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前列腺癌非编码 RNA1 在前列腺癌诊断与鉴别诊断中的价值

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[摘　要]　**目的：**探讨前列腺癌(prostate cancer，PCA)非编码RNA1(PRNCR1)在PCA早期诊断及与前列腺增生(benign prostate hyperplasia，BPH)鉴别诊断中的价值。**方法：**选取血清PSA水平增高的65例患者为研究对象，其中29例确诊PCA患者，31例BPH患者，5例PCA术后患者。应用实时荧光定量聚合酶链反应(quantitative polymerase chain reaction，qRT-PCR)检测血清中PRNCR1表达水平，分析PRNCR1在不同组别中的表达差异，探究其在PCA诊断中的临床应用价值。**结果：**PRNCR1在PCA组表达水平明显高于BPH组(P<0.05)，PCA组与PCA术后组表达差异无统计学意义(P>0.05)。ROC曲线分析结果显示：PSA的曲线下面积为0.808，敏感度为82.8%，特异度为61.3%；PRNCR1的曲线下面积为0.810，敏感度65.5%，特异度74.2%；两者联合诊断曲线下面积为0.899，敏感度为75.9%，特异度为96.8%。**结论：**PRNCR1在PCA中表达水平明显增高，提示PRNCR1在PCA诊断中具有潜在价值。

[关键词]　前列腺癌；前列腺癌非编码RNA1；qRT-PCR；诊断；鉴别诊断

Role of non-coding RNA1 in the diagnosis and differential diagnosis of prostate cancer

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Abstract　**Objective:** To evaluate the value of PRNCR1 in early diagnosis of prostate cancer and differential diagnosis of prostate hyperplasia. **Methods:** Sixty-five patients with elevated serum PSA levels were selected as the study subjects, including 29 patients with confirmed prostate cancer (PCA), 31 patients with prostate hyperplasia (BPH), and 5 patients with postoperative prostate cancer. Real-time fluorescence quantitative polymerase chain reaction (qRT-PCR) was used to detect the expression level of PRNCR1 in serum, analyze the expression difference of PRNCR1 in different groups, and explore its clinical application value in the diagnosis of prostate cancer. **Results:** The expression level of PRNCR1 in the PCA group was significantly higher than that in the BPH group (P<0.05), and there was no statistically significant difference between the prostate cancer group and the prostate cancer

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postoperative group (P>0.05). The ROC curve analysis results showed that the area under the PSA curve was 0.808, with a sensitivity of 82.8% and a specificity of 61.3%. The area under the PRNCR1 curve was 0.810, with a sensitivity of 65.5% and a specificity of 74.2%. The area under the combined diagnosis curve was 0.899, the sensitivity was 75.9%, and the specificity was 96.8%. **Conclusion:** PRNCR1 expression significantly increases in the prostate cancer, suggesting the potential value of PRNCR1 in the diagnosis of PCA.

Keywords prostate cancer; prostate cancer non-coding RNA1; qRT-PCR; diagnosis; differential diagnosis

近年来，在男性泌尿系统肿瘤疾病中，前列腺癌(prostate cancer，PCA)的发病率和病死率呈递增趋势[1]，欧美国家PCA发病率更是居于男性恶性肿瘤首位[2]。PCA患者早期临床症状不明显，出现明显临床表现时，多已至中晚期。早期PCA患者预后良好，5年生存率高达100%，而发生远端转移的患者预后较差，5年生存率仅为28%[3-4]。因此PCA早期诊断无论对于治疗还是预后的改善均具有重要意义。PCA特异性抗原(prostate specific antigen，PSA)作为早期诊断PCA的重要指标，虽广泛应用于临床，但由于干扰因素众多而缺乏肿瘤特异性[5]。故临床急需发展一种更有价值的PCA早期诊断新型标志物。最近有研究[6]发现：在8号染色体长臂2区4带“基因沙漠”区域存在一段长约13 kb的lncRNA，且在PCA组织与细胞中高表达，故被命名为PCA非编码RNA1(PRNCR1)。本研究旨在通过检测PRNCR1在PCA与前列腺良性疾病中的表达水平观察其临床意义。

