Pseudogene HSPA7 is a poor prognostic biomarker in Kidney Renal Clear Cell Carcinoma (KIRC) and correlated with immune infiltrates

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Abstract

Background: Pseudogenes played important roles in tumorigenesis, while there are nearly no reports about the expression and roles of HSPA7 in the cancer.

Methods: Firstly, we used Logistic regression, the KS test, the GEPIA database, UALCAN database and qRT-PCR to analyze the expression level of HSPA7 in KIRC, then we used the Cox regression and the Kaplan–Meier curve to analyze the overall survival (OS) of KIRC patients with different Clinico-pathological parameters. Thirdly, we used the multivariate Cox analysis of influencing factors to compare the correlation between the HSPA7 expression level and the clinical parameters. Finally, we used multi-GSEA analysis and the Tumor Immunoassay Resource (TIMER) database to explore the functional role of HSPA7 in KIRC.

Results: The HSPA7 is highly expressed in KIRC tumor tissues, and its expression is related to clinico-pathological features and survival in KIRC patients. GSEA analysis displayed the high expression of HSPA7 in KIRC were related to several tumor-related and immune-related pathways. With the TIMER database analysis we showed that HSPA7 levels were correlated with the CD4+ T cells, neutrophils and Dendritic Cell.

Conclusions: Our study showed that HSPA7 is very important in the tumor progression and may act as a poor prognostic biomarker for KIRC tumor by modulating immune infiltrating cells.

Keywords: Kidney Renal Clear Cell Carcinoma (KIRC), HSPA7, Immune, Prognostic biomarker, Tumor

Introduction

The morbidity of renal cell carcinoma (RCC) is about 4.2% of all newly-appeared cancer cases, which make RCC become one of the most frequent malignances worldwide. According to a recent survey, there were about 73,820 new cases of RCC and 14,770 deaths occurred in United States in 2019 [1]. Kidney Renal Clear Cell Carcinoma (KIRC) is the most common kidney cancer subtype [2]. At present, the primary treatment of KIRC is surgery, while 30% of the patients who underwent surgery still experience metastasis [3], and the currently used drugs are not effective and have relatively great side effects. Early identification and diagnosis of KIRC patients can help with more precise clinical treatment. Therefore, it is urgent to discover new and reliable markers to predict the prognosis of patients.

Pseudogenes are non-coding genes lacking of protein-coding ability, and were once labeled as junk genes. However, there is growing evidence indicated that pseudogenes can influence the regulatory mechanisms of many human cancers and pseudogene expression is treated as a novel marker and used in a variety of cancer
types to stratify patients subtypes [4, 5] and is therefore taken into account in cancer survival prognostic factors. For example, the pseudogene PRELID1P6 can promote glioma progression through the hnHNP-H1-Akt/mTOR pathway [6]. OCT4 abnormally activated pseudogene 5 (OCT4-pg5) can enhance cell proliferation by competing with miR-145 in endometrial carcinoma via upregulating OCT4 expression [7]. High expression of the pseudogene ANXA2P2 has been found to be related to a worse prognosis pseudogene in hepatocellular carcinoma [8]. LDHAP5 was associated with the poor prognosis of ovarian serous cystadenocarcinoma [9]. The Pseudogene HSPA7 (HSP70B) belongs to the HSPA70 family (HSPA), discovered in 1985 and encoded near the highly homologous HSPA6 (HSP70B′) on chromosome 1, although mRNA can be expressed after thermal stimulation, it cannot transcribe a functional protein [10]. Numerous investigations have shown that HSPA6 plays an important role in multiple human cancers, including esophageal cancer [11, 12], glioma [13], lung cancer [14], hepatocellular carcinoma [15] and leukemia [16]. However, little has been reported about the expression and role of HSPA7 in cancer. In this study, we reported that high expression of HSPA7 can indicate the poor prognosis of KIRC.

Our study examined the expression and prognostic value of HSPA7 in KIRC patients in the Cancer Genome Atlas (TCGA) and validated them in multiple independent cohorts. Moreover, GSEA [17] and Tumor Immuno-assay Resource (TIMER) database [18] were used to assay the potential mechanisms of HSPA7 in KIRC. Our results implied that the functional role of HSPA7 in KIRC may through regulating immune cell infiltration.

