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Prognostic potentials of miRNA-19a-3p and PDCD5 in nasopharynx carcinoma

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Abstract. – OBJECTIVE: To determine expressions of MicroRNA-19a-3p (miRNA-19a-3p) and PDCD5 in nasopharyngeal carcinoma (NPC) tissues, and their prognostic potentials in NPC.

PATIENTS AND METHODS: Expressions of miRNA-19a-3p and PDCD5 in NPC tissues and controls were determined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The correlation between expressions of miRNA-19a-3p and PDCD5 in NPC was evaluated by Pearson correlation test. Furthermore, potential influences of miRNA-19a-3p and PDCD5 on clinical features of NPC patients were assessed. Through 5-year follow-up, survival analysis in NPC patients was conducted by Kaplan-Meier method. Finally, factors influencing prognosis of NPC were determined using the Cox regression model.

RESULTS: MiRNA-19a-3p was upregulated and PDCD5 was downregulated in NPC tissues. Pearson correlation test uncovered a negative correlation between expression levels of miRNA-19a-3p and PDCD5 in NPC tissues. MiRNA-19a-3p level was correlated with N classification and clinical stage in NPC patients, while PDCD5 level was correlated with T classification, pathological grade and clinical stage. Survival analysis showed poor prognosis in NPC patients expressing high level of miRNA-19a-3p or low level of PDCD5. Cox regression analysis illustrated that N2+3 classification, clinical stage III+IV, high level of miRNA-19a-3p and low level of PDCD5 were independent risk factors for the prognosis of NPC.

CONCLUSIONS: MiRNA-19a-3p is upregulated and PDCD5 is downregulated in NPC tissues. High level of miRNA-19a-3p and low level of PDCD5 are unfavorable for the prognosis of NPC.

Key Words:
Nasopharynx carcinoma, MiRNA-19a-3p, PDCD5, Clinical features, Prognosis.

Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor occurring on the side or top of the nasopharyngeal cavity. NPC mainly occurs in Southeast Asia and Southern China, presenting a certain regionality[1]. With the application of comprehensive therapeutic strategies, the 5-year survival of NPC patients significantly increases. However, about 20%-30% of NPC patients develop tumor recurrence or distant metastasis, both of which are the leading causes of treatment failure and death[2]. Therefore, searching for effective biomarkers that could predict the prognosis of NPC is of significance in improving clinical outcomes.

MiRNAs are of significance in regulating life activities[3,4]. MiRNA-19a-3p belongs to the miRNA-17-92 gene cluster. It locates on human chromosome 13q31.3 and can encode miRNA-18a, miRNA-17-5p, miRNA-19a-3p, miRNA-20a-3p, miRNA-19b-3p and miRNA-92, showing important biological functions[5]. MiRNA-19a-3p stimulates the metastasis and proliferation of gastric cancer cells by regulating socs1 and MXD1[6,7].

PDCD5, also known as TFAR19 (TF-1 cell apoptosis related gene-19), is a newly discovered gene by Chinese scholars. It is responsible for regulating cell apoptosis[8]. PDCD5 is widely expressed in human body, and its mRNA expression level is markedly lower in embryonic tissues than that in adult tissues[9]. PDCD5 is downregulated in tumor patients, indicating its participation in tumor development[10-12]. Qiao et al[13] have shown that PDCD5 is regulated by miRNA-19a-3p and can be served as a biomarker for predicting the prog-

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nosis of gastric cancer. In this paper, we for the first time examined expressions of miRNA-19a-3p and PDCD5 in NPC tissues, and their prognostic potentials in NPC were assessed as well.

