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Enhanced expression of SRPK2 contributes to aggressive progression and metastasis in prostate cancer

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ABSTRACT

Serine/Arginine-Rich Protein-Specific Kinase-2 (SRSF protein kinase-2, SRPK2) is up-regulated in multiple human tumors. However, the expression, function and clinical significance of SRPK2 in prostate cancer (PCa) has not yet been understood. We therefore aimed to determine the association of SRPK2 with tumor progression and metastasis in PCa patients in our present study.

The expression of SRPK2 was detected by some public datasets and validated using a clinical tissue microarray (TMA) by immunohistochemistry. The association of SRPK2 expression with various clinicopathological characteristics of PCa patients was subsequently statistically analyzed based on the The Cancer Genome Atlas (TCGA) dataset and clinical TMA. The effects of SRPK2 on cancer cell proliferation, migration, invasion, cell cycle progression, apoptosis and tumor growth were then respectively investigated using in vitro and in vivo experiments.

First, public datasets showed that SRPK2 expression was greater in PCa tissues when compared with non-cancerous tissues. Statistical analysis demonstrated that high expression of SRPK2 was significantly correlated with a higher Gleason Score, advanced pathological stage and the presence of tumor metastasis in the TCGA Dataset (all $P < 0.01$). Similar correlations between SRPK2 and a higher Gleason Score or advanced pathological stage were also identified in the TMA ($P < 0.05$). Kaplan-Meier curve analyses showed that the biochemical recurrence (BCR)-free time of PCa patients with SRPK2 high expression was shorter than for those with SRPK2 low expression ($P < 0.05$). Second, cell function experiments in PCa cell lines revealed that enhanced SRPK2 expression could promote cell proliferation, migration, invasion and cell cycle progression but suppress tumor cell apoptosis in vitro. Xenograft experiments showed that SRPK2 promoted tumor growth in vivo.

In conclusion, our data demonstrated that SRPK2 may play an important role in the progression and metastasis of PCa, which suggests that it might be a potential therapeutic target for PCa clinical therapy.

1. Introduction

Prostate cancer (PCa) has become one of the most frequently diagnosed non-cutaneous tumors and is a great threat to male health. The incidence and mortality of PCa in developed countries, especially America, continuously increased during the last two decades [1]. Upward trends in incidence rates and mortality rates were also observed in Chinese PCa patients [2]. The longevity of the population, impact of an increasingly westernized lifestyle, regular implementation of prostate-specific antigen (PSA) screening and improved tumor biopsy techniques are potential major factors that have driven the increase in cancer. However, to some extent, the above major factors have also led to overdiagnosis and overtreatment. Therefore, PCa is indeed a clinically heterogeneous and multifocal cancer that needs to be addressed [3,4].

There are many treatments for PCa; for example, radical prostatectomy can cure indolent PCa (localized PCa), and endocrine therapy is used to treat advanced PCa. However, a minority of PCa patients will experience biochemical recurrence (BCR) and eventually develop

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castration-resistant PCa (CRPC) or metastasis, which are often fatal [5,6]. Besides cancer carcinogenesis, progression, recurrence, metastasis and castration resistance, the precise mechanism underlying this malignancy has not been fully understood, which make the natural history of PCa difficult to predict [7]. Therefore, it is important to identify more molecular mechanisms underlying PCa progression and metastasis and provide valuable knowledge for preventing disease progression and improving diagnosis.

Serine/Arginine-rich (SR) proteins are pre-mRNA processing proteins that can regulate alternative splicing and have been reportedly associated with splicing dysregulation in various cancers. For example, the prototypical SR protein, alternative splicing factor/splicing factor 2 (ASF/SF2), has been found to be oncogenic and affect cellular transformation in several malignancies such as lung cancer, breast cancer and PCa [8–11]. Furthermore, SR Protein-Specific Kinases (SRSF protein kinases, SRPKs), such as SRPK1 and SRPK2, can phosphorylate ASF/SF and regulate the cell cycle [12–15]. A previous study found that inhibiting SRPK1 could suppressed tumor growth and had subsequent negative effects on tumor angiogenesis by regulating VEGF isoform expression in PCa [16]. For SRPK2, in addition to its major role in cell cycle regulation and apoptosis [14,15], it can also promote cancer cell growth and migration [17]. The functions above indicated that SRPK2 might play an important role in tumor progression and metastasis. Upregulation of SRPK2 has also been observed in leukemia, colon cancer, lung cancer, hepatocellular carcinoma and head and neck squamous cell carcinoma [14,17–20]; however, there are no previous reports on the involvement of SRPK2 in PCa. We therefore aimed to investigate the function and clinical significance of SRPK2 in PCa and to evaluate its roles in tumor progression and metastasis in our present study.