Methods

Data mining and data collection

The KIRC data of TCGA consists of 72 normal tissues and 539 tumor samples, was acquired from the TCGA data portal (https://tcga-data.nci.nih.gov/tcga/). Clinical data pertaining to patients’ age, gender, survival, grade, stage, and recurred/progressed outcome were also acquired from the data portal. The dataset including mRNA expression counts and survival data with clinical information. The samples with missing expression data were excluded from the study. The dataset contains survival data with clinical information and mRNA expression counts. The samples with missing expression data were excluded from our study.

Data analysis

The R-3.6.2 project was used to analysis the acquired data. Firstly, we used the Logistic regression and the KS test to analyze the relation between the HSPA7 gene expression and Clinico-pathological features. Then we used the Cox regression and the Kaplan–Meier curve to analyze the overall survival of KIRC patients with different Clinico-pathological parameters from TCGA data. Finally, we used the multivariate Cox analysis of influencing factors to compare the correlation between the HSPA7 expression level and the clinical parameters, such as age, gender grade, stage, T classification, N classification, and M classification, related to survival. The Cut-off Finder.2 was used to determine the cut-off value of HSPA7 expression.

Gene set enrichment analysis (GSEA)

Gene Set Enrichment Analysis (GSEA) is a computational method that determines whether an a priori defined set of genes shows statistically significant between two biological expression states [17]. In our study, an ordered list of genes based on the pathways related to the HSPA7 expression level were generated by the GSEA, and then the significant differences between the high and low-level expression groups of HSPA7 were annotated. The multi-GSEA results and signaling pathway enrichment analysis of phenotypes and were ranked by normalized enrichment score (NES) and the nominal p-value.

Analysis of TIMER database

The TIMER (https://cistrome.shinyapps.io/timer/) database is designed for analysing immune cell infiltrates in multiple cancers. This database can estimate tumour immune infiltration by macrophages, dendritic cells, CD4/CD8+ T cells, neutrophils, and B cells [19]. We used the TIMER database to assess the HSPA7 differential expression levels in particular tumours, and then we explored the correlation between HSPA7 expression level and the degree of infiltration in particular immune cell subsets. We further explored the differences in patient survival as a function of gene expression or immune cell infiltration by Kaplan–Meier curve analyses.

Analysis of GEPIA and UALCAN database

The GEPIA (http://gepia.cancer-pku.cn/index.html) database and UALCAN (http://ualcan.path.uab.edu) database can explore the association of mRNA expression level with overall survival (OS). We used these two database to explore the correlation between the HSPA7 expression and patient overall survival in KIRC.

RNA extraction and qRT-PCR analysis

A total of 20 primary KIRC cancer tissues was collected from patients who had undergone surgery at the First Affiliated Hospital of Nanjing Medical University and the Second Affiliated Hospital of Nanjing Medical University. The study was approved by the Ethics Committee of Nanjing Medical University (Nanjing, Jiangsu, PR China),
and it was performed in compliance with the Declaration of Helsinki Principles. The clinical information of the 20 KIRC patients was shown in Additional file 1: Table S1). Written informed consent was obtained for all patient samples. RNA extraction and qRT-PCR of the KIRC cancer tissues were performed as the product manual described (Cat# R312-01, Cat# Q131-02, Vazyme, China). The primers used in this study are purchased from Genery (Shanghai, China) and listed as follows.

HSPA7-R: CATCCCAAAGGTGCAGAAGT;  
HSPA7-F: ACCATCCCTCCACCTCCT;  
GAPDH-R: GGGAGCCAAAAGGGTCAT;  
GAPDH-F: GAGTCCCTCCACGATAACAA.

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**Results**

**Characteristics of the of the patients**

537 patients’ clinical data were acquired from TCGA, including the age, gender, Histological grade, TNM classification of KIRC (Table 1).