Patients and Methods

Clinical Data

A total of 150 NPC patients who were diagnosed in The First Affiliated Hospital of Gannan Medical University from June 2016 to December 2018 were enrolled. Patient inclusion criteria: (1) patients newly diagnosed with type 2 or 3 NPC of WHO; (2) readjusted to III-IVb (T1- 2N2-3M0 and T3-4N0-3M0, 8th edition based on American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) staging system) radiography or computed tomography (CT), abdominal ultrasound or CT, whole-body bone scan or [^{18}F]-fluorodeoxyglucose positron emission Tomography combined with computed tomography (PET/CT); (3) Intensity Modulated Radiation Therapy (IMRT) plus concurrent chemotherapy alone. Patients were excluded if they received anti-cancer treatment before our hospital diagnosis, or were pregnant or breastfeeding, or were diagnosed with synchronous/synchronous cancer lesions during or before treatment or follow-up. In the meantime, a total of 150 nasopharyngeal tissues were harvested from those diagnosed with chronic mucosal inflammation by nasopharyngeal biopsy. Tissue samples were washed in normal saline, placed in 1.5 Eppendorf (EP; Hamburg, Germany) tubes containing RNA preservation solution and preserved in liquid nitrogen. Patients and their families in this study have been fully informed. This study was approved by Ethics Committee of The First Affiliated Hospital of Gannan Medical University.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Nasopharyngeal tissues were lysed for isolating total RNA using TRIzol method (Invitrogen, Carlsbad, CA, USA). RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA) using PrimeScript RT Reagent (TaKaRa, Otsu, Shiga, Japan), and applied for qRT-PCR using the All-in-OneTM miRNA qRT-PCR kit (GeneCopoeia Inc., Rockville, MD, USA). U6 was used as the internal reference. Primer sequences were listed as follows: U6 F: 5'-CTCGCTTCG-GCAGCACA-3'; R: 5'-AACGCTTCACGAATTTGCGT-3'; miRNA-19a-3p F: 5'-CAATCCTCTCAGGCTCAGTCC-3'; R: 5'-TATGCTTGTTCTCGTCTCTGTGTC-3'; PDCD5 F: 5'-CGACTGATCCAGAACTTGGG-3'; R: 5'-GAGCTGCAGGCCAAACAC-3'.

Follow-Up

Every patient was followed up through outpatient visit or telephone call for at least 5 years. The follow-up was conducted once every three months in the first year and once every six months since after. The last follow-up was conducted in the year of 2018. The death of patient was recorded as the end point of follow-up.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM Corp., Armonk, NY, USA) was used for data analyses. Measurement data and enumeration data were analyzed by the *t*-test and χ^2 test, respectively. Kaplan-Meier method was introduced for survival analysis, followed by Log-rank test for comparison between curves. The correlation between levels of miRNA-19a-3p and PDCD5 in NPC tissues was evaluated by Pearson correlation test. Potential factors influencing prognosis of NPC were analyzed by Cox regression model. $p<0.05$ was considered as statistically significant.

Results

Upregulated MiRNA-19a-3p and Downregulated PDCD5 in NPC

Compared with control tissues, miRNA-19a-3p was upregulated (Figure 1A) and PDCD5 was downregulated in NPC tissues (Figure 1B). It is suggested that miRNA-19a-3p and PDCD5 were involved in the progression of NPC.

Correlation Between MiRNA-19a-3p and PDCD5 in NPC

Pearson correlation test uncovered a negative correlation between expressions of miRNA-19a-3p and PDCD5 in NPC tissues (r=-0.7177, $p<0.001$, Figure 2). It is indicated that miRNA-19a-3p could negatively regulate PDCD5 level in NPC.

Correlation Between Expressions of MiRNA-19a-3p and PDCD5 with Clinical Features of NPC

Based on the median level of miRNA-19a-3p (7.02±2.99) in the enrolled NPC patients, they were assigned into high-level group (n=91) and low-level group (n=59). High level of miRNA-19a-3p was

