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# The impact of TRAIL on proliferation of secretory prostate cancer PC-3 cell and LMO2 gene expression

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**Abstract. – OBJECTIVE: To determine the expressions of TRAIL protein and LMO2 gene in prostate cancer tissues with different differentiation degree and identify the influence of TRAIL on prostate cancer PC-3 cell proliferation.**

**PATIENTS AND METHODS: Surgical specimens from a total of 30 prostate cancer patients with radical prostatectomy were collected. The subjects were divided into three groups according to the different degrees of differentiation. TRAIL positive rate was detected by immunohistochemistry (IHC). LMO2 expression was assessed by Real-time PCR and Western-blot. PC-3 cell proliferation was determined by CCK-8 assay.**

**RESULTS: The positive rate of TRAIL protein was significantly higher in moderately differentiated group (80%) and well differentiated group (100%) compared with that in poorly differentiated group (54.55%), respectively ($\chi^2$ = 27.33, $p < 0.05$; $\chi^2$ = 40.12, $p < 0.01$). Streptavidin-peroxidase (SP) assay showed that TRAIL protein expression in well-differentiated group was significantly higher than that in moderately differentiated group and poorly differentiated group. qRT-PCR result demonstrated that LMO2 mRNA levels in moderately and well-differentiated group were significantly increased compared to that in poorly differentiated group ($p < 0.001$). Also, the proliferation rate of PC-3 cells in well-differentiated group was significantly higher than that in well-differentiated and moderately differentiated groups ($p < 0.05$).**

**CONCLUSION: Our data indicated that the positive rate of TRAIL protein increased in a prostate cancer differentiation dependent manner.**

*Key Words:*
Prostate cancer, TRAIL protein, PC-3 cells, LMO2 gene.

## Introduction

Prostate cancer (PC) is a sort of common malignant tumor frequently occurred in the elderly male population[1]. Early diagnosis and surgery are the main effective methods for PC. However, due to the stealthiness of PC, most patients were suffered from local metastasis or infiltration and missed the best treatment method. In addition, the molecular biology study also confirmed that the occurrence and development of PC was accompanied by enhancement of cell proliferation[2]. Meanwhile, there were multiple genes involved in PC occurrence and development. Therefore, it is of great significance to develop novel gene therapy for PC as an alternative way to promote the effects of endocrine therapy, radiotherapy, and chemotherapy. As a member of the tumor necrosis factor superfamily, TRAIL plays a key role in tumorigenesis and apoptosis. Previous finding showed apparent difference of the expression of apoptosis-related factor TRAIL protein in PC tissue compared with non-cancerous tissue[3]. LMO2 gene is one of the important oncogenes and has been widely investigated in hematological tumors. Recent study[4] demonstrated that LMO2 gene con-



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tributed to the regulation in the development of PC. Therefore, this study tested the expression of TRAIL protein and LMO2 gene in PC tissue and cells at different differentiation stages.

## Patients and Methods

Surgical specimens from a total of 30 prostate cancer patients receiving radical prostatectomy in ZiBo Central Hospital (Shandong China) from January 2015 to January 2017 were enrolled. The study protocol was approved by the Research Ethics Committee of ZiBo Central Hospital (Shandong China), and all patients gave their informed consent before study commencement.

### Reagents

The reagents used in this study were listed in Table I.

### Sample Treatment

The samples were divided into three groups according to the different degree of differentiation, including 11 cases of poorly differentiated group, 10 of moderately differentiated group, and 9 of well-differentiated group. The sample was routinely dehydrated and paraffin-embedded for detection of the positive expression of TRAIL protein by SP method. Another sample was primary cultured to determine LMO2 gene expression via qRT-PCR and Western blot. The proliferation of PC-3 cells was detected by CCK-8 cell assay.

### SP Detection of TRAIL Positive Expression Rate

The obtained samples were routinely dehydrated and embedded in paraffin. After being dewaxed, the sample was treated by 3% hydrogen peroxide ($H_2O_2$) solution to remove endogenous peroxidase and was blocked in normal sheep serum. Next, the sample was incubated in TRAIL solution at 4°C overnight and further incubated in biotin labeled secondary antibody at room temperature for 30 min. Next, the sample was added with horseradish enzyme-labeled streptavidin solution at room temperature for 30 min. At last, the sample was developed by DAB, re-dyed by hematoxylin, and sealed by neutral gel. Brown color was considered as positive. TRAIL protein was expressed mainly in cytoplasm and occasionally in nucleus. The percentage of positive cells in five high power fields was counted and positive cells > 10% were considered as positive.

