Revista Argentina de Clínica Psicológica
2021, Vol. XXX, N°2, 727-731
DOI: 10.24205/03276716.2020.4075

RELATIONSHIP BETWEEN APOPTOSIS-RELATED PROTEIN BL-X AND CAPASE-3 AND LMO2 GENES IN PROSTATE CANCER

Dewang Fu[a], Huamao Jiang[a], Liu Ben[a], Tongyi Men[b]

Abstract

Our study intends to investigate the expressional profile of Bcl-xl proteins, Caspase-3 protein expression in different prostate tissues, along with tissue expression of LMO2 gene. A total of 25 cancer tissue samples were collected from surgical removal of prostate cancer from January 2015 to January 2017, along with collection of cancer adjacent tissues and benign prostate hyperplasia tissues. Immunohistochemistry staining was used to measure expression of Bcl-xl and Caspase-3. LMO2 gene expression was quantified by real-time fluorescent PCR. Bcl-xl showed 88% positive expression in cancer tissues, which was significantly higher than cancer adjacent tissues (32%) and benign prostate hyperplasia (45%). Caspase-3 protein presented 16% positive rate in cancer tissues and significantly lower than cancer adjacent tissues (72%) and benign prostate hyperplasia (60%). Bcl-xl presented higher expression whilst caspase-3 had lower expression in cancer tissues, compared to those in benign hyperplasia or cancer adjacent tissues ($p<0.05$). RT-qPCR showed similar LMO2 mRNA level in cancer adjacent tissues (1.038-fold) compared to benign hyperplasia group ($p>0.05$), whilst cancer tissues had 897.317-fold higher LMO2 mRNA level ($p<0.001$). In conclusion, abnormal expression of bcl-xl and caspase-3 proteins are closely correlated with the prostate cancer. LMO2 gene plays a crucial role in the progression of prostate cancer.

Keywords: Prostate cancer, Bcl-xl protein, Caspase-3 protein, LMO2 gene

1. INTRODUCTION

Prostate cancer is commonly occurred in middle aged males and is a popular malignant tumor in clinics [1]. As most patients are already at terminal stage at the time of diagnosis, the opportunity of surgery may be missed. Therefore, currently treatment approaches mainly include chemo-/radiotherapy combined with hormonal treatment, which, however, cannot obtain satisfactory treatment efficiency. Some studies [2] believed that apoptosis played crucial roles in the occurrence and progression of prostate tumor. Nowadays clinical practice is paying more attention to proteins and genes related with cell apoptosis and tumor occurrence or progression to identify treatment approaches for prostate cancer with higher efficiency. Bcl-xl belongs to Bcl-2 family and can exert inhibitory role in programmed cell death and are an important protein in tumor occurrence and progression [3]. A previous study [4] also believed that over-expression of Bcl-xl is closely correlated with tumor invasion and metastasis. Caspase family is a hydrolase that plays important roles in cell apoptosis. Studies [5, 6] showed gradually decreased caspase-3 expression in non-proliferative mucosa, adenoma, and cancer tissues, indicating the involvement of abnormal expression of caspase-3 in tumor occurrence and progression. LMO2 gene is an important oncogene and exerts important roles in the onset and progression of prostate cancer [7]. Therefore, this study measured the expression of Bcl-xl and caspase-3 and LMO2 gene expression to assess their role in the development and pathogenesis of prostate cancer.

[a] Department of Urology Surgery, First Affiliated Hospital of Jinzhou Medical University, Jinzhou, Liaoning, 121000, China
b. Department of Urology Surgery, Shandong University, Qianfoshan Hospital of Shandong Province, Qianfoshan, Jinan, Shandong, 250000, China
Correspondence author: Dr. Tongyi Men, Department of Urology Surgery, Shandong University, Qianfoshan Hospital of Shandong Province, No. 16766, Jingshi Road, Lixia District, Qianfoshan, Jinan, Shandong, 250000, China. Tel: +86-0531-82969995; Fax: +86-0531-82969995; E-mail: yayezhang5@163.com.