1 对象与方法

1.1 对象

收集2017年10月至2018年8月在徐州医科大学附属医院泌尿外科经病理活检确诊的患者血清标本。其中PCA组29例，年龄50~89(72.4±8.3)岁；前列腺增生(benign prostate hyperplasia，BPH)组31例，年龄50~89(72.2±8.3)岁；PCA术后组5例，年龄66~82(75.0±6.3)岁。为排除其他因素对lncRNA表达的影响，本试验研究对象均未患有其他疾病。本研究中所有患者知情同意并获得徐州医科大学附属医院医学伦理委员会批准。所有收集到的临床血清标本在实验前均保存于−80 ℃。

1.2 主要仪器与试剂

TRIzol RNA分离试剂购自美国Thermo Fisher公司；TonkBio第一链合成试剂盒购自上海同科生物科技有限公司；Peqlab* PerfectSpin 24R小型台式冷冻离心机购自奥然科技有限公司；热循环PCR仪购自美国Bio-Rad公司；SMA1000超微量紫外分光光度计购自美林恒通技术有限责任公司；TaqMan Gene Expression Master Mix，ABI 7500实时定量PCR仪购自美国Applied Biosystems公司。

1.3 方法

1.3.1 RNA 提取

将存放于−80 ℃的血清样本室温溶解，取患者血清250 μL置于无酶EP管中，采用TRIzol试剂盒提取样本总RNA，测吸光度值。qRT-PCR对RNA进行反转录生成cDNA(反应总体系为10 μL)。反转录反应条件为42 ℃ 15 min，85 ℃ 5 s，之后保持12 ℃。

1.3.2 qRT-PCR

对扩增后的cDNA进行qRT-PCR检测，以1 μL cDNA为模板，总反应体系20 μL。反应条件如下：95 ℃孵育10 min，95 ℃ 15 s，60 ℃ 1 min，45个循环。每个样本重复3次，以*PRNCR1*为目的基因，*β-actin*为内参基因，所有反应在ABI 7500实时定量PCR仪上完成。按照已知PRNCR1，*β-actin*基因序列，采用Oligo 7设计目的引物，并通过Primer Blast进行同源性比较分析，确定其可行性与特异性，引物由中国Sangon Biotech公司合成(表1)。

表1 PRNCR1和β-actin的引物序列

Table 1 Primer sequences of PRNCR1 and β-actin

基因	引物序列
PRNCR1	上游：5'-CCAGATTCCAAGGGCTGATA-3'
	下游：5'-GATGTTTGGAGGCATCTGGT-3'
β-actin	上游：5'-GAAATCGTGCGTGACATTAA-3'
	下游：5'-AAGGAAGGCTGGAAGAGTG-3'

采用$2^{-\Delta\Delta Ct}$方法计算PRNCR1的相对定量表达水平，ΔCt = Ct(目的) – Ct(内参)，ΔΔCt = 实验组ΔCt – 对照组ΔCt。

1.4 统计学处理

采用SPSS 16.0统计软件进行数据分析。采用两独立样本*t*检验；绘制受试者工作曲线(receiver operating characteristic，ROC)，对PRNCR1诊断PCA的价值进行评估，并报告曲线下面积。以*P*<0.05为差异具有统计学意义。

2 结果

2.1 一般资料比较

3组年龄差异无统计学意义(*P*>0.05)。PCA组与BPH组，术后组PSA水平差异均具有统计学意义(*P*<0.05，表2)。

表2 一般资料比较

Table 2 Comparison of general information

组别	*n*	年龄/岁	PSA/(ng·mL^{-1})
PCA组	29	72.4 ± 8.3	21.5 ± 24.9
BPH组	31	72.2 ± 8.3	2.2 ± 2.6
术后组	5	75.0 ± 6.3	12.8 ± 24.3
P		>0.05	<0.05

2.2 PRNCR1 表达水平比较

PCA组PRNCR1表达水平(5.55±10.78)较BPH组(0.63±0.68)明显提高，差异有统计学意义(*P*<0.05)。PCA组(1.10±2.13)与术后组(0.94±1.76)差异无统计学意义(*P*>0.05)。

