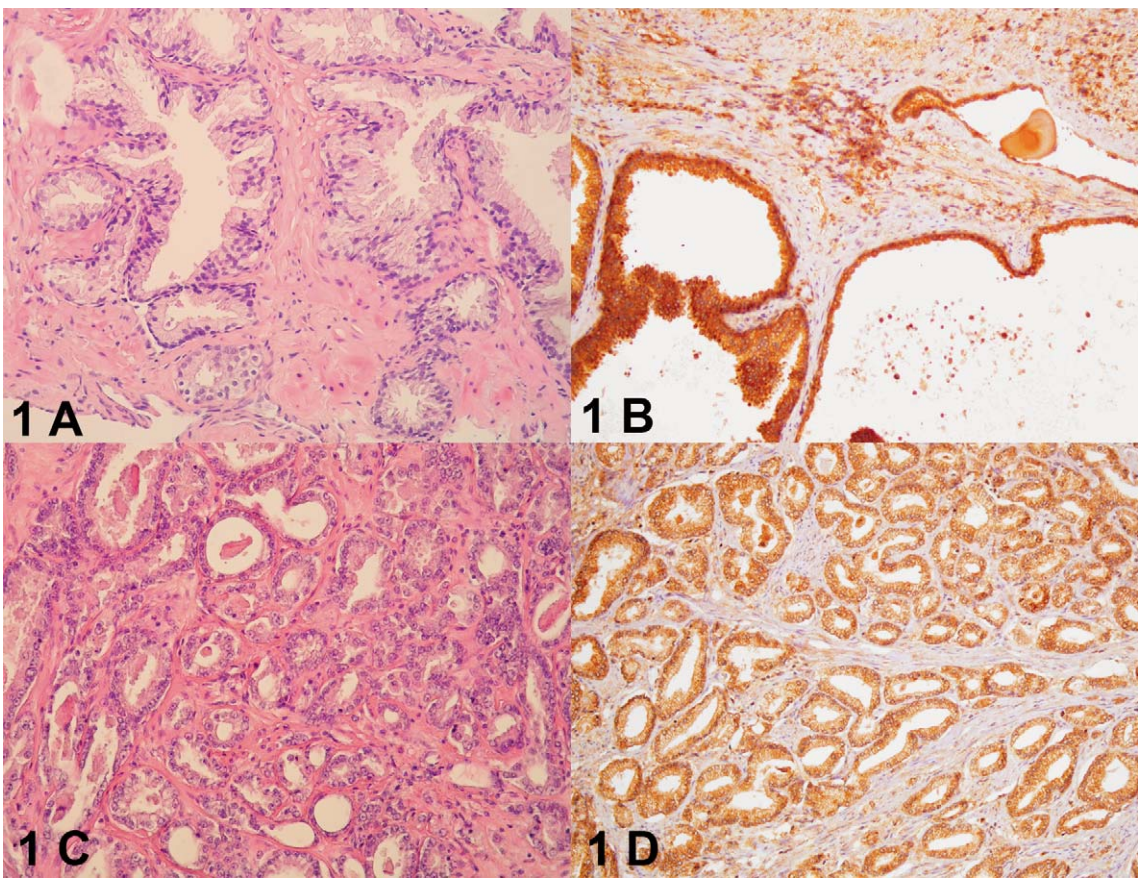

Fig. 1. A) Microscopic appearance of benign prostate hyperplasia (HEx200) and B) strong epithelial positive staining for LMO2 with less positive stromal cells (200x LMO2). C) Microscopic appearance of prostate cancer with D) strong positive cancer epithelial staining and weak stromal staining (200x LMO2)

glandular expression in BPH (ρ = -0.255, P = 0.021). A positive correlation was found between stromal expression in BPH and stromal expression in carcinomas (ρ = 0.306, P = 0.005).