2. MATERIALS AND METHODS

2.1 Sources of experimental materials

A total of 25 patients who received surgical resection of prostate cancer in Shandong University, Qianfoshan Hospital of Shandong Province between January 2015 and January 2017 were recruited in this study and the tumor adjacent tissue samples.

and 20 benign prostate hyperplasia tissues were collected. All patients received confirmed diagnosis of prostate cancer or benign hyperplasia. This study has been approved by the ethical committee of Shandong University, Qianfoshan Hospital of Shandong Province.

2.2 Experimental reagents

Reagents involved in this study were shown in Table 1.

2.3 Sample processing

All tissue samples were assigned into benign prostate hyperplasia, cancer adjacent tissue and cancer tissue groups. Each sample was divided into two parts: one was dehydrated, embedded in paraffin to measure Bcl-xl and Caspase-3 protein expression by immunohistochemistry staining (SP approach). The other part was used to isolate cells, which were cultured and measured for LMO gene expression by real-time fluorescent quantitative PCR as described below.

2.4 IHC staining for measuring Bcl-xl and caspase-3 expression

All acquired samples were dehydrated and embedded in paraffin. Samples were tested by two-step IHC staining using DAB-H_2O_2 labelling. Semi-quantitative integrity approach was used to calculate positive expression rate of proteins. Positive expression was deducted using the product of staining cell percentage and cell staining intensity score. In brief, (1) Staining cell percentage: 1 point was assigned for less than one third of stained cells; 2 points were remarked when staining percentage locates between 1/2 and 2/3; Those tissues with higher than 2/3 positively stained cells were denoted as 3 points. (2) For cell staining intensity index, 0 denoted no stain, 1 point referred to light yellow, 2 points refers to dark yellow, and 3 points identified dark brown color. The product of staining percentage and intensity score was used to identify positive expression and 0~3 points were denoted as negative expression, and 4~9 points refer to positive expression.

2.5 RT-qPCR for LMO2 expression

Prostate matrix cells were extracted from tissue samples and inoculated into RPMI-1640 medium containing 10% fetal bovine serum (FBS) at 37°C with 5% CO_2. Total RNA was extracted using TRIzol (Invitrogen, US) reagent, followed by reverse transcription to obtain cDNA which was used for RT-qPCR. Using GAPDH as the internal reference, SYBR Green I fluorescence approach was used to measure LMO2 mRNA levels in all groups. LMO forward primer: 5'-CTGAA GGCCA TCGAC CAGTA-3'; reverse primer: 5'-TTGTC ACAGG ATGCG CAGAG-3'; GAPDH forward primer: 5'-GAAGG TCGGA GTCAA CGGAT T-3'; Reverse primer: 5'-CGCTC CTGGA AGATG GTGAT-3'. The relative expression of LMO2 mRNA was calculated in all groups.

2.5 Statistics

SPSS19.0 software was used for analyzing data which were presented as mean ± standard deviation (SD) and assessed by student t-test for measurement data. Enumeration data were presented as ratios and compared by chi-square test. Spearson approach was used to analyze the correlation. $p<0.05$ indicates a statistical significance.

3. RESULTS AND DISCUSSION

3.1 Positive expression of Bcl-xl and Caspase-3 proteins

Table 2 showed elevated expression of Bcl-xl protein in cancer adjacent tissues, benign prostate hyperplasia group and cancer tissues, which had a positive expression rate of 32%, 45% and 88%, respectively. Among those, Bcl-xl showed significantly higher positive rate in cancer tissues compared to those in adjacent and benign hyperplasia tissues ($p<0.05$). Caspase-3, on the other hand, presented a decreasing trend in cancer adjacent tissues (72%), benign hyperplasia group (60%) and cancer tissues (16%). Casapse-3 presented significantly lower expression rate in cancer tissues than adjacent tissues and benign prostate hyperplasia group ($p<0.05$).

3.2 Immunohistochemistry staining of Bcl-xL and Casapse-3 proteins

As shown in Figure 1, Bcl-xL protein expression was significantly higher in cancer tissues (Figure 1c) than benign hyperplasia (Figure 1a) and cancer adjacent tissues (Figure 1b, $p<0.05$). Casapse-3 protein, on the other hand, showed significantly lower expression in cancer tissues (Figure 2c) than benign hyperplasia (Figure 2a) and cancer adjacent tissues (Figure 2b, $p<0.05$).