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**High HSPA7 mRNA expression in KIRC**

First, we assessed the differences in HSPA7 expression between KIRC tumor tissues and adjacent tissues via differential expression scatter plots and paired difference analyses. We find that the expression level of HSPA7 was significantly higher in KIRC tumor tissues ($p = 6.183 \times 10^{-35}$) and in paired cancer tissues ($p = 3.311 \times 10^{-18}$) compared with adjacent tissues (Fig. 1A, B). Then, the expression level of HSPA7 in KIRC tumor tissues and adjacent tissues were verified by GEPIA [20] (Fig. 1C) database, UALCAN database (Fig. 1D) [21] and qRT-PCR analysis (Fig. 1E). The clinical data of 20 patients’ used in qRT-PCR were shown in Additional file 1: Table S1.

**Correlation between HSPA7 expression level and clinico-pathological features in KIRC tumors**

As the Table 2 shown the expression of HSPA7 was highly statistically significantly correlated with clinical stage ($p = 0.044$) and distant metastasis (positive vs. negative, $p = 0.049$).

**Correlation between KIRC patients survival and HSPA7 expression**

To evaluate the effect of HSPA7 expression on KIRC patients survival, the log-rank test and Kaplan–Meier survival analysis were used to estimate the correlation between HSPA7 expression and KIRC patients prognosis. The patients with high HSPA7 expression level displayed relatively poor survival ($p = 1.176 \times 0.04$; Fig. 2A). The clinical subgroup analysis implied that the patients in Histological grade (G1–2 vs. G3–4), clinical stage (Stage I vs. Stage IV), M classification and T classification (the T1-2 vs. T3-4) with HSPA7 expression also had significantly poor overall survival (OS) (Fig. 2B–E), whereas not in the N classification (Fig. 2F). We performed the univariate analysis with the variables and listed in the Table 3. We also performed Multivariate analysis with the Cox proportional hazards model and the results implied that the expression of HSPA7 (HR = 1.304605, $p = 0.005187$) is a potential prognostic factor for KIRC patients (Table 4). Then we performed the forest plot analysis (Fig. 3), the outcome of KIRC patients are statistically significant correlation with age ($p < 0.001$), histological grade ($p = 0.002$), clinical stage ($p = 0.019$) and the expression of HSPA7 ($p < 0.001$). In conclusion, HSPA7 is a reliable and effective independent prognostic biomarker of KIRC patients.

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**Table 1 Clinical characteristics of TCGA KIRC patients (n = 537)**

| Clinical characteristic | Total (537) | %   |
|-------------------------|-------------|-----|
| Age                     |             |     |
| < 60                    | 247         | 46.00|
| ≥ 60                    | 290         | 54.00|
| Gender                  |             |     |
| Female                  | 190         | 35.38|
| Male                    | 347         | 64.62|
| Histologic grade        |             |     |
| G1–2                    | 244         | 45.44|
| G3–4                    | 285         | 53.07|
| Gx                      | 5           | 0.93 |
| NA                      | 3           | 0.56 |
| Stage                   |             |     |
| I–II                    | 326         | 60.71|
| III–IV                  | 208         | 38.73|
| NA                      | 3           | 0.56 |
| T classification        |             |     |
| T1–2                    | 344         | 64.06|
| T3–4                    | 193         | 35.94|
| N classification        |             |     |
| N0                      | 240         | 44.69|
| N1                      | 17          | 3.17 |
| Nx                      | 280         | 52.14|
| M classification        |             |     |
| M0                      | 426         | 79.33|
| M1                      | 79          | 14.71|
| Mx                      | 30          | 5.59 |
| NA                      | 2           | 0.37 |
| Vital status            |             |     |
| Deceased                | 170         | 31.66|
| Living                  | 367         | 68.34|

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**Fig. 1** HSPA7 is overexpressed in KIRC. A) HSPA7 mRNA expression in KIRC based on TCGA Data. B) Paired difference analysis of HSPA7 mRNA expression in KIRC based on TCGA Data. C) HSPA7 protein expression comparison between normal and tumor tissues obtained from the UALCAN web tool. D) HSPA7 protein expression comparison between normal and tumor tissues obtained from the GEPIA expression in KIRC based on TCGA Data. E) HSPA7 expression was inspected by qRT-PCR and normalized to GAPDH expression in human KIRC tissues compared with corresponding non-tumor tissues (n=20) (*p < 0.05, log-rank test)