2.3 ROC 曲线分析

ROC曲线分析结果显示：PRNCR1能够作为鉴别PCA和BPH的较好指标。PSA区分PCA和BPH的曲线下面积为0.808(95%CI 0.699~0.917，*P*<0.001)，敏感度为82.8%，特异度为61.3%(图1)；PRNCR1区分PCA和BPH的曲线下面积为0.810(95%CI 0.705~0.916，*P*<0.001)，敏感度65.5%，特异度74.2%(图2)；PSA联合PRNCR1的曲线下面积为0.899(95%CI 0.821~0.977，*P*<0.001)，敏感度为75.9%，特异度为96.8%(图3)。

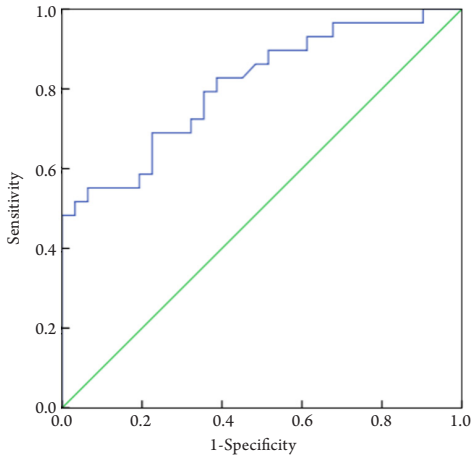

图1 PSA诊断PCA的ROC曲线分析

Figure 1 ROC curve analysis of PSA diagnosis of prostate cancer

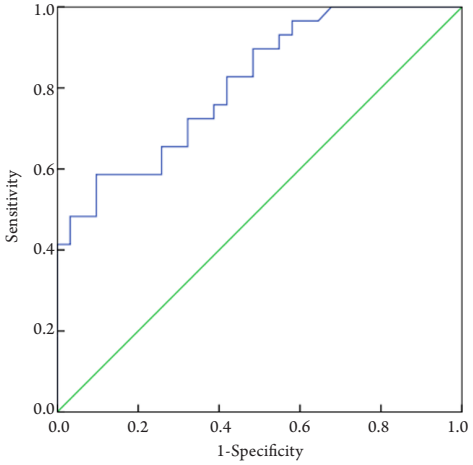

图2 PRNCR1诊断PCA的ROC曲线分析

Figure 2 ROC curve analysis of PRNCR1 diagnosis of prostate cancer

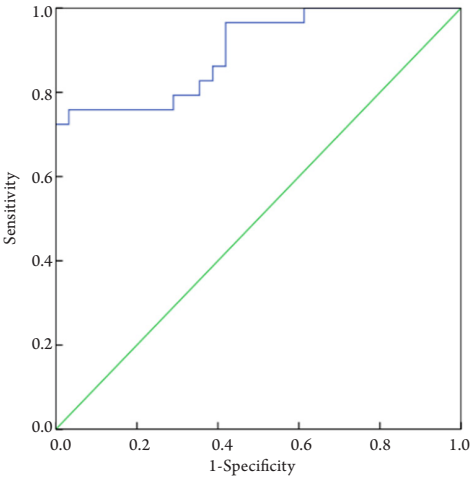

图3 PSA联合PRNCR1诊断PCA的ROC曲线分析

Figure 3 ROC curve analysis of PSA combined with PRNCR1 for diagnosis of prostate cancer

3 讨论

LncRNA是近些年用于肿瘤早期诊断的新型标志物。大量研究[7-10]报道证实：lncRNA与细胞增殖、分化、凋亡密切相关，且通过调控基因表达促进肿瘤发生发展。有研究[11]报道：lncRNA主要通过3种机制促进肿瘤发生发展，包括致癌基因或其调节因子、启动子、抑癌基因。目前PCA的早期诊断主要依赖于PSA[12]，但因其缺乏PCA特异性[13]，前列腺良性疾病也可引起血清PSA水平升高，确诊部分PCA仍需进行前列腺穿刺活检，因此临床迫切需要一种前列腺特异性新型标志物。