3.3 LMO2 gene expression profile

RT-qPCR results (Table 3) showed that, LMO2 mRNA level in cancer adjacent tissues was 1.038-fold higher than that in benign hyperplasia group, without a statistical significance ($p>0.05$). Cancer tissues had 897.317-fold higher of LMO2 mRNA compared to control group, with a significant difference ($p<0.001$).

3.4 Discussion

Multiple diseases are closely correlated with cell apoptosis. Current molecular biology scholars [8] believed that decreased cell apoptosis plays a crucial role in disease progression of prostate tumors. As an important anti-apoptosis gene, Bcl-xl has been found to be over-expressed in multiple human tumor tissues [9, 10]. Other study also showed Bcl-xl over-expression in PC-3 cells which was correlated with the differentiation grade of prostate cancer. In this study, we showed a significantly elevated expression of Bcl-xL in cancer adjacent tissues, benign prostate hyperplasia group, and cancer tissues, with a positive expression rate at 32%, 45% and 88%. Among those Bcl-xL proteins showed a significantly higher positive expression rate than cancer adjacent tissues and benign prostate hyperplasia group ($p<0.05$). Bcl-xL protein expression was significantly higher in cancer tissues compared to those in benign prostate hyperplasia and cancer adjacent tissues ($p<0.05$). These results indicated the close correlation of Bcl-xl protein over-expression with prostate cancer onset possibly through inhibition of cell apoptosis. In this study, we found a decrease of the expression of Caspase-3 protein in cancer adjacent tissues, benign prostate hyperplasia group and cancer group, which had a positive rate of 72%, 60% and 16%, respectively. Caspase-3 showed a significantly lower positive expression rate in cancer tissues compared to adjacent or benign hyperplasia groups ($p<0.05$), indicating significantly down-regulation of caspase-3 protein in cancer tissues compared to benign hyperplasia and tumor adjacent tissues ($p<0.05$). These data suggested a potential involvement of Casapase-3 in the onset and progression of prostate cancer, and especially for the regulation of the apoptosis of adenoma cells. As a critical proteinase at the downstream of caspase-3 to facilitate cell apoptosis [11]. Our results are inconsistent with previous literatures [12, 13] demonstrating a significantly higher Caspase-3 protein in benign prostate hyperplasia tissues than prostate cancer tissue. Other studies [14, 15] believed that Bcl-xl can prevent cell apoptosis via suppressing Casapse-3 activation, regulating ROS toxicity, and maintaining normal membrane potential. In addition, our results were similar with previous studies [16, 17]. Clinical data showed that the expression of oncogene LMO2 was strongly and positively correlated with the aggravation and metastasis of prostate cancer [18]. Oncogene LMO2 can affect the malignancy and metastasis via transcriptional inhibition targeting E-cadherin [19, 20]. In the present study, our results showed that LMO2 mRNA level in cancer adjacent tissues was 1.038-fold higher than that in benign prostate hyperplasia. Cancer tissues, however, had 897.317-fold higher of LMO2 mRNA compared to control group ($p<0.001$). In this study, we found a significantly elevated LMO2 expression, whilst Bcl-xL and Caspase-3 proteins were closely related with the onset and progression of prostate cancer. These data indicated important roles of LMO2 in the development and pathogenesis of prostate cancer. However, the exact mechanism by how LMO2, Bcl-xL and Casapae-3 involves in the pathogenesis of prostate cancer was not assessed in our study, which is a main study limitation. In the future, we plan to assess this in our further study. Another study limitation is the limited number of patient samples in our study. A large cohort study is required to confirm the findings in the future.

Acknowledgments

This work was supported by the Project is sponsored by Liaoning BaiQianWan Talents Program.

Disclosure of conflict of interest

None.

4. CONCLUSION

Our results showed the close relationship of abnormal expression of Bcl-xl protein and Caspase-3 protein with the onset of prostate cancer. Moreover, LMO2 gene plays a crucial role in progression of prostate cancer. Our study indicates that they might be used as an indicator for the prognosis of prostate cancer.

References and Notes

[1] P.G. Pilie*, et al.*, Germline genetic variants in men with prostate cancer and one or more additional cancers. Cancer 123(20) (2017), 3925-3932.

[2] X. Guo*, et al.*, Upregulation of the Kank1 gene-induced brain glioma apoptosis and blockade of the cell cycle in G0/G1 phase. Int J Oncol 44(3) (2014), 797-804.