**Table 2** Correlation between the clinicopathologic characteristics and HSPA7 mRNA expression (logistic regression)

| Clinical characteristics                  | Total (N) | Odds ratio in HSPA7 expression | p-value |
|------------------------------------------|-----------|--------------------------------|---------|
| Age (≥ 60 vs. < 60)                       | 537       | 0.985 (0.700–1.386)            | 0.931   |
| Grade (G1–2 vs G3–4)                      | 529       | 1.185 (0.840–1.673)            | 0.334   |
| Stage (I–IV)                              | 349       | 1.678 (1.018–2.794)            | 0.044   |
| Local invasion (T1–2 vs. 3–4)             | 445       | 1.017 (1.002–1.032)            | 0.205   |
| Distant metastasis (positive vs. negative)| 505       | 1.636 (1.005–2.697)            | 0.049   |
| Lymph nodes (positive vs. negative)        | 257       | 1.008 (0.360–2.825)            | 0.987   |

Bold values indicate statistically significant, p < 0.05
HSPA7-related signaling pathway was identified by GSEA

The differentially regulated pathways between high and low expression of HSPA7 groups were identified by GSEA and then the activated signaling pathways in KIRC were founded. The results with significant differences in enrichment (FDR < 0.25, NOM p < 0.05) in the MSigDB gene set (c2.cp.kegg.v6.2.symbols.gmt) were selected based on the NES and listed in Table 5. Figure 4 showed that renin angiotensin system, primary immunodeficiency, O-glycan biosynthesis, JAK-STAT signaling pathway, hematopoietic cell lineage, intestinal immune network for IgA production, glycerophospholipid metabolism, cytokine-cytokine receptor interaction, cytosolic DNA sensing pathway, autoimmune thyroid disease and asthma. GSEA analysis displayed
the high expression of HSPA7 in KIRC were related to several tumor- and immune-related pathways.

**HSPA7 expression correlated with immune cell infiltration in KIRC**

Previous studies showed that lymph node metastasis and survival are independently predicted by the frequency of lymphocytes infiltrating in cancer patients. Also GSEA analysis displayed the high expression of HSPA7 in KIRC were related to immune-related pathways. Using TIMER database we investigated whether HSPA7 expression was correlated with six main infiltrating immune cells in KIRC. The result implied that expression of HSPA7 associated with CD4⁺ T cells (r=0.395, p-value=1.24e−18),

**Table 3** Univariate cox regression of overall survival and clinicopathologic characteristics in TCGA KIRC patients

| Clinical characteristics (univariate cox regression) | Hazard ratio | HR (95% CI) | p-value |
|-----------------------------------------------------|--------------|-------------|---------|
| Age                                                 | 1.022636     | 1.004910–1.040674 | 0.012106 |
| Gender                                              | 1.013224     | 0.666050–1.541361 | 0.951059 |
| Grade                                               | 2.242118     | 1.682289–2.988246 | 3.61E−08 |
| Stage                                               | 1.862243     | 1.540797–2.250751 | 1.26E−10 |
| T                                                    | 1.943172     | 1.537502–2.455879 | 2.69E−08 |
| M                                                    | 4.073388     | 2.635544–6.300444 | 2.76E−10 |
| HSPA7                                               | 1.046542     | 1.017951–1.075936 | 0.001287 |

Bold values indicate statistically significant, p < 0.05

**Table 4** Multivariate analyses of overall survival and clinicopathologic characteristics in TCGA KIRC patients

| Clinical characteristics (univariate cox regression) | Hazard ratio | HR (95% CI) | p-value |
|-----------------------------------------------------|--------------|-------------|---------|
| Age                                                 | 1.031079     | 1.011672–1.050858 | 0.001594 |
| Gender                                              | 1.329053     | 0.843200–2.094854 | 0.220453 |
| Grade                                               | 1.439203     | 1.025090–2.020609 | 0.035457 |
| Stage                                               | 1.485765     | 0.894490–2.467884 | 0.126193 |
| T                                                    | 0.962168     | 0.600494–1.541677 | 0.872618 |
| M                                                    | 1.700401     | 0.758832–3.810280 | 0.197200 |
| HSPA7                                               | 1.304605     | 1.082694–1.572000 | 0.005187 |