2. Materials and methods

2.1. Cell line culture and transfections

Three PCa cell lines (LNCaP, DU145 and PC3) were purchased from American Type Culture Collection (ATCC, USA) and cultured in 1640 medium (HyClone, USA; Cat.No:SH30809-01B) and DMEM medium (HyClone, USA; Cat.No:SH30022.01B) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA; Cat.10270-106), 100 U/mL penicillin and 100 Ug/mL streptomycin. The cells were placed in a humid incubator containing 5% CO_2 at 37℃. The stable cell line over-expressing SRPK2 and the control cell line were made by HYY Med Company (Guangdong, China) using a lentivirus containing the ORF sequence of SRPK2. Cells that appeared to be growing robustly after lentivirus infection were selected for further experiments in this study.

2.2. Patients and tissue samples

For immunohistochemistry analysis, a tissue microarray (TMA) with samples from 80 cases, including 73 cases of prostate cancer with radical prostatectomy, 1 case of prostate hyperplasia and 6 normal prostate tissue samples, was purchased from Creative Bioarray (USA. Catalog number: PRTMA053). Detailed clinical information was included in this TMA. Patients who had been administered chemotherapy or radiotherapy prior to surgery were all excluded from this study.

For further evaluation of the clinical relevance and survival analysis of SRPK2, a publicly available dataset of mRNA microarray expression data and relevant clinical information was obtained from The Cancer Genome Atlas (TCGA) (GEO: GSE21032, including 498 PCa tissues but no normal prostate tissue).

2.3. Immunohistochemistry

Subcellular localization and protein expression levels of SRPK2 in PCa and benign prostate tissue specimens were detected by immunohistochemistry. A primary antibody against SRPK2 (HPA015522, Sigma-Aldrich Co. LLC, USA) was used. The clinical specimens were instantly fixed in 10% neutral buffered formalin and embedded in paraffin after surgery. Tissues were cut at 5 μm thickness for further H&E or peroxidase (DAB) immunohistochemistry staining. Briefly, following a proteolytic digestion and a peroxidase blocking, the tissue slides were incubated with the primary antibody against SRPK2 at a dilution of 1:150 at 4℃ overnight. After washing the tissue slides, a peroxidase-conjugated secondary antibody and chromogen substrate were employed to visualize the protein staining. Negative controls were carried out by omitting the primary antibody in each immunohistochemistry experiment. Following a hematoxylin counterstaining, the immunostaining score was determined by two independent pathologists who were blinded to the clinicopathological information. The scores were compared, and any discrepant scores were resolved by re-examining the staining to achieve a consensus score. Given the homogenicity of protein staining, tumor specimens were scored in a semi-quantitative assessment. As previous studies have indicated [21,22], the number and percentage of positively stained cells among ten representative microscopic fields were calculated. SRPK2 protein levels were determined by percentage of staining (i.e., 0 (0–5%), 1 (6-25%), 2 (26-50%), 3 (51-75%), and 4 (> 75%)) and intensity of staining (i.e., 0 (negative), 1 (weak), 2 (moderate) and 3 (strong)) in each sample. A final immunoreactivity score (IRS) was obtained by adding the percentage and intensity scores.

2.4. Western blot analysis

The expression levels of the SRPK2 protein in LNCaP, DU145 and PC3 cell lines with transfection of an SRPK2-overexpressing plasmid and corresponding control were detected by Western blot analysis according to our previous reports [21–23]. Proteins (20 μg) were extracted for Western blot analyses 48 hours after transfection. The extracted protein was then isolated by way of SDS-PAGE and transferred onto Hybond nitrocellulose membranes (GE Healthcare, USA). The membrane was shaken in PBST and blocked with 5% skimmed milk and probed with antibody. An anti-SRPK2 antibody (HPA015522, Sigma-Aldrich Co. LLC, USA) was used to detect SRPK2 expression, and an anti-GAPDH antibody (M30008, Abmart, CHINA) was used as an internal loading control. The results were visualized using the Super-Signal West PICO chemiluminescent detection system (Pierce Biotechnology).

2.5. Cell proliferation, migration and invasion assays

According to the protocol of our previous reports [22,23], the effects of SRPK2 on cell proliferation, migration and invasion in LNCaP, DU145 and PC3 cells transfected with an SRPK2-overexpression plasmid and corresponding control cell lines were detected by CCK-8 assay, wound-healing assay and transwell assay.

For the CCK-8 (cell proliferation) assay, cell suspensions of approximately 5×10^3 cells/200 μL were seeded into each well of a 96-well plate. After culturing for 24–72 hours, the cells were incubated with 20 μL of CCK-8 solution (Beyotime, China; Cat No: C0039) for 0-4 hours in an incubator. The absorbance of each cell was measured at 450 nm with a spectrophotometer (Thermo Scientific, MA, USA). For the wound-healing (cell migration) assay, cell suspensions were seeded into each well of a 6-well plate. A scratch on the middle of plates was made with a 10 μL sterile pipette tip when the cells reach approximately 80% confluence. After being incubated with mitomycin for 0-48 hours, the cells that migrated from the wound edge were counted, and representative photos were taken using a phase-contrast microscope. For the transwell (cell invasion) assay, according to our previous studies [22,23], the surface of the upper chamber of the 24-well transwell culture plates (Corning, USA) was previously coated with 20 μL matrigel (BD company) diluted 8-fold with serum-free cell medium. Cell suspensions (5×10^4 cells in 200 μL) were placed in the upper