



# The Prognosis Value of PSPC1 Expression in Nasopharyngeal Cancer


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**Background:** Paraspeckle component 1 (PSPC1) is overexpressed in various cancer and correlated with poor survival in the patients. However, little is known about its expression and role in the progression of nasopharyngeal carcinomas (NPC). The purpose of this study is to examine PSPC1 expression in NPC and explore its role in clinical prognosis of radiation therapy.

**Methods:** The association of PSPC1 expression with clinicopathological features of 109 NPC patients was examined using partial correlation analysis. Cancer tissues were obtained prior to clinical treatment. All cases were diagnosed and pathologically confirmed to be poorly differentiated or undifferentiated NPC without distant metastasis. The patients were then treated with radiation and followed-up. Survival analysis was performed.

**Results:** Partial correlation analysis revealed that the PSPC1 expression in NPC was correlated with N classification, recurrence, prognosis and radiosensitivity in NPC patients, but not with the gender, age, pathohistological pattern, clinical stage, and T classification. The overexpression of PSPC1 was detected in 64 samples (58.72%). Kaplan–Meier survival analysis revealed that the overall survival (OS) was longer in NPC patients with PSPC1 low expression than that in those with PSPC1 high expression. Moreover, patients with the overexpression of PSPC1 had a low progression-free survival and distant metastasis-free survival rate, compared to those who had a low expression of PSPC1. Although not statistically significant, patients with high expression of PSPC1 had a lower locoregional recurrence-free survival rate than those with low expression, and the curves between the two groups was well separated.

**Conclusion:** PSPC1 overexpression was associated with poor prognosis for NPC, which might be a novel useful biomarker to predict the response of NPC to radiation therapy and its clinical outcome.

**Keywords:** nasopharyngeal carcinoma, paraspeckle component 1, PSPC1, clinical prognosis, radiation, overexpression

## Introduction

Nasopharyngeal carcinoma (NPC) is the most common primary malignancy in the nasopharynx<sup>1</sup> and highly prevalent in southern China, reaching 20 cases per 100,000 people.<sup>2,3</sup> The radiotherapy is currently the first choice of treatment of NPC.<sup>4,5</sup> With the development of novel radiotherapy, such as intensity modulated radiation therapy (IMRT), the regional control rate of NPC with IMRT has reached nearly 90%, with relatively satisfying progression-free survival (PFS) and overall survival (OS) for newly diagnosed patients with early stages.<sup>6–9</sup> Unfortunately, about 20% of the patients suffer from radio-resistance and

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recurrence,<sup>10</sup> the leading cause of NPC treatment failure.<sup>11,12</sup> The mechanisms underlying NPC radioresistance have not been completely understood.<sup>13</sup> There is no specific biomarker that could precisely predict the radiosensitivity for NPC.<sup>14</sup>

Paraspeckles are nuclear bodies, which located in the interchromatin space of the cell nucleus near to speckles.<sup>15</sup> These bodies are mainly composed of three proteins, including paraspeckle component 1 (PSPC1), splicing factor glutamine- and proline-rich, (PSF) and non-POU-domain containing octamer-binding protein (p54nrb), and the scaffolding long noncoding RNA Neat1.<sup>16</sup> Knockdown of PSPC1 does not prevent paraspeckle formation, but PSF and p54nrb do.<sup>17</sup>

PSPC1, a paraspeckle biomarker identified in an aberrant 13q12.11 locus, plays a role in DNA damage,<sup>18</sup> RNA editing,<sup>19</sup> adipocytes differentiation<sup>20</sup> and gene regulation.<sup>21–23</sup> PSPC1 upregulation has been identified in several cancers, including hepatocellular carcinoma,<sup>24</sup> lung cancer and breast cancer.<sup>16</sup> It has been confirmed that PSPC1 overexpression commonly occurred in the higher stage of liver, breast and lung cancers and associated with poor patient prognosis.<sup>16</sup> However, the expression level of PSPC1 and its clinical value in NPC have not yet been investigated. Here, we examined the PSPC1 expression in tumor samples obtained from NPC patients prior to treatment, and conducted a retrospective study to assess the value of PSPC1 expression as a novel biomarker in the NPC prognosis and response to radiation therapy.