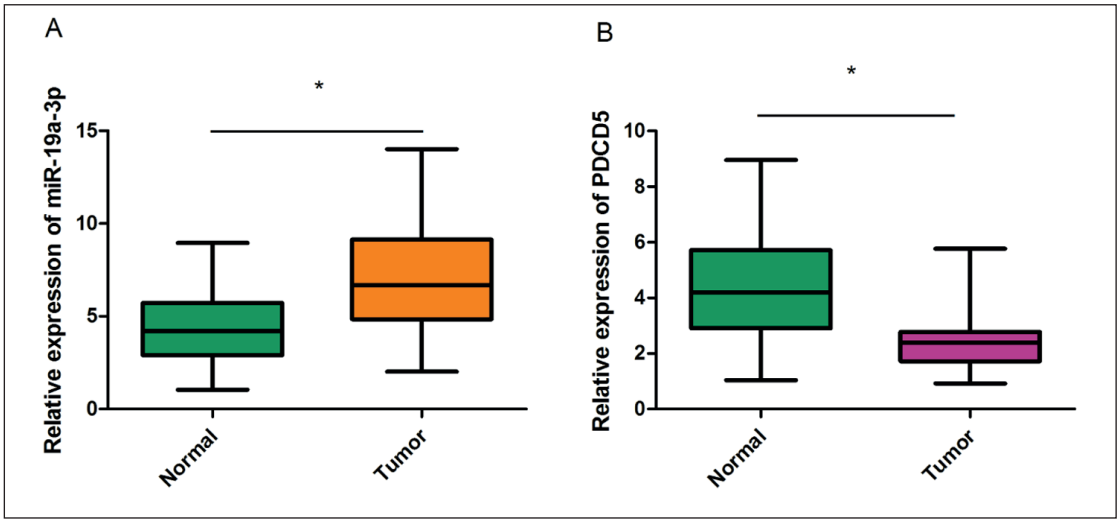

Figure 1. Upregulated miRNA-19a-3p and downregulated PDCD5 in NPC. **A-B**, Relative levels of miRNA-19a-3p (**A**) and PDCD5 (**B**) in NPC tissues and control tissues.

correlated with N_{2+3} classification and clinical stage III+IV. Similarly, NPC patients were assigned into high-level group (n=66) and low-level group (n=84) based on the median level of PDCD5 (2.37±0.95). Low level of PDCD5 was correlated with T_{3+4} classification, medium to high level of tumor differentiation and clinical stage III+IV (Table I).

Potential Influences of MiRNA-19a-3p and PDCD5 on Survival of NPC

To elucidate potential influences of miRNA-19a-3p and PDCD5 on survival of NPC, we conducted 5-year survival in NPC patients. Kaplan-Meier curves revealed poor prognosis in NPC patients expressing high level of miRNA-19a-3p (HR=13.48, p<0.001, Figure 3A) or low level of PDCD5 (HR=6.716, p=0.0096, Figure 3B). It is suggested that miRNA-19a-3p was unfavorable and PDCD5 was favorable to prognosis of NPC.

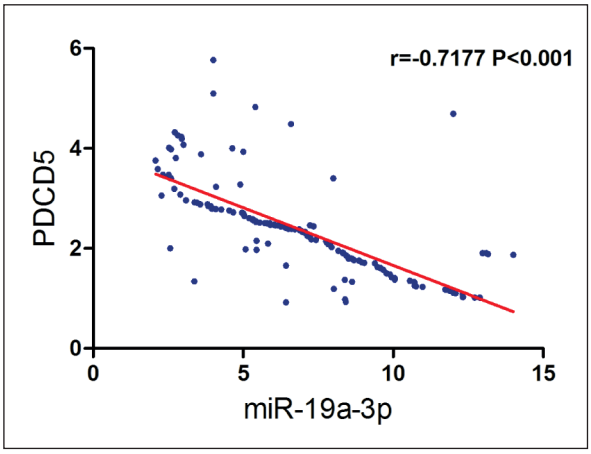

Figure 2. Correlation between miRNA-19a-3p and PDCD5 in NPC. Pearson correlation test showed a negative correlation between expression levels of miRNA-19a-3p and PDCD5 in NPC tissues (r=-0.7177, p<0.001).

Cox Regression Analysis on Factors Influencing Survival of NPC

To further clarify factors affecting survival in NPC patients, Cox regression model was applied. It is shown that N_{2+3} classification (HR=1.944, 95%CI=1.031-2.549), clinical stage III+IV (HR=2.081, 95%CI=1.312-4.148), high level of miRNA-19a-3p (HR=2.861, 95%CI=1.971-4.713) and low level of PDCD5 (HR=1.911, 95%CI=1.323-3.165) were independent risk factors for influencing prognosis of NPC (Table II).