Bold values indicate statistically significant, p < 0.05

Fig. 3 Forest map analysis of expression and clinicopathologic characteristics
**Table 5** GSEA identifies an HSPA7-related signaling pathway

| NAME                                                      | NES   | NOM p-val | FDR q-val |
|-----------------------------------------------------------|-------|-----------|-----------|
| KEGG_O_GLYCAN_BIOSYNTHESIS                               | 2.17  | 0.00      | 0.02      |
| KEGG_RENIN_ANGIOTENSIN_SYSTEM                            | 2.21  | 0.00      | 0.02      |
| KEGG_ALDOSTERONE_REGULATED_SODIUM_REABSORPTION           | 1.92  | 0.00      | 0.09      |
| KEGG_PROXIMAL_TUBE_BICARBONATE_RECLAMATION              | 1.93  | 0.00      | 0.09      |
| KEGG_ARRHYMOMEGIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC  | 1.87  | 0.01      | 0.10      |
| KEGG_TIGHT_JUNCTION                                      | 1.89  | 0.01      | 0.10      |
| KEGG_STEROID_BIOSYNTHESIS                               | 1.94  | 0.00      | 0.11      |
| KEGG GLYCOLYSIS_GLUCONEOGENESIS                          | 1.85  | 0.02      | 0.11      |
| KEGG_CITRATE_CYCLE_TCA_CYCLE                            | 1.95  | 0.00      | 0.12      |
| KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS            | 1.81  | 0.01      | 0.12      |
| KEGG_VALINE_LEUCINE_AND_ISO LEUCINE_DEGRADATION          | 1.79  | 0.04      | 0.12      |
| KEGG_N_GLYCAN_BIOSYNTHESIS                              | 1.73  | 0.04      | 0.13      |
| KEGG_PYRUVATE_METABOLISM                                | 1.77  | 0.04      | 0.13      |
| KEGG_THYROID_CANCER                                     | 1.71  | 0.03      | 0.13      |
| KEGG_PROPA NOATE_METABOLISM                             | 1.79  | 0.04      | 0.13      |
| KEGG_VIBRIO_CHOLERAE_INFECTION                          | 1.73  | 0.05      | 0.13      |
| KEGG_ADHERENS_JUNCTION                                  | 1.97  | 0.02      | 0.13      |
| KEGG_SPHINGOLIPID_METABOLISM                            | 1.73  | 0.02      | 0.14      |
| KEGG_PENTOSE_PHOSPHATE_PATHWAY                          | 1.74  | 0.00      | 0.15      |
| KEGG_VASOPRESSIN_REGULATED_WATER_REABSORPTION           | 1.65  | 0.04      | 0.16      |
| KEGG_MELANOMA                                           | 1.61  | 0.03      | 0.18      |
| KEGG_LONG_TERM_DEPRESSION                               | 1.56  | 0.03      | 0.19      |
| KEGG_GLYCEROPHOSPHOLIPID_METABOLISM                     | 2.26  | 0.00      | 0.01      |
| KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION       | 2.15  | 0.01      | 0.03      |
| KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY                      | 2.12  | 0.00      | 0.03      |
| KEGG_ASTHMA                                              | 2.05  | 0.01      | 0.03      |
| KEGG_AUTOIMMUNE_THYROID_DISEASE                         | 2.05  | 0.01      | 0.03      |