## Materials and Methods

### Antibodies and Reagents

Anti-PSPC1 polyclonal antibody was purchased from Abcam (cat#: ab184123, Cambridge, USA). Elivision™ super HRP (Mouse/Rabbit) IHC kit (cat#: Kit-9922), EDTA buffer (pH 9.0) (cat#: MVS-0099) and Antibody Diluent buffer (cat#: ABD-0030) were purchased from Maixin (Fuzhou, China).

### Ethical Statement

This study was approved by the Ethical Review Committee of Fujian Cancer Hospital. Written informed consent with a detailed description of the purpose of the study was obtained from all participants. All subjects involved in this study agreed to publish related demographic and clinical features. All procedures were conducted in accordance with the Helsinki declaration. All

experimental procedures described in this study were conducted in accordance with national and local laws, regulations and guidelines.

## Samples and Patients

The studied cohort includes 109 paraffin-embedded NPC samples from patients (92 males and 17 females, with a median age of 47 years old, ranged 15–89) diagnosed between April and November in 2012 in Fujian Cancer Hospital, Fuzhou, Fujian China. All cases were clinically diagnosed and confirmed pathologically as poorly differentiated or undifferentiated primary NPC without distant metastasis at the time of the diagnosis. Pretreatment evaluation consisted of a detailed patient history and physical examination, whole blood counts, flexible fiberoptic endoscopic examination, urinalysis, blood chemistries, electrocardiogram, chest X-ray, bone emission computed tomography (ECT) scans, magnetic resonance imaging (MRI) scans of the head and neck, and ultrasound of liver and abdominal lymph nodes. CT scans and Positron emission tomography (PET) scans of the abdomen/chest were performed when clinically recommended. All cases were reclassified according to the 2017 American Joint Committee on Cancer (AJCC, 8th edition). All tumor samples were obtained by biopsy prior to radiotherapy or chemoradiation therapy. At the same time, 9 normal nasopharyngeal epithelia tissues as control.

## Immunohistochemical Analysis

Immunohistochemical (IHC) analysis was performed to examine the expression levels of PSPC1 protein. After deparaffinization, the tissue sections were immersed in ethylenediaminetetraacetic acid (EDTA) buffer (pH 9.0) and subjected to 121 °C treatment for 1.5 min for antigen retrieval. The samples were subsequently immersed in 3% H<sub>2</sub>O<sub>2</sub> for 10 min to block endogenous peroxidase, and then incubated with anti-PSPC1 primary antibody diluted in antibody diluent buffer at 4°C overnight. The slides were processed using an Elivision™ super Horseradish Peroxidase (HRP) (Mouse/Rabbit) IHC kit (cat#: Kit-9922, Maixin) according to the manufacturer's protocol, and counterstained using haematoxylin.

The expression level of PSPC1 was scored by assessing both percentage of positive cell population and staining intensity. Immunohistochemically stained sections were scored independently by two pathologists blinded to the clinical parameters. The immunoreactive scores of

PSPC1 staining were calculated as reported previously.<sup>25</sup> The staining intensity was scored as: no staining (score 0), light-yellow (score 1), yellowish-brown (score 2) and brown (score 3). Percentage of positive cells was graded as following: < 5% (score 0), 5–25% (score 1), > 25–50% (score 2), > 50–75% (score 3) and > 75% - 100% (score 4). The combination (IHC score) of the intensity score and percentage score was used to represent the expression level of PSPC1 in the tissues. According to the IHC score, the expression level of PSPC1 is divided into - (negative), + (weak), ++ (moderate), +++ (strong). Finally, we defined IHC score -, + as low level of PSPC1 expression (Low PSPC1 group) and ++, +++ as high level of the expression (High PSPC1 group).

