BRITISH JOURNAL OF BIOMEDICAL SCIENCE
2018, VOL. 75, NO. 3, 145–148
https://doi.org/10.1080/09674845.2018.1424499

Taylor & Francis
Taylor & Francis Group

BIOMEDICAL SCIENCE IN BRIEF

Serum relaxin-2 as a novel biomarker for prostate cancer

Y Xu[a], Q Yu[b] and Y Liu[a]

[a]Department of Urology, The Central Hospital of Linyi, Yishui, China; [b]Department of Urology, The Affiliated Hospital of Qingdao University, Qingdao, China

ARTICLE HISTORY Received 6 November 2017; Accepted 29 December 2017

KEYWORDS Relaxin-2; prostate cancer; prostate-specific antigen

Prostate cancer is a common neoplasia in men and its incidence continues to rise in many countries. Some patients never experience symptoms or disease progression, whereas others will have a rapid progression to a life-threatening disease [1]. Prostate-specific antigen (PSA) testing has revolutionarily improved early prostate cancer detection [2]. The PSA level at diagnosis is associated with tumour volume, stage and Gleason grade, and in combination with other factors can risk-stratify patients upon diagnosis [3]. However, the use of PSA has resulted in significant prostate cancer over-diagnosis [4], since elevated levels are often detected in non-malignant conditions such as benign prostatic hyperplasia (BPH). Therefore, there is a need for improved biomarkers.

Relaxin is a short circulating peptide hormone that enhances the motility of sperm in semen [5]. Two highly homologous genes (*RLN1*and *RLN2)*, on human chromosome 9 encode relaxin-1 and relaxin-2 peptides with 82% identity at amino acid level. Relaxin-2 is produced in the prostate by males [6] and corpus lutea in females [7]. *RLN1* is a pseudogene that does not translate a functional peptide. Thus, the major stored and circulatory form of relaxin in humans is relaxin-2, and so is the only circulating form of relaxin detected in peripheral blood.

Elevated relaxin-2 serum concentrations are present in metastatic breast cancer [8], oesophageal squamous cell carcinoma and osteosarcoma, correlate with disease metastasis and short survival rate [9,10], and may be a clinically useful indicator for diagnostic and prognostic evaluation in ovarian cancer [11]. A previous study has reported that relaxin mRNA expression is significantly higher in recurrent and low-grade prostatic intraepithelial neoplasia lesions prostate cancer samples [12], but a role for circulating relaxin-2 has not been reported. In the present study, we hypothesized increased serum relaxin-2 in prostate cancer that is linked to tumour pathology and outcome.

We prospectively recruited 131 consecutive patients with prostatic adenocarcinoma who were diagnosed and treated at the central hospital of Linyi, Yishui, China. Clinical data were extracted from medical records by study personnel. Operative and pathology reports are obtained by study personnel from the office of the diagnosing physician. From these reports, prostate cancer stage, grade, histologic type, size of tumour and extent of surgical treatment were verified. All patients were staged by digital rectal examinations and transrectal ultrasound for local disease and by bone scanning and CT of the abdomen and pelvis for metastatic disease according to the 2002 American Joint Committee on Cancer (AJCC) TNM classification criteria. All patients had histopathological confirmation of the diagnosis of prostate cancer based on examination of tissues obtained by TRUS-guided prostate biopsy or by TURP. The histologic grade of the tumour was determined using the Gleason's system. Of the 131 patients, 75 had clinically localized prostate cancer and were treated by retropubic radical prostatectomy (RP). The remaining 56 were diagnosed with locally advanced (n = 24) or metastatic prostate cancer (n = 32). The metastatic disease included lymph node metastases in 12 patients, distant metastases to bone in 21 and to the liver in 2. All 56 patients received androgen-deprivation therapy (ADT) as the sole treatment – chemical [i.e. luteinizing hormone-releasing hormone (LHRH) agonists plus antiandrogen agents] in 31 and surgical (i.e. bilateral orchidectomy plus antiandrogen agents) in the remaining 25. No patient had evidence of active infection or inflammatory disease, and none were treated before collecting blood samples. We included two control groups: 66 normal healthy men and 48 men with BPH.