| KEGG_JAK_STAT_SIGNALING_PATHWAY                         | 2.01  | 0.00      | 0.04      |
| KEGG_PRIMARY_IMMUNODEFICIENCY                           | 2.00  | 0.01      | 0.04      |
| KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION             | 2.00  | 0.00      | 0.03      |
| KEGG_HEMATOPOIETIC_CELL_LINEAGE                        | 1.95  | 0.01      | 0.04      |
| KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY          | 1.92  | 0.01      | 0.05      |
| KEGG_HOMOLOGOUS_RECOMBINATION                           | 1.90  | 0.01      | 0.05      |
| KEGG_RIG_1 LIKE RECEPTOR_SIGNALING_PATHWAY               | 1.87  | 0.02      | 0.06      |
| KEGG_FC_EPSILON_RI_SIGNALING_PATHWAY                    | 1.82  | 0.02      | 0.07      |
| KEGG_NOD_LIKE RECEPTOR_SIGNALING_PATHWAY                 | 1.81  | 0.03      | 0.07      |
| KEGG_ALLOGRAFT_REJECTION                                | 1.80  | 0.04      | 0.07      |
| KEGG_SNARE_INTERACTIONS_IN_VESICULAR_TRANSPORT          | 1.77  | 0.02      | 0.08      |
| KEGG_ALPHA_LINOLENIC_ACID_METABOLISM                    | 1.76  | 0.00      | 0.08      |
| KEGG_GNRH_SIGNALING_PATHWAY                             | 1.75  | 0.01      | 0.08      |
| KEGGETHER_LIPID_METABOLISM                              | 1.70  | 0.01      | 0.09      |
| KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM              | 1.67  | 0.04      | 0.09      |
| KEGG_ABC_TRANSPORTERS                                   | 1.61  | 0.03      | 0.11      |
| KEGG_LINOLEIC_ACID_METABOLISM                           | 1.59  | 0.03      | 0.12      |
| KEGG_HEDGEHOG_SIGNALING_PATHWAY                         | 1.53  | 0.05      | 0.14      |
| KEGG_ARACHIDONIC_ACID_METABOLISM                        | 1.50  | 0.04      | 0.14      |
| KEGG_VEGF_SIGNALING_PATHWAY                             | 1.49  | 0.04      | 0.14      |
Family can play a causal role in cancer initiation. Evi-
pancreatic cancer cells [29] and serve as a molecular
proliferation. HSPA8 could regulate the cell viability in
[16] and baldder cancer’s [28] migration, invasion and
found associated with the lung cancer [14], leukemia
ing cell apoptosis [27]. The expression of HSPA6 were
ANXA1 and repress PSAT1 expression, which inhib-
with HSPA2 can promote tumor growth and progres-
station and activating IGF1Rβ [25]. RNF144A interacted
cell-like properties via regulating β-Catenin transcrip-
dence showed that HSPA1L can enhance cancer stem
[23, 24]. Accumulating data indicated that HSP70
HSPA12A, HSPA12B HSPA9, HSPA13 and HSPA14
HSPA1L, HSPA2, HSPA5, HSPA6, HSPA7, HSPA8,
family is composed of about 13 members, including
HSP70 which is transcribed in response to stress, but now sug-
that immune infiltration may serve as a important
role in KIRC patient outcomes, and HSPA7 could modu-
le immun infiltrating cells into KIRC tissues.