## Radiotherapy

All patients were treated primarily with IMRT according to an IRB-approved institutional treatment protocol.<sup>26</sup> The nodal masses and gross volumes (GTV) of primary tumors were obtained by MRI and/or CT. The high-risk clinical tumor volume (CTV) included the GTV plus 5–10 mm margin, encompassing the entire nasopharyngeal mucosa plus 5 mm sub-mucosal volume. CTV for potentially involved regions included the posterior part of pterygopalatine fossae, parapharyngeal space, nasal cavity, posterior ethmoid sinus, maxillary sinuses, skull base, cervical vertebra and anterior third of the clivus, inferior sphenoid sinus and the retropharyngeal lymph nodal and cavernous sinuses and regions from the base of skull to the cranial edge of the second cervical vertebra. CTV of the neck nodal regions were outlined according to the recommendations by the consensus CTV delineation protocol for head and neck malignancies. An additional 3-mm margin was added to create the planning tumor volume (PTV). Spinal cord, brainstem, optic nerves, parotid glands, chiasm, globes, lens, temporomandibular joints, temporal lobes, pituitary gland and mandible were contoured and set as organs at risk during optimization. Cumulative doses of the primary lesion ranged from 69.2 to 75.25 Gy.<sup>27</sup>

## Efficacy Evaluation

Efficacy was evaluated according to RECIST (Response Evaluation Criteria in Solid Tumors) V.1.1 criteria (<https://recist.eortc.org/recist-1-1-2/>). The patients with complete remission (CR) or partial remission (PR) after radiotherapy were classified as RS-NPC, and the patients with stable disease (SD) and disease progression (PD) as RR-NPC.

## Patient Follow-Up

All cases in this study have been followed up with established protocol till June 2016 or decease. The median follow-up time was 45.7 months (range, 2–51 months). After the completion of the radiotherapy, patients were followed up at 3-month intervals during the first 2 years, 6-month intervals from the 2nd year until the end of the fifth year, and annually thereafter or till the patients deceased. The follow-up includes flexible fiber optic endoscopy, ultrasound of liver and abdomen, chest X-ray and basic serum chemistry. Either CT and/or MRI of the head and neck was performed after the completion of treatment and then every 6 months.

## Statistical Analysis

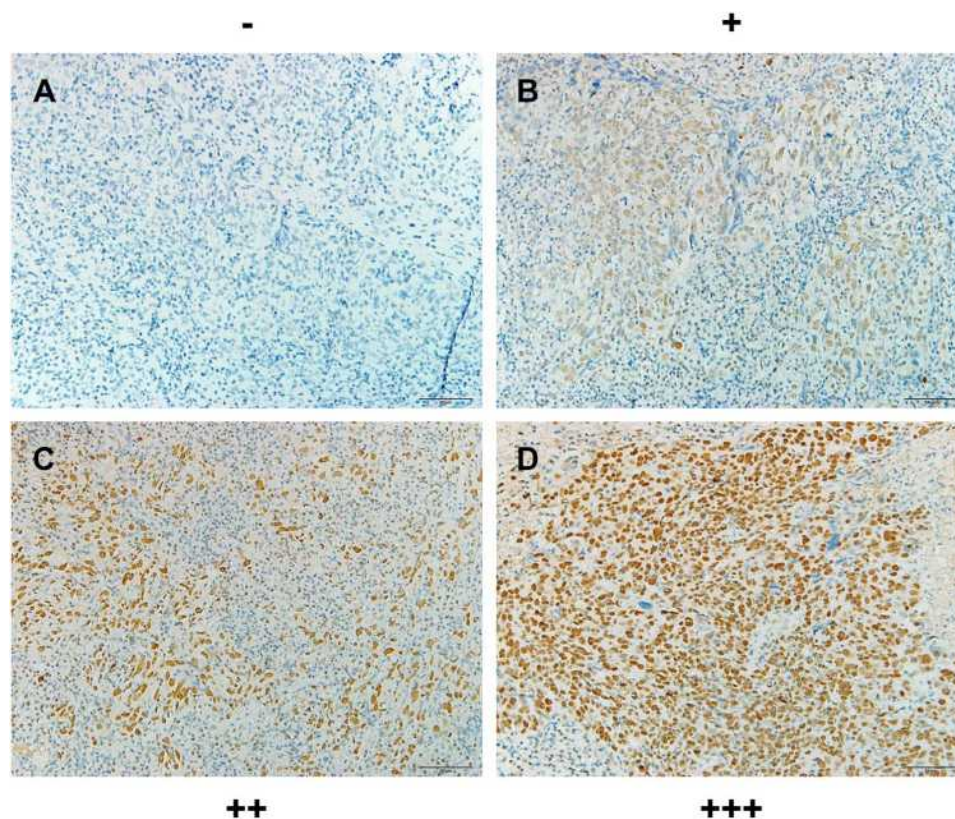
All statistical analyses were performed using the statistical software SPSS version 18.0 (SPSS, Inc.; Chicago, IL, USA). Comparisons of the distributions of socio-demographic and clinical stages of different PSPC1 expression groups were calculated by chi-square test. The overall survival (OS) time, progression-free survival (PFS) time, distant metastasis-free survival (DMFS) time and locoregional recurrence-free survival (LRRFS) time was estimated with the Kaplan-Meier method and compared using the Log rank test. Multivariate analysis using the Cox semiparametric method (proportional hazard model) was performed. *P* values of statistical significance are represented as \**P* < 0.05, \*\**P* < 0.01 and \*\*\**P* < 0.001.