### LMO2 Gene Expression and PC-3 Cell Proliferation

The tissue was cut to maintain the volume of about 1 $mm^3$ size and then digested with collagenase to facilitate separation. Differential gradient centrifugation was used to separate epithelial cells and stromal cells. The isolated stromal cells

**Table I.** Reagents and manufacture

| Reagent | Manufacture |
|---|---|
| TRAIL antibody | Sigma-Aldrich (Waltham, MA, USA) |
| SP immunohistochemical kit | ZSbio (Beijing, China) |
| FBS | Gibco (Rockville, MD, USA) |
| RPMI-1640 medium | HyClone (South Logan, UT, USA) |
| 0.25% enzyme-EDTA | HyClone (South Logan, UT, USA) |
| PC-3 cell | ATCC (Manassas, VA, USA) |
| TRIzol | Invitrogen (Carlsbad, CA, USA) |
| One-step qRT-PCR kit | Takara (Otsu, Shiga, Japan) |
| RIPA | Beyotime (Shanghai, China) |
| LMO2 antibody | Abcam (Cambridge, MA, USA) |
| GAPDH antibody | Santa Cruz Biotechnology (Santa Cruz, CA, USA) |
| HRP labeled secondary antibody | Cell Signaling Technology (Danvers, MA, USA) |
| Transwell chamber (6.5 mm diameter, 0.4 μm aperture) | Corning (Corning, NY, USA) |
| CCK-8 solution | Dojindo (Shanghai, China) |
| LKB-NOVA ultra microtome | LKB (Bromma, Sweden) |
| BX51F32H01 optical microscope | Olympus (Tokyo, Japan) |
| DYY-8C electrophoresis apparatus | Beijing Liuyi (Beijing, China) |
| PCR amplifier | Bio-Rad (Hercules, CA, USA) |
| Tanon-GIS 2016 gel imaging system | Tanon (Shanghai, China) |

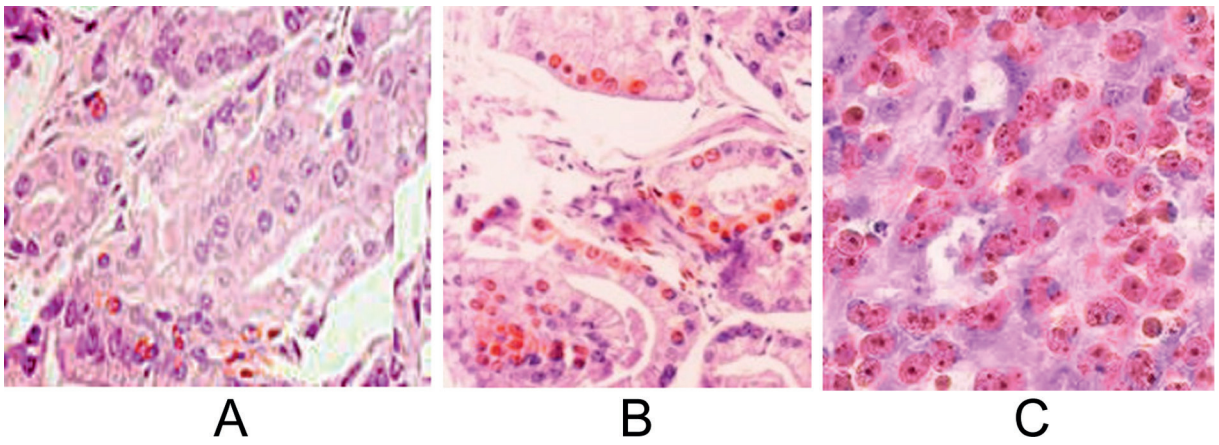


**Figure 1.** TRAIL protein expression in PC tissue (SP, ×400). ***(A)*** TRAIL protein expression in poorly differentiated PC tissue. ***(B)*** TRAIL protein expression in moderately differentiated PC tissue. ***(C)*** TRAIL protein expression in well-differentiated PC tissue.

were inoculated in Roswell Park Memorial Institute-1640 (RPMI-1640) medium containing 10% fetal bovine serum (FBS) and cultured at 37°C and 5% $CO_2$.