Discussion
Our study first reported that pseudogene HSPA7 was
expressed highly in KIRC patients and can predict a poor
prognosis. We showed that the up-regulated HSPA7 had
statistical correlation with histological grade, clinical
stage, M classification, T classification and overall sur-
vival in KIRC.

HSPA7 belongs to the heat shock protein 70 (HSP70)
family, has long been considered as being a pseudogene
which is transcribed in response to stress, but now sug-
st as a high homology to HSPA6 [22]. The HSP70
family is composed of about 13 members, including
HSPA1L, HSPA2, HSPA5, HSPA6, HSPA7, HSPA8,
HSPA12A, HSPA12B HSPA9, HSPA13 and HSPA14
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ing cell apoptosis [27]. The expression of HSPA6 were
found associated with the lung cancer [14], leukemia
[16] and baldder cancer’s [28] migration, invasion and
proliferation. HSPA8 could regulate the cell viability in
pancreatic cancer cells [29] and serve as a molecular
target in human hepatocellular carcinoma [30]. Over-
expression of HSPA12A can suppresses renal carci-
noma cell migration while promotes hepatocellular
carcinoma growth [31]. Overexpression of HSPA12B
induce cisplatin resistance in non-small-cell lung
cancer (NSCLC) [32]. HSPA9 is associating with sur-
vival and proliferation of thyroid carcinoma cells [33,
34]. Less information is available for HSPA7, HSPA13
and HSPA14 representing more distally related mem-
ers of the HSP70 family. In our research we explored
that highly-expressed HSPA7 is related to clinicopatho-
logical features of KIRC. Most importantly, univariate
and multivariate Cox analyse demonstrated that HSPA7
expression is an independent prognostic indicator of
KIRC survival and may be a promising biomarker for
clinical applications. Through GSEA analysis, we found
that the high expression of HSPA7 in KIRC may related
to several immune pathways. HSPA7 expression was
found to correlate with the degree of immune infiltra-
in KIRC through the TIMER database. Knowledge
of the immune components has increased over the past
decade. Several studies have reported that immune cells
from infiltrating tumors are capable of acting as tumor
suppressors or promoters in the tumor microenvi-
ronment. CD8+ T cells were reported to correlate with the
improved survival of cancer patients [35, 36], while regu-
latory T cells and tumor-associated macrophages were
correlated with the promotion of tumor development
[37, 38]. Few studies have shown that the HSP70 family
members can serve as immunes signature for prognosis
of cancers [11]. And the role of Hsp70 in cell immune
modulation has remained contentious, only several
studies have shown that the HSP70 family members
may related to the cell immune. For example, HSPA2 is
related to the responses of bone marrow derived den-
dritic cells to LPS [39], HSPA8 is central at different key
steps in the presentation of peptide antigens to CD4+
T cells, with a potential to regulate T and B cell acti-
vation and the final secretion of antibodies by plasma
cells [40]. HSPA13 is critical for plasma cells develop-
ment and may be a new target for eliminating patho-
logic plasma cells [41]. Our research showed that the
expression of HSPA7 was significant correlated with
macrophage, CD4+ T cells, neutrophils and dendritic
cell infiltrating. With the subsequent Kaplan–Meier
analysis we found that CD4+ T cells and macrophage
cells can predict the KIRC patients prognosis.

Conclusions
In summary, we explored that the pseudogenes HSPA7
is highly expressed in KIRC tumors and is correlated
with tumor survival and progression. We implied
that the expression level of HSPA7 was moderately
Fig. 5  HSPA7 expression is correlated with the level of immune infiltration in KIRC. A HSPA7 expression is correlated with the level of immune infiltration in KIRC. B Kaplan–Meier plots of immune infiltration and HSPA7 expression levels in KIRC.
positively associated with degree of macrophage, neutrophil, CD4+ T cells and DC infiltration, and weakly positively correlated with the degree of B cells and CD8+ T cells infiltration in KIRC tumor tissues. The pseudogene are believed as therapeutic targets or potential prognostic markers for KIRC tumor patients, while the detailed mechanism of pseudogene affect the KIRC patients prognosis is still to be explored.

**Abbreviations**

KIRC: Kidney Renal Clear Cell Carcinoma; HSPA7: Heat Shock Protein Family A (Hsp70); Member 7; TIMER: Tumor Immunomassay Resource; RCC: Renal cell carcinoma; TCGA: The Cancer Genome Atlas; GSEA: Gene Set Enrichment Analysis; NES: Normalized enrichment score; OS: Overall survival; DC: Dendritic cell.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12935-021-02141-1.

**Additional file 1: Table S1.** Clinical characteristics of KIRC patients (n = 20).

**Acknowledgements**

Not applicable.

**Authors' contributions**

CD and RH collected and analyzed the data and were the major contributors to writing the original draft of the manuscript. ZD and JW designed the experiment and analyzed the data. ZD and JW reviewed and edited the manuscript. JZ coordinated the study. All authors have agreed to the published version of the manuscript. All authors read and approved the final manuscript.

**Funding**

Not applicable.

**Availability of data and materials**

The data generated or analyzed during this study are included in this article, or if absent are available from the corresponding author upon reasonable request.

**Declarations**

**Ethics approval and consent to participate**

A total of 20 primary KIRC cancer tissues was collected from patients who had undergone surgery at the First Affiliated Hospital of Nanjing Medical University and the Second Affiliated Hospital of Nanjing Medical University. The study was approved by the Ethics Committee of Nanjing Medical University (Nanjing, Jiangsu, PR China), the approval number from the Ethical Committee is 2016-SRFA-011, and it was performed in compliance with the Declaration of Helsinki Principles. Written informed consent was obtained for all patient samples.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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Received: 11 May 2021 Accepted: 10 August 2021

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