## Results

### PSPC1 Overexpression is Associated with NPC

The expression levels of PSPC1 in 109 NPC tissues and 9 normal nasopharyngeal epithelial samples were determined with IHC. We firstly set up the IHC score criteria of the level of PSPC1 expression. According to the IHC score, the expression level of PSPC1 is divided into - (negative), + (weak), ++ (moderate), +++ (strong) (Figure 1).

In 109 tumor samples, the positive rate of PSPC1 in tumor tissues is 93.58% (102/109), and only one out of 9 normal nasopharyngeal tissues (11.11%,  $\chi^2 = 51.181$ , *P* = 0.000) was found positive for PSPC1. Among 109 cases of NPC, 64 were found to be PSPC1 high expression (score + + ~ +++), accounting for 58.72% (64/109) of cases studied, while in normal nasopharyngeal epithelial tissue, 0 were found to be PSPC1 high expression, the high



**Figure 1** Scoring criteria for PSPC1 expression in IHC.

**Notes:** Pictures of IHC processed at identical conditions for PSPC1 expression were taken and scored. (A) – (negative). (B) + (weak). (C) ++ (moderate). (D) +++ (strong). Images were taken under light microscope (200 $\times$ ). Scale bar=50  $\mu$ m.

expression rate is 0% (0/9) ( $\chi^2 = 11.547$ ,  $P = 0.001$ ), suggesting that PSPC1 overexpression is associated with NPC (Figure 2).

### PSPC1 Expression is Closely Related with Radiosensitivity of NPC

To determine the correlation of PSPC1 expression with radiosensitivity of NPC, normal nasopharyngeal epithelial tissues (Normal), radiosensitive NPC (RS-NPC) and radio-resistant NPC (RR-NPC) slides were compared to find the difference of their PSPC1 expression. As showed in Figure 3, the RS-NPC had a low expression level of PSPC1, while RR-NPC had a high expression level of PSPC1, and Normal hardly expressed PSPC1. Moreover, it was found that the PSPC1 expression was correlated with radiosensitivity ( $P = 0.010$ ) in the NPC patients (Table 1).

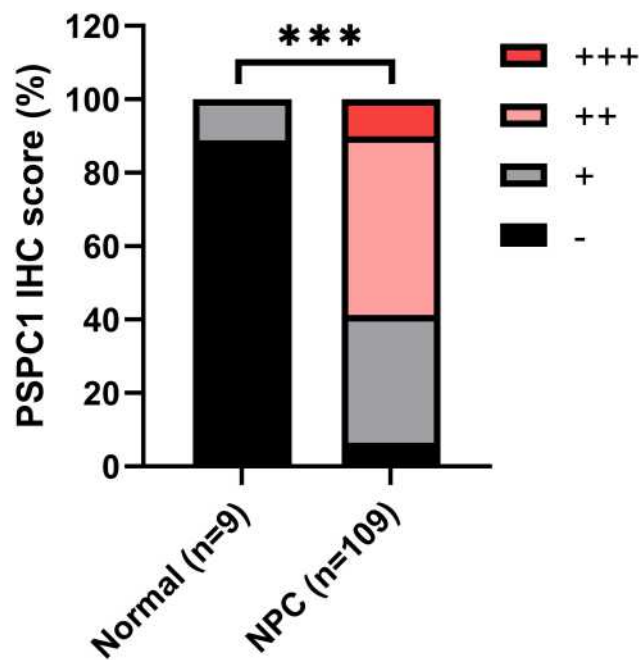
### Correlation of PSPC1 Expression with Clinical Parameters

To examine the correlation between PSPC1 expression levels and clinical parameters of NPC, the 109 cases

were grouped based on different clinical parameters. It was found that the PSPC1 expression was correlated with N classification ( $P = 0.038$ ), recurrence ( $P = 0.014$ ), prognosis ( $P = 0.040$ ) and radiosensitivity ( $P = 0.010$ ) in the NPC patients, but not with the gender ( $P = 0.299$ ), age ( $P = 0.220$ ), pathohistological pattern ( $P = 0.500$ ), clinical stage ( $P = 0.469$ ), T classification ( $P = 0.689$ ) (Table 1).