Blood samples were prospectively collected from prostate cancer patients on the morning of the scheduled day of RP or on the morning of the starting day of ADT after a pre-treatment overnight fast. For patients who underwent TRUS-guided needle biopsy of the prostate, the blood samples were drawn at least 4 weeks after the biopsy. The blood samples from 66 normal healthy

CONTACT Y Liu qyfywang@163.com

men were also drawn on the morning of the scheduled day of visiting after an overnight fast. The samples were collected into evacuated tubes, serum was separated within 1 h of blood collection after centrifuging at 3000 rpm for 10 min at room temperature and stored immediately at −80 °C until analysis. Studies were performed after receiving approval from the central hospital of Linyi, Yishui, China. All patients and healthy control gave full written consent for their samples to be stored and used for research.

ELISA kits for total serum PSA and relaxin-2 were purchased from ALPCO Diagnostics (Shanghai, China). Detection limits are > 1 pg/mL for PSA and 0.05 ng/mL for relaxin-2. The intra-assay and inter-assay coefficients of variation were 3.5% and 2.8%, respectively. All data with a normal distribution was presented as mean ± SD. For non-normal distribution, the data were presented as median/IQR (range). Chi-square test or Fisher's exact test were performed to determine the relationship between serum relaxin-2 and clinicopathological parameters. Mann–Whitney U test or the Kruskal–Wallis with Dunn's tests were used to investigate inter-group differences. The Pearson correlation test was applied to evaluate possible correlations between the serum relaxin-2 and PSA. Receiver operating curve (ROC) analysis was performed for evaluation of specificity and sensitivity of serum relaxin-2 and PSA levels for discriminating prostate cancer patients from controls. Diagnostic accuracy for combination of biomarkers was also determined by calculating weight coefficients for every biomarker obtaining the largest possible area under the curve (AUC) in ROC analysis. Overall, survival (OS) was calculated as the interval between surgery and death or last clinical evaluation. Disease-free survival (DFS) was measured from the time of surgery to initial tumour relapse (local recurrence or distant) or death from any cause. Kaplan–Meier curves of DFS and OS were compared by the log-rank test. Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, 13.0). *p*-values < 0.05 were considered statistically significant.

The groups were matched for age: 68.4 ± 6.7 years in patients with prostate cancer, 68.8 ± 7.4 in BPH and 67.3 ± 8.4 in healthy controls ($p = 0.214$). All tumours were acinar-type adenocarcinoma: Gleason score ≤ 7 was present in 72 cases, >7 in 59 cases. Extra-prostatic capsule extension was present in 39, a positive surgical margin in 30, lymphovascular invasion in 12, perineural invasion in 67, seminal vesicle invasion in 14, clinically localized disease in 75, locally advanced disease in 24, lymph node metastases in 12 and distant metastasis in 23.

Levels of relaxin-2 in prostate cancer group, BPH group and healthy control groups were 2.0 (0.2–3.7) ng/ml, 0.4 (0.3–0.7) ng/ml and 0.4 (0.2–0.6) ng/ml, respectively (overall, $p < 0.001$: prostate cancer vs. BPH, $p = 0.004$; prostate cancer vs. healthy, $p = 0.0012$; BPH vs. control, $p = 0.143$). Levels of PSA in prostate cancer group, BPH group and healthy control group were 26 (1–284) ng/ml, 4 (0.8–48) ng/ml and 3 (0.7–36) ng/ml, respectively (overall, $p < 0.001$: prostate cancer vs. BPH, $p = 0.0013$; prostate cancer vs. healthy, $p = 0.0028$; BPH vs. control, $p = 0.287$). Relaxin-2 failed to correlate significantly with age or PSA level ($r = 0.03$, $p = 0.426$ and $r = 0.14$, $p = 0.073$, respectively).

Associations between serum relaxin-2 and clinicopathological characteristics showed that serum relaxin-2 was linked to perineural invasion ($p < 0.001$), distant metastasis ($p = 0.001$) and biochemical recurrence ($p < 0.001$). However, no significant association was observed between serum relaxin-2 and Gleason score ($p = 0.124$), extra-prostatic capsule extension ($p = 0.837$), surgical margin status ($p = 0.132$), seminal vesicle invasion ($p = 0.547$), lymph node metastases ($p = 0.336$) and tumour stage ($p = 0.447$). PSA was linked to tumour stage ($p = 0.001$), lymph node metastases ($p = 0.014$), distant metastasis ($p < 0.001$) and biochemical recurrence ($p = 0.003$). However, no significant association was observed between PSA and surgical margin status ($p = 0.294$), distant metastasis ($p = 0.173$), biochemical recurrence ($p = 0.076$) and Gleason score ($p = 0.254$).