### qRT-PCR

Total RNA was extracted by TRIzol and reverse transcribed to cDNA. qRT-PCR was used to test LMO2 mRNA expression. GAPDH was selected as internal control.

The primer sequences were listed as follows: LMO2, forward, 5'-CTGAAGGCCATCGACCAGTA-3', reverse, 5'-TTGTCACAGGATGCGCAGAG-3'; GAPDH, forward, 5'-GAAGGTCGGAGTCAACGGATT-3', reverse, 5'- CGCTCCTGGAAGATGGTGAT-3'.

### Western Blot

The cells were lysed by RIPA and total protein was extracted. A total of 50 μg protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane at 300 mA for 2.5 h. Next, the membrane was blocked in 5% skim milk at room temperature for 2 h, and further incubated in primary antibody (1:1000) at 4°C overnight. At last, the membrane was incubated in HRP-labeled secondary antibody (1:2000) at room temperature for 1.5 h and developed by enhanced chemiluminescence (ECL).

### CCK-8 Assay

The prostate cancer cells were seeded in 96-well plate at the density of $5\times10^3$ cells/well, while PC-3 cells were seeded in 24-well plate at $5\times10^3$ cells/well. Co-culture system was established and the lower chamber was changed to 500 μl RPMI-1640 medium without phenol red. At last, 50 μl CCK-8 solution were added to each well and incubated for 3 h. The plate was tested at 450 nm to obtain the absorbance.

### Statistical Analysis

All data were analyzed by SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). The measurement data were presented as mean ± standard deviation and tested by *t*-test after normal distribution and homogeneity test of variance. Continuous data were analyzed by using one-way ANOVA, with the Tukey's post-hoc test. The enumeration data were compared by $\chi^2$-test. $p < 0.05$ was depicted as statistical significance.

## Results

### TRAIL Positive Expression in Different Groups

The positive rate of TRAIL protein was significantly higher in moderately differentiated group (80%) and well differentiated group (100%) compared with poorly differentiated group (54.55%), respectively ($\chi^2 = 27.33$, $p < 0.05$; $\chi^2 = 40.12$, $p < 0.01$, Table II). SP assay showed that TRAIL protein expression in well differentiated group was significantly higher than that in moderately differentiated group and poorly differentiated group, while the protein expression was also markedly higher in moderately differentiated group than that in poorly differentiated group ($p < 0.05$, Figure 1).

**Table II.** TRAIL positive rate comparison.

| Group | Cases | TRAIL | Positive rate (%) | $X^2$ | *p* | |
|---|---|---|---|---|---|---|
| Poorly differentiated group | 11 | 6 | 5 | 54.55 | / | / |
| Moderately differentiated group | 10 | 8 | 2 | 80 | 27.33 | < 0.05 |
| Well-differentiated group | 9 | 9 | 0 | 100 | 40.12 | < 0.01 |

**Table III.** LMO2 mRNA relative expression.

| Group | Cases | Relative expression | *p* |
|---|---|---|---|
| Poorly differentiated group | 11 | 1 | / |
| Moderately differentiated group | 10 | 1.03 | >0.05 |
| Well-differentiated group | 9 | 97.33 | <0.001 |

### *LMO2 Gene Expression in Different Groups*

The result from qRT-PCR demonstrated that LMO2 mRNA level in moderately differentiated group was 51.03-fold higher than that in poorly differentiated group ($p < 0.05$), while it was 97.33-fold higher in well-differentiated group than that in poorly differentiated group ($p < 0.001$, Table III). Western-blot data exhibited that there was no significant difference in the expression of LMO2 protein between poorly and moderately differentiated groups, while LMO2 expression in well-differentiated group was apparently higher than that in poorly and moderately differentiated groups ($p < 0.05$, Figure 2).