### Correlation of PSPC1 Expression with Three-Year Outcome of NPC

Clinically, four metrics are currently used to describe the outcomes of NPC: overall survival (OS), progression-free survival (PFS), locoregional recurrence-free survival (LRRFS) and distant metastasis-free survival (DMFS). The 3-year OS rate of the whole cohort was 88.9%, and PFS, LRRFS and DMFS were 80.6%, 93.3% and 86.7%, respectively in these NPC cases. Compared with PSPC1 high expression group, the PSPC1 low expression group had significantly higher OS (95.6% vs 84.3%.  $P = 0.034$ ), PFS (93.3% vs 71.9%.  $P = 0.012$ ) and DMFS rate (95.6% vs 80.5%.  $P = 0.029$ ). Although LRRFS rate (97.3% vs



**Figure 2** Percentage distribution of PSPC1 IHC score in NPC tissues versus normal tissues.

**Notes:** The PSPC1 staining intensity was scored and the distribution of IHC score was expressed as the percentage in NPC and normal tissues. Chi-square test was used to compare IHC scores in NPC tissues versus normal tissues. \*\*\* $P < 0.001$ .

88.6%.  $P = 0.085$ ) showed not any statistically significant difference, the curves between the two groups were well separated (Figure 4 and Table 2).

## Independent Risk Factors Affect the Outcomes of NPC

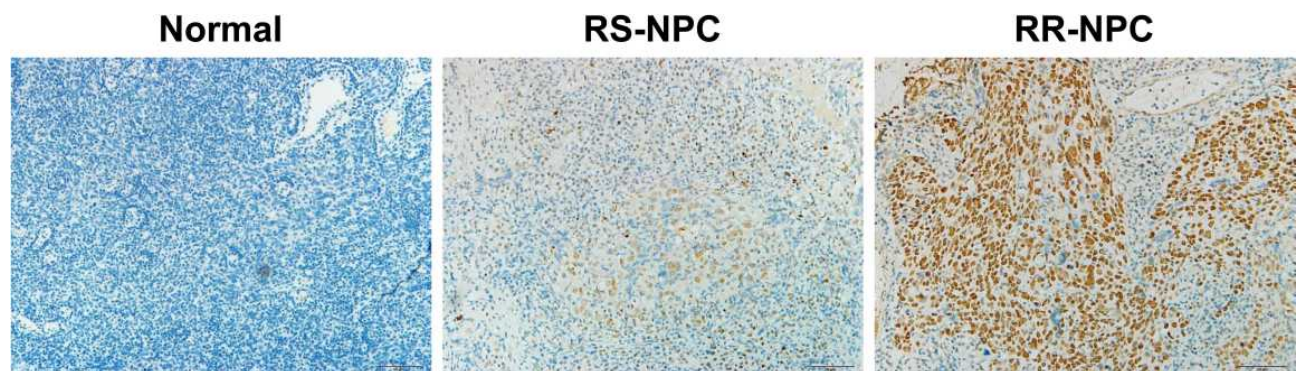
Potential prognostic factors, including PSPC1 expression level, gender, age, pathohistological pattern, clinical stage, T classification, N classification were analyzed by using

the Log rank test. As shown in Table 3, the gender, age, pathohistological pattern, clinical stage, T classification, N classification have not prognostic value for OS, PFS, LRRFS and DMFS ( $P > 0.05$ , respectively), while PSPC1 expression was found to be a potential predictor for OS, PFS and DMFS ( $P = 0.034$ ,  $0.012$  and  $0.029$ , respectively), but not for LRRFS ( $P = 0.085$ ).