有研究[14-16]报道：PRNCR1在消化道恶性肿瘤中表达增高。蔡毅[14]研究发现：通过siRNA下调胃癌细胞中PRNCR1的表达，可抑制癌细胞的增殖和侵袭转移能力。Li等[15]证实PRNCR1中的单核苷酸多态性(single nucleotide polymorphisms，SNPs)与胃癌发生的风险相关，提示PRNCR1中的SNPs可能是胃癌病因学的生物标志物。Yang等[16]报道PRNCR1在结直肠癌组织与细胞中表达明显上调。上述研究结果提示PRNCR1具备新型肿瘤标志物潜能。

目前有关PRNCR1在PCA中的表达及其临床价值报道较少。有学者[17]通过聚合酶链反应-限制性片段长度多态性进行基因分型测定，发现伊朗人群中PCA患者PRNCR1异常表达，其中PRNCR1 rs13252298，rs1456315和rs7841060多态性与伊朗人群中PCA风险增加显著相关。刘红等[18]对45例PCA患者和88例BPH患者进行PRNCR1表达水平比较，结果PRNCR1检测灵敏度为84.4%(38/45)，特异度为90.9%(80/88)。本研究通过qRT-PCR检测PCA，BPH，PCA术后患者血清中PRNCR1的相对表达水平，结果显示：PCA组与PCA术后组差异无统计学意义，PCA组血清中PRNCR1表达显著增高，而BPH组表达相对较低，这表明PRNCR1具有较高的癌特异性。这与上述研究结果一致。

王丽娟等[19]应用qRT-PCR检测C4-2细胞中PRNCR1的表达情况，以RNA干扰技术沉默PRNCR1，进而用蛋白印迹法技术检测细胞中AR的变化，用MTT实验检测对细胞增殖的影响。结果发现PRNCR1在C4-2细胞中高表达，可明显增加PCA细胞中AR的表达，促进癌细胞增殖。以上研究提示PRNCR1很可能成为临床PCA早期诊断的新型标志物。本研究通过ROC曲线分析PSA、PRNCR1区分PCA和BPH的价值，结果PSA诊断的灵敏度和特异度分别为82.8%和61.3%，AUC为0.810，这表明PSA诊断PCA虽具有较高的灵敏度但特异性差。PRNCR1诊断PCA的敏感性和特异性分别为65.5%和74.2%，AUC为0.810。相对于PSA来说，PRNCR1具有更高的特异度，但灵敏度比PSA略差，临床应用其诊断PCA的价值不大。但本研究的入组样本有限，关于PRNCR1早期诊断PCA的价值，还有待进一步探究[20]。

本研究利用ROC曲线进一步分析PSA联合PRNCR1诊断PCA的价值，结果AUC为0.899，敏感度为75.9%，特异度为96.8%。这些结果表明PSA和PRNCR1联合诊断具有较好的灵敏度和特异性，对于PCA筛查、PCA诊断与鉴别诊断具有较高的临床价值。

参考文献

1. De Groat WC, Griffiths D, Yoshimura N. Central neural control of the lower urinary tract[J]. Compr Physiol, 2015, 5(1): 327-396.
2. Ferlay J, Shin H, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008[J]. Int J Cancer, 2010, 127(12): 2893-2917.
3. Wu JN, Fish KM, Evans CP, et al. No improvement noted in overall or cause-specific survival for men presenting with metastatic prostate cancer over a 20-year period[J]. Cancer, 2014, 120(6): 818-823.
4. Humphreys MR, Fernandes KA, Sridhar SS. Impact of age at diagnosis on outcomes in men with castrate-resistant prostate cancer (CRPC)[J]. J Cancer, 2013, 4(4): 304-314.
5. 钟婉媚, 杨其运, 黎桂源, 等. PSA对前列腺癌诊断价值(附833例病例分析)[J]. 实用医学杂志, 2018, 34(21): 3655-3656.
ZHONG Wanmei, YANG Qiyun, LI Guiyuan, et al. Diagnostic value of PSA for prostate cancer (analysis of 833 cases)[J]. Journal of Practical Medicine, 2018, 34(21): 3655-3656.
6. Chung S, Nakagawa H, Uemura M, et al. Association of a novel long non-coding RNA in 8q24 with prostate cancer susceptibility[J]. Cancer Sci, 2011, 102(1): 245-252.
7. Esteller M. Non-coding RNAs in human disease[J]. Nat Rev Genet, 2011, 12(12): 861-874.
8. Gibb EA, Brown CJ, Lam WL. The functional role of long non-coding RNA in human carcinomas[J]. Mol Cancer, 2011, 10: 38.
9. Novikova IV, Hennelly SP, Sanbonmatsu KY. Sizing up long noncoding RNAs: do lncRNAs have secondary and tertiary structure?[J]. Bioarchitecture, 2012, 2(6): 189-199.
10. Spitale RC, Tsai MC, Chang HY. RNA templating the epigenome: long