Discussion

NPC is a common malignant tumor of the head and neck. Comprehensive therapies based on radiotherapy and chemotherapy are preferred for NPC patients. In recent years, tumor immunotherapy has shown a promising application[14]. However, improvements on prognosis of NPC are limited. Dysfunctional oncogenes and tumor-suppressor genes would result in malignant progression of tumors, including malignant growth, metastasis and treatment resistance[15]. Therefore, searching for molecular hallmarks and intervention targets associated with tumor progression

Table I. Correlation between expression levels of miRNA-19a-3p and PDCD5 with clinical features of NPC.

Variable	n	miRNA-19a-3p		p	PDCD5		p
		High (n=91)	Low (n=59)		High (n=84)	Low (n=66)	
Sex							
Male	107	60	47	0.069	57	50	0.288
Female	43	31	12		27	16	
Age							
<55	98	54	44	0.055	56	42	0.356
≥55	52	37	15		28	24	
T classification							
T1+2	80	48	32	0.858	47	33	0.006
T3+4	70	43	27		37	33	
N classification							
N0+1	58	13	45	0.030	25	33	0.861
N2+3	92	78	14		59	33	
Pathological grade							
Low	30	12	18	0.075	21	9	0.001
Medium+High	120	79	41		63	57	
Clinical stage							
I +II	45	20	25	0.008	27	18	0.010
III +IV	105	71	34		57	48	

contributes to the monitoring of the progression and the improvement of the prognosis.

In our study, miRNA-19a-3p was upregulated in NPC tissues. After analyzing clinical features of NPC patients, it is shown that miRNA-19a-3p level was correlated to N classification and clinical stage. MiRNA-19a-3p could be an oncogene that was responsible for promoting the malignant progression of NPC. MiRNA-19a-3p is closely linked to tumor progression and autophagy[16,17]. Besides, Zhu et al[18] found enhanced serum level of miRNA-19a-3p in rectal cancer patients. We believed that miRNA-19a-3p might be an excellent hallmark for monitoring the progression of NPC.

PDCD5 exerts the regulation on cytokine-dependent apoptosis[19]. When cells undergo apoptotic

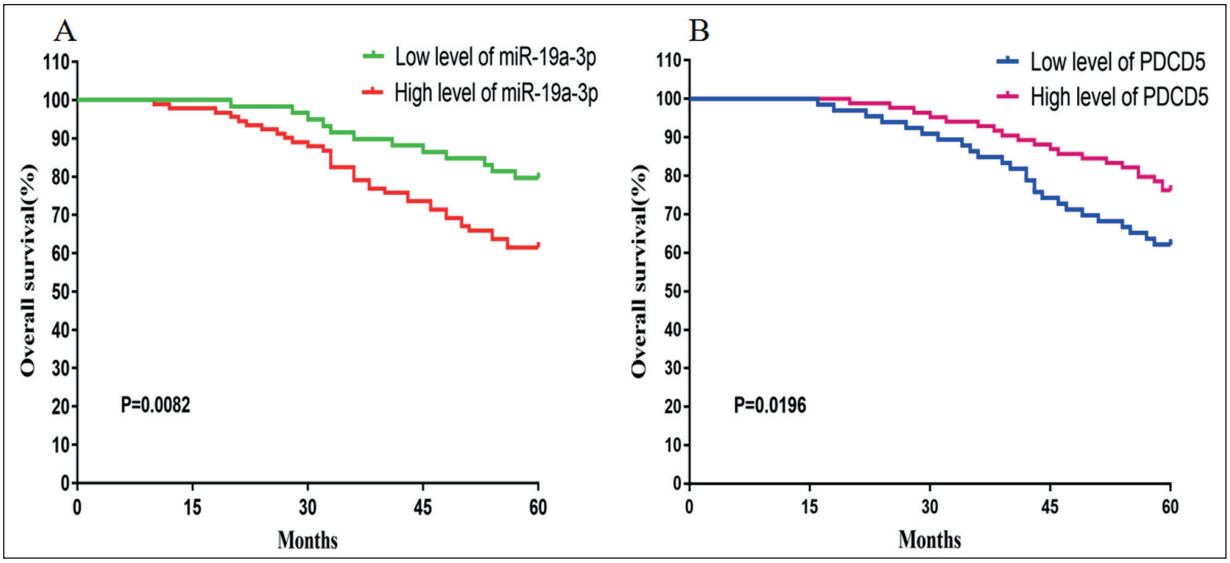

Figure 3. Potential influences of miRNA-19a-3p and PDCD5 on survival of NPC. **A**, Survival analysis in NPC patients based on miRNA-19a-3p level (HR=13.48, $p<0.001$). **B**, Survival analysis in NPC patients based on PDCD5 level (HR=6.716, $p=0.0096$).