[3] A. Zaanan*, et al.*, The Mutant KRAS Gene Up-regulates BCL-XL Protein via STAT3 to Confer Apoptosis Resistance That Is Reversed by BIM Protein Induction and BCL-XL Antagonism. J Biol Chem 290(39) (2015), 23838-49.

[4] D. Trisciuoglio*, et al.*, BCL-XL overexpression promotes tumor progression-associated properties. Cell Death Dis 8(12) (2017), 3216.

[5] I. Dolka, M. Krol, and R. Sapierzynski, Evaluation of apoptosis-associated protein (Bcl-2, Bax,

cleaved caspase-3 and p53) expression in canine mammary tumors: An immunohistochemical and prognostic study. Res Vet Sci 105((2016), 124-33.
[6] G. Borghetti, *et al.*, Fish oil administration mediates apoptosis of Walker 256 tumor cells by modulation of p53, Bcl-2, caspase-7 and caspase-3 protein expression. Lipids Health Dis 14((2015), 94.
[7] X. Peng, Z. Ye, and Y. Gao, [Regulation of E-cadherin promoter activity by LMO2 impact on the progression and metastasis of prostate cancer]. Zhongguo Ying Yong Sheng Li Xue Za Zhi 29(5) (2013), 385-9.
[8] U. Beyer, *et al.*, Rare ADAR and RNASEH2B variants and a type I interferon signature in glioma and prostate carcinoma risk and tumorigenesis. Acta Neuropathol 134(6) (2017), 905-922.
[9] K. Setoguchi, *et al.*, Antisense Oligonucleotides Targeting Y-Box Binding Protein-1 Inhibit Tumor Angiogenesis by Downregulating Bcl-xL-VEGFR2/-Tie Axes. Mol Ther Nucleic Acids 9((2017), 170-181.
[10] I. Killcciouglu, *et al.*, Proteasome and HDAC Inhibition Changes the Expression Levels of Bcl-2, Bcl-XL, Bim and Bik Proteins in Androgen-Independent PC-3 Cell Line. Gazi Medicial Journal 26(4) (2015), 155-157.
[11] T. Mitupatum, *et al.*, Hep88 mAb-mediated paraptosis-like apoptosis in HepG2 cells via downstream upregulation and activation of caspase-3, caspase-8 and caspase-9. Asian Pac J Cancer Prev 16(5) (2015), 1771-9.
[12] Y. Qu, *et al.*, Pim-3 is a Critical Risk Factor in Development and Prognosis of Prostate Cancer. Med Sci Monit 22((2016), 4254-4260.
[13] H.C. Cai, *et al.*, Effects of Xialiqi Capsules on the expressions of PCNA and caspase-3 in rats with benign prostatic hyperplasia. National Journal of Andrology 23(8) (2017), 728-733.
[14] R.A. Kirkland and J.L. Franklin, Bax and caspases regulate increased production of mitochondria-derived reactive species in neuronal apoptosis: LACK of A role for depletion of cytochrome c from the mitochondrial electron transport chain. Biochem Biophys Rep 4((2015), 158-168.
[15] S. Deng, *et al.*, Furazolidone induces apoptosis through activating reactive oxygen species-dependent mitochondrial signaling pathway and suppressing PI3K/Akt signaling pathway in HepG2 cells. Food Chem Toxicol 75((2015), 173-86.
[16] X. Chen, *et al.*, Suppression effect of recombinant adenovirus vector containing hIL-24 on Hep-2 laryngeal carcinoma cells. Oncol Lett 7(3) (2014), 771-777.
[17] Q.Q. Gao, H. Chen, and Y. Chen, Expressions of Bcl-xl and caspase-3 in the prostate cancer tissue and their correlation. National Journal of Andrology 29(5) (2016), 385.
[18] J.J. Yu, *et al.*, [Effect of prostate peripheral zones stromal cells on the proliferation of prostate cells by overexpression of LMO2 gene]. Zhonghua Yi Xue Za Zhi 96(2) (2016), 91-4.
[19] J.R. Cerhan, *et al.*, LIM domain only 2 protein expression, LMO2 germline genetic variation, and overall survival in diffuse large B-cell lymphoma in the pre-rituximab era. Leuk Lymphoma 53(6) (2012), 1105-12.
[20] C.N. Bui, *et al.*, Budget Impact of Enzalutamide for Chemotherapy-Naive Metastatic Castration-Resistant Prostate Cancer. J Manag Care Spec Pharm 22(2) (2016), 163-70.