To access whether relaxin-2 can be used as diagnostic biomarker for prostate cancer, we measured the sensitivity and specificity using receiver operating characteristic (ROC) curve analysis. The relaxin-2 had most accurate discrimination (AUC = 0.81, 95% CI: 0.70–0.92, $p = 0.0014$) of cancer patients and BPH control subjects (Figure 1). At the optimal cut-off values of 0.4 ng/ml, sensitivity was 80.4% and specificity was 70.8%. Relaxin-2 outperformed PSA serum levels (AUC = 0.71, 95% CI: 0.61–0.85, $p = 0.012$) with 74.3% sensitivity and 51.4% specificity at a cut-off value of 10 ng/mL (Figure 1). When combining relaxin-2 and PSA as prostate cancer predictors, diagnostic efficiency was not improvement compared to the relaxin-2 alone, with the AUC = 0.83, 95% CI: 0.71–0.93, $p = 0.0012$, sensitivity 86.2%, specificity 88.3% and diagnostic accuracy 91.2% (Figure 1).

The median follow-up period was 45 months (range, 12–148 months). In Kaplan–Meier log-rank test analysis, the patients with relaxin-2 levels ≥ 0.4 ng/ml had shorter OS ($p = 0.004$) (Figure 2) and DFS ($p = 0.003$) than those with levels < 0.4 ng/ml. Cox proportional hazard model confirmed that relaxin-2 (for OS: RR 5.32, 95% CI, 1.38–12.6, $p = 0.014$; for DFS: RR 5.54, 95% CI, 1.33–11.83, $p = 0.013$) was an independent prognostic factor for unfavourable survival.

Despite recent advances in treatment and diagnosis, prostate cancer remains the second leading cause of cancer-related deaths among men. Detecting prostate cancer at earlier curable stages has been facilitated by the measurement of the PSA in serum. Traditionally, PSA values > 4 ng/mL trigger a suspicion for prostate cancer. Men with serum PSA > 10 ng/mL have an increased risk