Table II. Cox regression analysis on factors influencing survival of NPC.

Variable	HR (95% CI)	p
N classification (N0+1, N2+3)	1.944 (1.031-2.549)	0.022
Clinical stage (I +II, III +IV)	2.081 (1.312-4.148)	0.031
miRNA-19a-3p (Low, High)	2.861 (1.971-4.713)	0.009
PDCD5 (High, Low)	1.911 (1.323-3.165)	0.016

HR=hazard ratios, CI=confidence interval.

process, cytoplasmic PDCD5 rapidly translocates to the nucleus and exerts a pro-apoptotic effect[20]. The role of PDCD5 in human diseases has been extensively studied. Low expression of PDCD5 in ovarian cancer, glioma and prostate cancer is positively associated with tumor grade and prognosis[21,22]. Our findings revealed that PDCD5 was downregulated in NPC tissues. PDCD5 level was correlated with T classification, pathological grade, and clinical stage in NPC patients. Overall survival of NPC was negatively correlated with PDCD5 level. Qiao et al[13] have reported that miRNA-19a-3p can regulate PDCD5 level in gastric cancer, which promotes the malignant progression and predicts a poor prognosis. In this analysis, a negative correlation was identified between the expression levels of miRNA-19a-3p and PDCD5 in NPC tissues.

Conclusions

Collectively, miRNA-19a-3p is upregulated and PDCD5 is downregulated in NPC tissues. They were closely linked to prognosis of NPC patients, and can be utilized as prognostic hallmarks.

Conflict of Interests

The authors declare that they have no conflict of interest.

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References

1) Yu WM, Hussain SS. Incidence of nasopharyngeal carcinoma in Chinese immigrants, compared with Chinese in China and South East Asia: review. J Laryngol Otol 2009; 123: 1067-1074.
2) Levendag PC, Keskin-Cambay F, de Pan C, Idzes M, Wildeman MA, Noever I, Kolkman-Deurloo IK, Al-Mamgani A, El-Gantiry M, Rosenblatt E, Teguh DN. Local control in advanced cancer of the nasopharynx: is a boost dose by endocavitary brachytherapy of prognostic significance? Brachytherapy 2013; 12: 84-89.
3) Du T, Zamore PD. MicroPrimer. The biogenesis and function of microRNA. Development 2005; 132: 4645-4652.
4) Inui M, Martello G, Piccolo S. MicroRNA control of signal transduction. Nat Rev Mol Cell Biol 2010; 11: 252-263.
5) Fuziwara CS, Kimura ET. Insights into regulation of the miR-17-92 cluster of miRNAs in cancer. Front Med (Lausanne) 2015; 2: 64.
6) Wu Q, Yang Z, An Y, Hu H, Yin J, Zhang P, Nie Y, Wu K, Shi Y, Fan D. MiR-19a/b modulate the metastasis of gastric cancer cells by targeting the tumour suppressor MXD1. Cell Death Dis 2014; 5: e1144.
7) Qiao F, Gong P, Song Y, Shen X, Su X, Li Y, Wu H, Zhao Z, Fan H. Downregulated PITX1 Modulated by miR-19a-3p promotes cell malignancy and predicts a poor prognosis of gastric cancer by affecting transcriptionally activated PDCD5. Cell Physiol Biochem 2018; 46: 2215-2231.
8) Liu H, Wang Y, Zhang Y, Song Q, Di C, Chen G, Tang J, Ma D. TFAR19, a novel apoptosis-related gene cloned from human leukemia cell line TF-1, could enhance apoptosis of some tumor cells induced by growth factor withdrawal. Biochem Biophys Res Commun 1999; 254: 203-210.
9) Dobson AT, Raja R, Abeyta MJ, Taylor T, Shen S, Haqq C, Pera RA. The unique transcriptome through day 3 of human preimplantation development. Hum Mol Genet 2004; 13: 1461-1470.
10) Li H, Wang Q, Gao F, Zhu F, Wang X, Zhou C, Liu C, Chen Y, Ma C, Sun W, Zhang L. Reduced expression of PDCD5 is associated with high-grade astrocytic gliomas. Oncol Rep 2008; 20: 573-579.
11) Spinola M, Meyer P, Kammerer S, Falvella FS, Boettger MB, Hoyal CR, Pignatiello C, Fischer R, Roth RB, Pastorino U, Haeussinger K, Nelson MR, Dierkesmann R, Dragani TA, Braun A. Association of the PDCD5 locus with lung cancer risk and prognosis in smokers. J Clin Oncol 2006; 24: 1672-1678.
12) Yang YH, Zhao M, Li WM, Lu YY, Chen YY, Kang B, Lu YY. Expression of programmed cell death 5 gene involves in regulation of apoptosis in gastric tumor cells. Apoptosis 2006; 11: 993-1001.
13) Qiao F, Gong P, Song Y, Shen X, Su X, Li Y, Wu H, Zhao Z, Fan H. Downregulated PITX1 modulated by miR-19a-3p promotes cell malignancy and predicts a poor prognosis of gastric cancer by affecting transcriptionally activated PDCD5. Cell Physiol Biochem 2018; 46: 2215-2231.