To more accurately analyze the covariates and to avoid the interference among variates included in this study, the multivariate survival analyses was carried out. As showed in Table 4, PSPC1 expression was also found to be one of the most significant prognostic factors for OS, PFS and DMFS ( $P = 0.032$ ,  $0.012$  and  $0.026$ , respectively), while LRRFS was not affected ( $P = 0.162$ ). The multivariate analyses also indicated that gender, age, pathohistological pattern, clinical stage, T classification, N classification have not prognostic value for OS, PFS, LRRFS and DMFS ( $P > 0.05$ , respectively). Both the univariate and multivariate models showed that PSPC1 expression was one significant prognostic factor for OS, PFS, and DMFS (Tables 3 and 4) in NPC patients.

## Discussion

Radiotherapy is the first choice for the treatment of NPC.<sup>4</sup> With the development of imaging and radiotherapy techniques, the progression rate, local recurrence rate and distant metastasis rate of NPC after radiotherapy have been significantly reduced.<sup>28</sup> Especially after utilizing the IMRT method, the 3-year LRRFS and DMFS can reach more than 90% and 80%, respectively.<sup>29</sup> However, there are patients still developing regional recurrence and distal



**Figure 3** Differences of PSPC1 expression among the normal tissues, radiosensitive NPC tissues and radioresistant NPC tissues.

**Notes:** Representative pictures of Normal (normal nasopharyngeal epithelial tissues), RS-NPC (radiosensitive NPC tissues) and RR-NPC (radioresistant NPC tissues) were taken after IHC under a light microscope. The staining intensity was compared. The image amplification was 200 $\times$ . Scale bar=50  $\mu$ m.

**Table I** Associations of PSPC1 Expression with Pathological Characteristics in NPC Patients

| Parameters                | Category | High PSPC1<br>(n = 64) | Low PSPC1<br>(n = 45) | $\chi^2$ | P      |
|---------------------------|----------|------------------------|-----------------------|----------|--------|
| Gender                    | Male     | 56                     | 36                    | 1.129    | 0.299  |
|                           | Female   | 8                      | 9                     |          |        |
| Age (years)               | ≤ 50     | 39                     | 33                    | 1.811    | 0.220  |
|                           | > 50     | 25                     | 12                    |          |        |
| Pathohistological pattern | WHO II   | 3                      | 1                     | 0.454    | 0.500  |
|                           | WHO III  | 61                     | 44                    |          |        |
| Clinical stage            | I        | 4                      | 1                     | 2.535    | 0.469  |
|                           | II       | 5                      | 3                     |          |        |
|                           | III      | 31                     | 18                    |          |        |
|                           | IV       | 24                     | 23                    |          |        |
| T classification          | T1–T2    | 24                     | 15                    | 0.200    | 0.689  |
|                           | T3–T4    | 40                     | 30                    |          |        |
| N classification          | N0       | 8                      | 1                     | 3.684    | 0.038* |
|                           | N1–N3    | 56                     | 44                    |          |        |
| Recurrence                | No       | 56                     | 45                    | 6.071    | 0.014* |
|                           | Yes      | 8                      | 0                     |          |        |
| Prognosis                 | Death    | 12                     | 2                     | 4.830    | 0.040* |
|                           | Survival | 52                     | 43                    |          |        |
| Radiosensitivity          | CR or PR | 55                     | 45                    | 6.898    | 0.010* |
|                           | SD or PD | 9                      | 0                     |          |        |