noncoding RNAs as molecular scaffolds[J]. Epigenetics, 2011, 6(5): 539-543.

11. Bensen JT, Xu Z, Smith GJ, et al. Genetic polymorphism and prostate cancer aggressiveness: a case-only study of 1,536 GWAS and candidate SNPs in African-Americans and European-Americans[J]. Prostate, 2012, 73(1): 11-22.
12. Hoskin PJ, Rojas AM, Ostler PJ, et al. Quality of life after radical radiotherapy for prostate cancer: longitudinal study from a randomised trial of external beam radiotherapy alone or in combination with high dose rate brachytherapy[J]. Clin Oncol (R Cool Radiol), 2013, 25(5): 321-327.
13. Zhang L, Ji G, Li X, et al. fPSA/tPSA ratio optimize the early diagnosis is in the prostatecancer[J]. Chin J Androl, 2004, 10(8): 582-585.
14. 蔡毅. 长链非编码RNA PRNCR1对胃癌细胞增殖、侵袭及转移的研究[J]. 中国现代医学杂志, 2017, 27(12): 35-39.
CAI Yi. Long non-coding RNA PRNCR1 promotes proliferation, invasion and migration of gastric cancer cells[J]. Chinese Journal of Modern Medicine, 2017, 27(12): 35-39.
15. Li L, Jia F, Bai P, et al. Association between polymorphisms in long non-coding RNA PRNCR1 in 8q24 and risk of gastric cancer[J]. Tumor Biol, 2016, 37(1): 299-303.
16. Yang L, Lin C, Jin C, et al. LncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs[J]. Nature, 2013, 500(7464): 598-602.
17. Sattarifard H, Hashemi M, Hassanzarei S, et al. Association between genetic polymorphisms of long non-coding RNA PRNCR1 and prostate cancer risk in a sample of the Iranian population[J]. Mol Clin Oncol, 2017, 7(6): 1152-1158.
18. 刘红, 陈益荣. PRNCR1在前列腺癌诊断及预后的价值[J]. 国际泌尿系统杂志, 2019, 39(1): 10-12.
LIU Hong, CHEN Yirong. The value of PRNCR1 in the diagnosis and prognosis of prostate cancer[J]. International Journal of Urology and Nephrology, 2019, 39(1): 10-12.
19. 王丽娟, 史圣甲, 王乐, 等. 前列腺癌非编码RNA1在雄激素非依赖去势抵抗型前列腺癌细胞中的作用[J]. 细胞与分子免疫学杂志, 2013, 29(8): 789-793.
WANG Lijuan, SHI Shengjia, WANG Le, et al. Role of PRNCR1 in the castration resistant prostate cancer[J]. Chinese Journal of Cellular and Molecular Immunology, 2013, 29(8): 789-793.
20. 徐枫, 赵雄碧, 娄坚江, 等. 不同肿瘤标志物在前列腺癌中的诊断价值[J]. 浙江临床医学, 2018, 20(8): 1435-1436.
XU Feng, ZHAO Xiongbi, LOU Jianjiang, et al. Diagnostic value of different tumor markers in prostate cancer[J]. Zhejiang Clinical Medical Journal, 2018, 20(8): 1435-1436.

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