Table 1. **Reagents and manufactures.**

Reagents	Manufactures
Bcl-xL antibody	Aobosen Biotech, China
Caspase-3 antibody	Aobosen Biotech, China
SP Immunohistochemistry staining kit	Zhongshan Corp, China
Fetal bovine serum	Gibco, US
RPMI-1640 culture medium	Hyclone, US
TRIzol	Invitrogen, US

Table 2. **Positive expression rats of Bcl-xl and Caspase-3 proteins.**

Group	N	Bcl-xL protein		Positive rate (%)	Capase-3 protein		Positive rate (%)
		+	-		+	-	
Benign prostate hyperplasia group	20	9	11	45	12	8	60
Cancer adjacent tissue group	25	8	12	32	18	7	72
Cancer tissue	25	22	3	88	4	21	16

Table 3. **LMO2 mRNA relative expression level in all groups.**

Group	N	Relative expression	P
Benign prostate hyperplasia	20	1	/
Cancer adjacent tissue	25	1.038	>0.05
Cancer tissue	25	897.317	<0.001

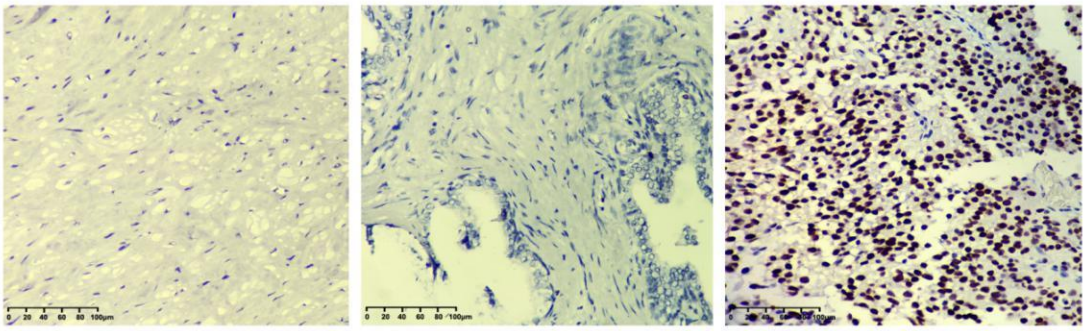

Figure 1. **Bcl-xL protein expression (SP, X400). (A) Bcl-xL protein expression in benign prostate hyperplasia tissues; (B) Bcl-xL protein expression in cancer adjacent tissues; (C) Bcl-xL protein expression in prostate cancer tissues.**

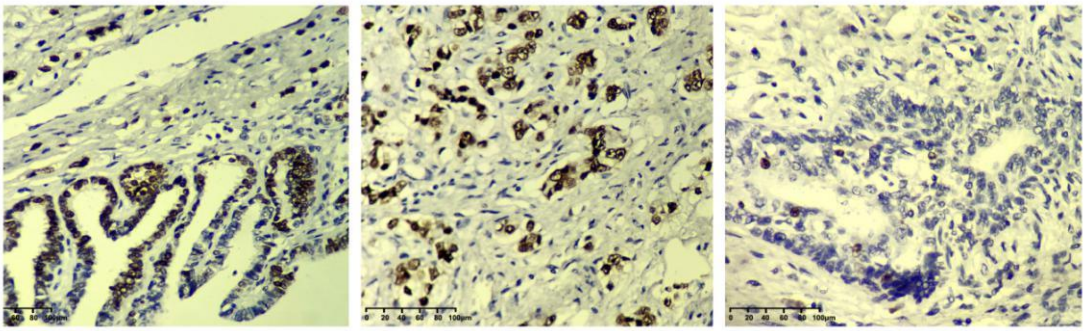

Figure 2. **Caspase-3 protein expression (SP, X400). (A) Caspase-3 protein expression in benign prostate hyperplasia tissues; (B) Caspase-3 protein expression in cancer adjacent tissues; (C) Caspase-3 protein expression in prostate cancer tissues.**