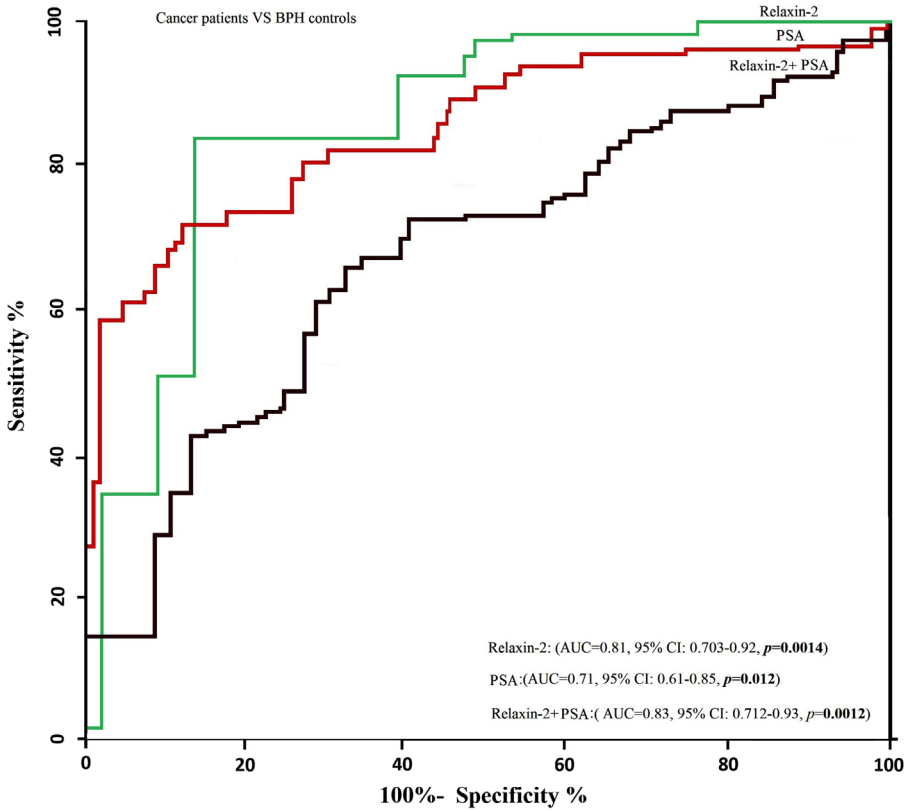

Figure 1. Diagnostic outcomes of serum relaxin-2 in the diagnosis of prostate cancer. ROC curve for serum relaxin-2, PSA and relaxin-2/PSA for patients with prostate cancer vs. BPH.

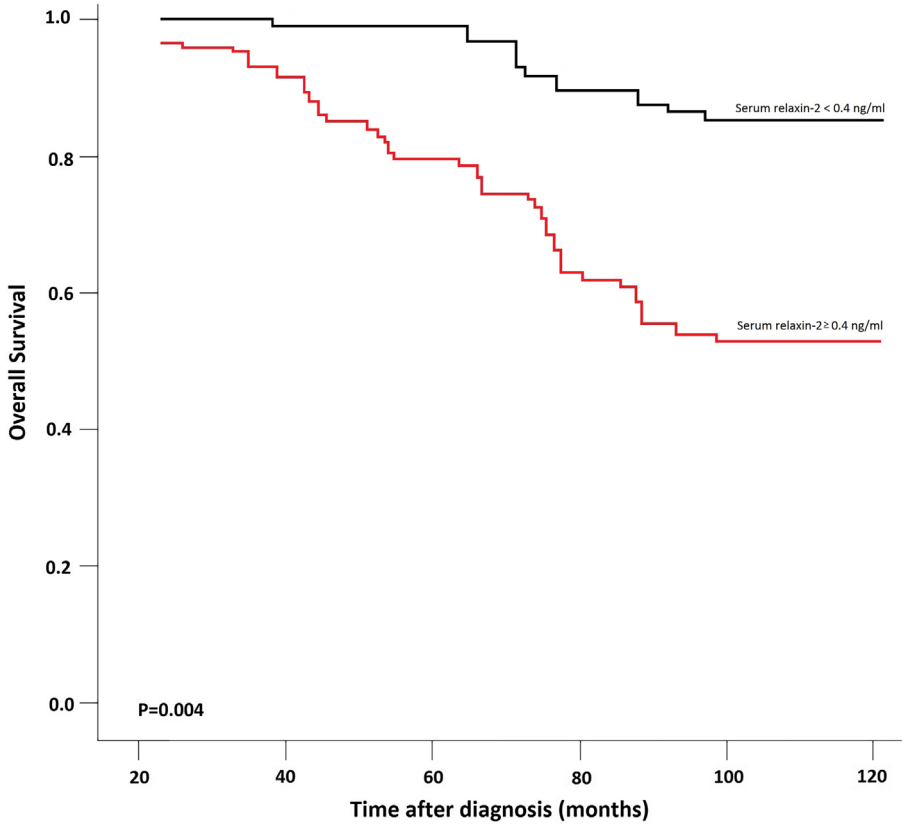

Figure 2. Kaplan–Meier survival analysis of serum relaxin-2 in 131 patients of prostate cancer (log-rank test). Relationship of serum relaxin-2 and patient overall survival ($P = 0.004$).

of non-organ-confined prostate cancer. However, only 30% of men with serum PSA levels between 4 and 10 are diagnosed with prostate cancer and up to 15% of patients with PSA values < 4 ng/mL carry the diagnosis [13]. In some cases of poorly differentiated disease, PSA levels are not significantly elevated. PSA is an imperfect marker for prostate cancer and that additional diagnostic and prognostic tools are needed. In the present study, we found that serum relaxin-2 is significantly higher levels in prostate cancer patients compared to BPH and healthy subjects. Hence, detection of serum relaxin-2 might be used to refine the PSA test to better determine the risk or prognosis of prostate cancer

It has demonstrated that PSA does not distinguish between stages of prostate cancer and, significantly, does not identify metastatic prostate cancer with the sensitivity and specificity required to make accurate therapeutic decisions [14]. It is now evident that the PSA test produces unacceptably high rates of false positive results and is not prognostic. We found that high serum relaxin-2 was associated with perineural invasion, distant metastasis and biochemical recurrence. In addition, serum relaxin-2 has higher accurate discrimination of cancer patients and BPH controls compared to the PSA, suggesting that relaxin-2 could be the better diagnostic biomarker for prostate cancer.