Discussion

Prostate is divided into peripheral and transitional zone, central zone and anterior fibromuscular stroma, by McNeal[11]. Histologic differences between these zones are well known, but different molecular features of prostatic zones have also been observed and reported[12, 13]. Alves *et al*. have recently shown a different proportion of collagen and elastic fibres, muscle and peripheral nerves in the peripheral and transitional zones[14]. Similarly, Tomas *et al*. described differences in the arrangement of reactive stroma, which occurs in prostatic carcinoma, using histochemical staining by Mallory method and confirmed by immunohistochemistry (desmin and vimentin)[15]. Studies on different human cancer specimens demonstrated activated stromal cell phenotypes, modified extracellular matrix (ECM) composition, and increased micro-vessel density similar to stromal changes at the site of wound repair[3]. Numerous molecules and cytokines are involved in cancerous stromal-epithelial interaction and are important in providing a suitable microenvironment for cancer progression. One of these molecules is LMO2[9-10,16].

Overexpression of LMO2 was strongly associated with advanced tumor stage and metastasis of prostate carcinoma[4,10]. Ma *et al*. compared the LMO2 expression in human prostatic tissue, prostate cancer cell lines, and xenografts and found that LMO2 may contribute to the development of prostatic adenocarcinoma by suppressing E-cadherin expression, a well-known ECM protein[10]. A recent study has also suggested that LMO2 is overexpressed in stromal cell and cancer-associated fibroblasts of the peripheral zone which may contribute to cancer promotion[4]. It was strongly associated with established features indicative of worse prognosis, such as higher preoperative PSA (p=0.011), higher Gleason score (p<0.001), positive surgical margins (p<0.003), and extraprostatic extension of disease (p<0.003). LMO2 expression was also associated with biochemical disease[4]. Authors suggest that this effect may be due to stimulation of secretion of IL-11, which can activate STAT3 signalling downstream via its receptor IL11Rα. This is a pathway for creating the so-called cancer promoting microenvironment[4,5].

The aim of our study was to analyse the expression of LMO2 in prostate cancer epithelial and stromal cells, as well as epithelial cells of normal glands and stromal cells of the adjacent prostatic parenchyma. Our results were similar in the sense of higher expression of LMO2 in cancer epithelial cells, as well as stromal cells when compared to BPH. Significant negative correlation between expression of LMO2 in carcinomatous glands and stromal expression in BPH was found (ρ = -0.238, P = 0.033), but also between stromal expression in carcinomas and glandular expression in BPH (ρ = -0.255, P = 0.021). Overexpression of LMO2 wasn't related to higher Gleason grade or PSA and extraprostatic extension.

In conclusion, we believe that LMO2 has a role in PCa progression and creation of cancer promoting microenvironment, but further investigation is needed for novel therapeutic possibilities that could offer a microenvironment-targeted strategy for PCa treatment.

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Sažetak

IZRAŽENOST LMO2 U KARCINOMU PROSTATE I PARENHIMU PROSTATE

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LMO2 *(LIM domain only)* pripada obitelji proteina transkripcijskih faktora, za koje je karakteristična cink vežuća LIM domena bogata cisteinom. Njegova izraženost u karcinomu prostate, kao i u okolnoj stromi određena je u jednoj od studija na kohorti od 83 bolesnika nakon radikalne prostatektomije zbog lokaliziranog karcinoma prostate. Povišena izraženost LMO2 bila je povezana s pokazateljima lošije prognoze (s višim preoperativnim PSA vrijednostima, višim Gleason zbrojem, pozitivnim kirurškim rubovima i širenjem tumora izvan prostate). Izraženost LMO2 bila je povezana i s biokemijskom progresijom bolesti u univarijantnim analizama. U našem istraživanju analizirana je imunohistokemijska izraženost LMO2 u karcinomu prostate, u epitelnim i stromalnim stanicama karcinoma, kao i u okolnom, netumorskom parenhimu prostate. Pronađena je značajna negativna korelacija između izraženosti LMO2 u epitelu karcinoma i stromalnoj izraženosti u benignoj hiperplaziji (BPH) (ρ = -0.238, p = 0.033), također i stromalne izraženosti u karcinomu i žlijezdama BPH (ρ = -0.255, P = 0.021). Pozitivna korelacija pronađena je između izraženosti LMO2 u stromi BPH i stromi karcinoma (ρ = 0.306, P = 0.005). Rezultati ovog istraživanja podupiru moguću ulogu LMO2 u karcinogenezi prostate i progresiji tumora. Nije dobivena povezanost ekspresije LMO-2 i dobi te Gleason zbroja.

Ključne riječi: *LMO2; Karcinom prostate; Reakcija strome*