**Note:** \*P < 0.05.

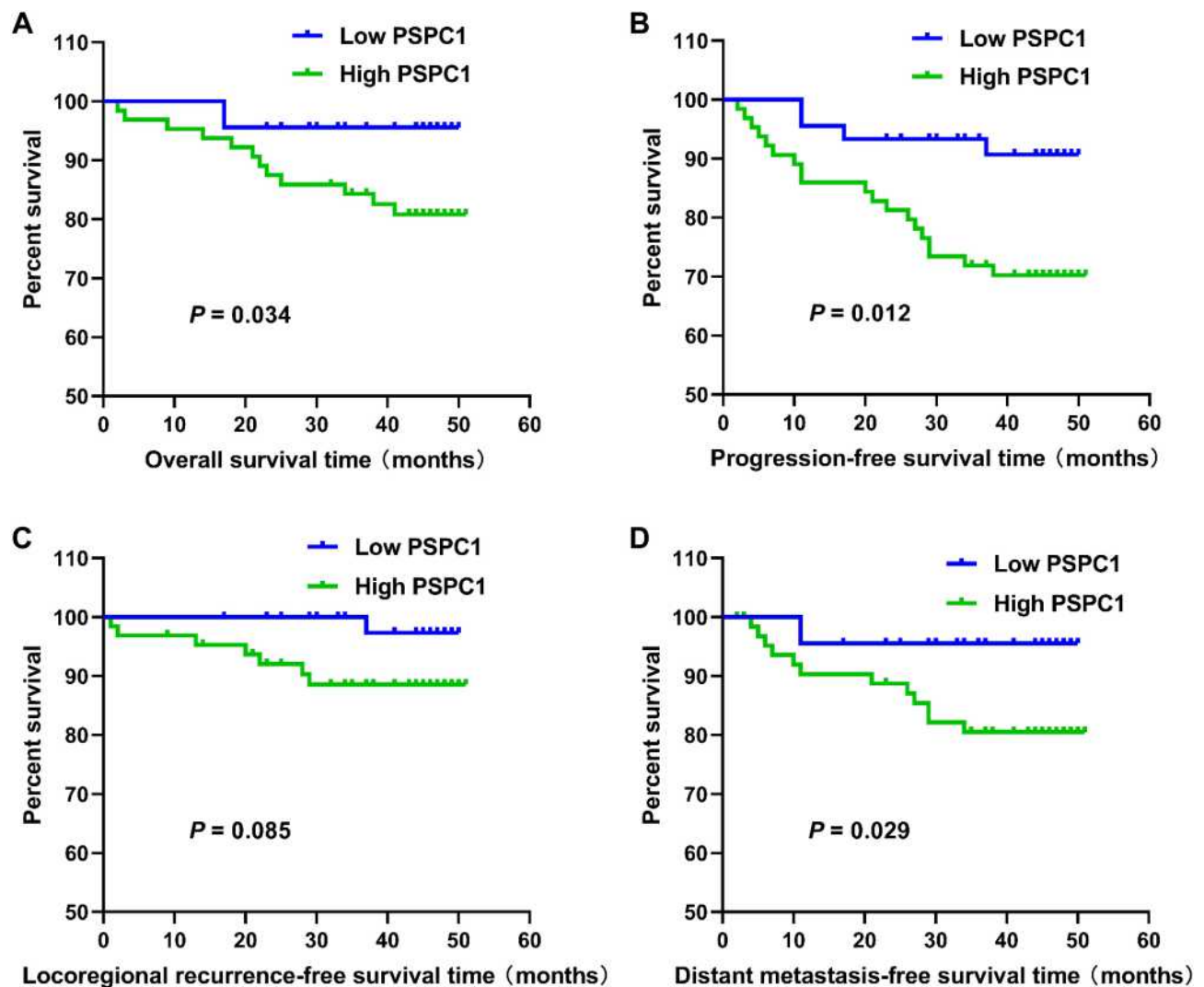
**Abbreviations:** WHO, World Health Organization; Low PSPC1, low level for PSPC1 expression; High PSPC1, high level for PSPC1 expression.

metastasis.<sup>14,26,30</sup> Radio-resistance has been ascertained as a major factor restricting the therapeutic efficacy of NPC.<sup>13,31</sup> Elucidation of the mechanisms of radio-resistance is therefore of great value to improve the therapeutic efficacy of NPC. On the other hand, identifying prognostic biomarker may facilitate the prediction of response prior to treatment and guide the choice of treatment approaches in clinic setting, leading to improvement in the survival rate and life quality of patients.

PSPC1 is overexpressed in numerous tumors and associated with cancer progression and metastasis, serving as a predictor of prognosis.<sup>16,32</sup> Whether PSPC1 is upregulated in NPC has not been investigated previously. In this study, we revealed for the first time that the expression level of PSPC1 was significantly higher in NPC than that in normal nasopharyngeal epithelia tissues. The 3-year OS, PFS and DMFS rate in the low PSPC1 group of NPC patients is significantly

higher than that in PSPC1 overexpression group, indicating that the expression levels of PSPC1 in NPC may be a potential prognostic biomarker to identify patients with potentially unfavorable outcomes prior to the treatment, and these patients may benefit from a more intensive therapeutic regimen.