14) Ferris RL, Blumenschein GJ, Fayette J, Guigay J, Colevas AD, Licitra L, Harrington K, Kasper S, Vokes EE, Even C, Worden F, Saba NF, Iglesias DL, Haddad R, Rordorf T, Kiyota N, Tahara M, Monga M, Lynch M, Geese WJ, Kopit J, Shaw JW, Gillison ML. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. N Engl J Med 2016; 375: 1856-1867.
15) Wellenstein MD, de Visser KE. Cancer-cell-intrinsic mechanisms shaping the tumor immune landscape. Immunity 2018; 48: 399-416.
16) Li Y, Luo T, Wang L, Wu J, Guo S. MicroRNA-19a-3p enhances the proliferation and insulin secretion, while it inhibits the apoptosis of pancreatic beta cells via the inhibition of SOCS3. Int J Mol Med 2016; 38: 1515-1524.
17) Zou M, Wang F, Gao R, Wu J, Ou Y, Chen X, Wang T, Zhou X, Zhu W, Li P, Qi LW, Jiang T, Wang W, Li C, Chen J, He Q, Chen Y. Autophagy inhibition of hsa-miR-19a-3p/19b-3p by targeting TGF-beta R II during TGF-beta1-induced fibrogenesis in human cardiac fibroblasts. Sci Rep 2016; 6: 24747.
18) Zhu M, Huang Z, Zhu D, Zhou X, Shan X, Qi LW, Wu L, Cheng W, Zhu J, Zhang L, Zhang H, Chen Y, Zhu W, Wang T, Liu P. A panel of microRNA signature in serum for colorectal cancer diagnosis. Oncotarget 2017; 8: 17081-17091.
19) Liu H, Wang Y, Zhang Y, Song Q, Di C, Chen G, Tang J, Ma D. TFAR19, a novel apoptosis-related gene cloned from human leukemia cell line TF-1, could enhance apoptosis of some tumor cells induced by growth factor withdrawal. Biochem Biophys Res Commun 1999; 254: 203-210.
20) Chen Y, Sun R, Han W, Zhang Y, Song Q, Di C, Ma D. Nuclear translocation of PDCD5 (TFAR19): an early signal for apoptosis? FEBS Lett 2001; 509: 191-196.
21) Li H, Wang Q, Gao F, Zhu F, Wang X, Zhou C, Liu C, Chen Y, Ma C, Sun W, Zhang L. Reduced expression of PDCD5 is associated with high-grade astrocytic gliomas. Oncol Rep 2008; 20: 573-579.
22) Du YJ, Xiong L, Lou Y, Tan WL, Zheng SB. Reduced expression of programmed cell death 5 protein in tissue of human prostate cancer. Chin Med Sci J 2009; 24: 241-245.