However, because of the limitation of small sample size, such as lymphovascular invasion, seminal vesicle invasion, lymph node metastases, the possible false negative in using relaxin-2 to diagnose prostate cancer may exist. Moreover, because records were not maintained for the purpose of research, there was missing information related to few variables in standard record maintained by hospital. No conclusive finding could be obtained about certain risk factors because of small sample in one of the comparison groups that rendered statistical analysis insignificant. Therefore, large sample size would be used in the future study.

The PSA test is currently the best biomarker for prostate cancer recurrence and it has undoubtedly been partly responsible for the increased awareness of this disease. However, no study to date has proven that screening with PSA reduces prostate cancer mortality. Our study reveals that high serum relaxin-2 has a short survival and bad prognosis for patients with prostate cancer.

This work represents an advance in biomedical science because it shows that serum relaxin-2 could discriminate prostate cancer patients from healthy or BPH controls, so may represent a novel prognostic and diagnostic markers for prostate cancer.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Mitchell RE, Chang SS. Current controversies in the treatment of high-risk prostate cancer. Curr Opin Urol. 2008;18(3):263–268.
[2] Damber JE, Aus G. Prostate cancer. Lancet. 2016; 371(9625):1710–1721.
[3] Lilja H, Ulmert D, Vickers AJ. Prostate-specific antigen and prostate cancer: prediction, detection and monitoring. Nat Rev Cancer. 2008;8:268–278.
[4] Welch HG, Albertsen PC. Prostate cancer diagnosis and treatment after the introduction of prostate-specific antigen screening: 1986–2005. J Natl Cancer Inst. 2009;101(19): 1325–1329.
[5] Sherwood OD. Relaxin's physiological roles and other diverse actions. Endocr Rev. 2004;25:205–234.
[6] Yki-Jarvinen H, Wahlstrom T, Seppala M. Immunohistochemical demonstration of relaxin in the genital tract of men. J Reprod Fertil. 1983;69:693–695.
[7] Shabanpoor F, Separovic F, Wade JD. The human insulin superfamily of polypeptide hormones. Vitam Horm. 2009; 80:1–31.
[8] Binder C, Simon A, Binder L, et al. Elevated concentrations of serum relaxin are associated with metastatic disease in breast cancer patients. Breast Cancer Res Treat. 2014;87(2):157–166.
[9] Ren P, Yu ZT, Xiu L, Wang M, Liu HM. Elevated serum levels of human relaxin-2 in patients with esophageal squamous cell carcinoma. World J Gastroenterol. 2013;19(15):2412–2418.
[10] Ma J, Niu M, Yang W, Zang L, Xi Y.. Role of relaxin-2 in human primary osteosarcoma. Cancer Cell Int. 2013;13(1):1–10.
[11] Guo X, Liu Y, Huang X, Wang Y, Qu J, Lv Y. Serum relaxin as a diagnostic and prognostic marker in patients with epithelial ovarian cancer. Cancer Biomark. 2017;21(1):81–87.
[12] Feng S, Agoulnik IU, Bogatcheva NV, Kamat A., Kwabi-Addo B, Li R, Ayala G, Ittmann MM, Agoulnik AI. Relaxin promotes prostate cancer progression. Clin Cancer Res. 2017;13(6):1695–1702.
[13] Raaijmakers R, Wildhagen MF, Ito K, Paez A, de Vries SH, Roobol MJ, Schroder FH. Prostate-specific antigen change in the European randomized study of screening for prostate cancer, section rotterdam. Urology. 2004;63:316–320.
[14] Hessels D, Schalken JA. Urinary biomarkers for prostate cancer: a review. Asian J Androl. 2013;6:341–346.