### *PC-3 Cell Proliferation*

From day 3 after culture, the proliferation rate of PC-3 cells in well-differentiated group was significantly increased compared to that in well-differentiated and moderately differentiated groups ($p < 0.05$). The proliferation rate of PC-3 cells in moderately differentiated group was also slightly enhanced, but showed no significant difference compared with that in the poorly differentiated group ($p > 0.05$, Figure 3).

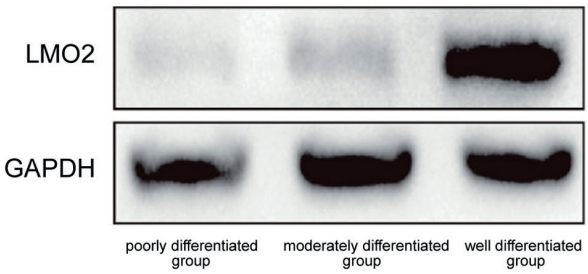


**Figure 2.** LMO2 protein expression.

## Discussion

TRAIL, also known as apolipoprotein II ligand (Apo-2L), regulates apoptosis by specifically binding to receptors on the surface of target cells, so as to activate Caspase system and NF-κB signaling[5-7]. It was showed that TRAIL and its receptors were expressed in multiple tissues, of which prostate is one with high expression of TRAIL[8]. In addition, it was also demonstrated that TRAIL can selectively induce the apoptosis of tumor cells and virus-infected cells without affecting normal cells[9]. Therefore, the role of TRAIL protein attracts extensive attention in cancer research and therapy. LMO2 gene is one of the important oncogenes involved in hematological tumors[10,11]. However, recent studies revealed that LMO2 gene plays a crucial role in the development of PC[12], gastric cancer[13], and hemangiomas[14]. In this report, the level of TRAIL protein was significant-

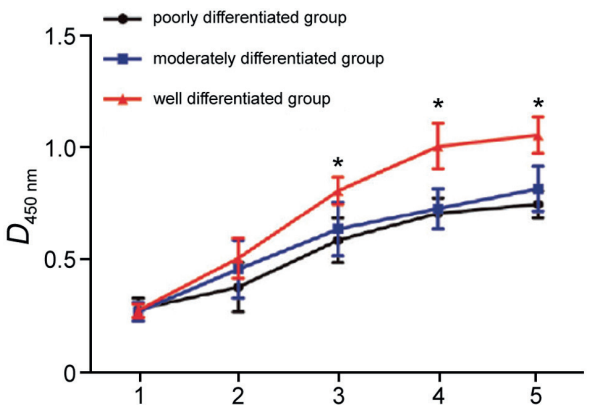


**Figure 3.** PC-3 cell proliferation. *, $p < 0.05$ compared to poorly differentiated group.

ly higher in moderately differentiated group and well differentiated group compared with that in poorly differentiated group, indicating that the expression of TRAIL was associated with the development of PC tissue, which was in accordance with the previous study[15]. SP assay also validated high levels of TRAIL protein in well differentiated group compared to that in moderately differentiated group and poorly differentiated group. It revealed that TRAIL protein expression enhanced following differentiation upgrade, which was similar to previous results[16,17]. Clinical investigation exhibited that the higher expression of proto-oncogene LMO2 aggravated the degree of PC exacerbation and metastasis, which showed a close positive correlation[18]. The proto-oncogene LMO2 can affect the progression and metastasis of prostate cancer by inhibiting the transcription of E-cadherin[4,19]. Recent evidence[20,21] showed that critical proteins such as SCCRO, ZEB1 participated in the regulation of the occurrence and development of prostate cancer. In this study, the results of qRT-PCR and Western-blot on the detection of LMO2 mRNA level showed that its expressions in moderately and well-differentiated group were remarkably enhanced compared to that in poorly differentiated group, suggesting that LMO2 expression has certain correlation with PC differentiation. This research established relationship between TRAIL protein and LMO2 with PC-3 cell differentiation. Our results demonstrated that TRAIL protein may be positively correlated with LMO2, which may provide new direction for the gene therapy. However, further investigation is needed to explore their specific relationship.

## Conclusions

We observed that TRAIL protein expression was increased in a PC differentiation dependent manner. The levels of TRAIL and LMO2 were associated with PC-3 cell proliferation.

### Conflict of Interest

The Authors declare that they have no conflict of interest.

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