Previous studies indicated that PSPC1 upregulation was associated with poor survival.<sup>16,33</sup> PSPC1 is frequently altered at the DNA level and upregulated at the RNA level in many cancer types, such as breast, lung and liver tumors. The expression of PSPC1 was correlated with poor patient outcomes and low survival rate.<sup>16,24</sup> Immunohistochemical analysis revealed that PSPC1-positivity in nuclear and cytoplasm was strongly associated with shorter overall survival of hepatocellular carcinoma patients.<sup>34</sup> In a HCC cohort study, the nuclear PSPC1 positivity was associated with the absence of liver cirrhosis and cytoplasmic positivity was



**Figure 4** High PSpC1 expression predicts inferior outcomes in NPC patients.

**Notes:** Survival data were analyzed and plotted using the Kaplan–Meier method. The NPC patients were classified into Low PSpC1 or High PSpC1 according to IHC score. (A) Overall Survival (OS). (B) Progression-free survival (PFS). (C) Locoregional recurrence-free survival (LRRFS). (D) Distant metastasis-free survival (DMFS).

associated with HCV infection. Interestingly, hepatitis delta virus (HDV) replication was shown to induce translocation of PSpC1 protein from the nucleus to the cytoplasm.<sup>21</sup> When PSpC1 was overexpressed in HCC cells, PSpC1 not only lost its sequestration of tumor suppression of PTK6 in the nucleus but also facilitated PTK6 cytoplasmic translocation to be an oncogene and  $\beta$ -catenin nuclear translocation to interact with PSpC1 for augmenting Wnt3a autocrine signaling and tumor progression.<sup>32</sup> In breast and ovarian cancer, downregulation of PSpC1 enhanced cellular proliferation and might play a significant role with long non-coding RNAs (lncRNAs) NEAT1 and LSINCT5 in multiple processes.<sup>35</sup>

In NPC, one of the major treatment failure is distant metastasis, which is linked to an unfavorable prognosis and survival.<sup>36,37</sup> Our results revealed that upregulation of PSpC1 was associated with poor patient's DMFS in NPC, the underlying mechanisms could be: 1) PSpC1 can enhance epithelial–mesenchymal transition (EMT), stemness and tumor growth through the activation of core transcription factors (TFs),<sup>16</sup> 2) PSpC1 is also the interacting partner of Smad2/3, which acts as a contextual determinant of TGF- $\beta$ 1 responses to switch the dichotomous TGF- $\beta$ 1 function from tumor suppressing in precancerous cells to pro-metastatic signaling in malignant cancer cells.<sup>16,38</sup>

Local recurrence is another major treatment failure for NPC patients.<sup>39</sup> PSpC1 is involved in both RNA synthesis

**Table 2** Correlation Between PSPC1 Expression and 3-Year Outcomes in NPC Patients

| Rate (%) | High PSPC1 | Low PSPC1 | $\chi^2$ | P      |
|----------|------------|-----------|----------|--------|
|          | (n = 64)   | (n = 45)  |          |        |
| OS       | 84.3       | 95.6      | 4.494    | 0.034* |
| PFS      | 71.9       | 93.3      | 6.365    | 0.012* |
| LRRFS    | 88.6       | 97.3      | 2.974    | 0.085  |
| DMFS     | 80.5       | 95.6      | 4.746    | 0.029* |

Note: \*P < 0.05.

**Abbreviations:** High PSPC1, high level for PSPC1 expression; Low PSPC1, low level for PSPC1 expression; OS, overall survival; PFS, progression-free survival; LRRFS, locoregional recurrence-free survival; DMFS, distant metastasis-free survival.

and DNA repair. Knockdown of PSPC1 led to severe radio-sensitivity and delayed resolution of DSB repair foci.<sup>40</sup> Our results suggested that patients with high expression of PSPC1 had a lower LRRFS than those of low expression. Further expansion of the number of cases and increased follow-up time might help to further verify the correlation between the PSPC1 overexpression and the patient's survival.

The univariate analysis results of this study indicated that gender, age, pathohistological pattern, clinical stage, T classification and N classification were not association

with OS, PFS, LRRFS and DMFS of NPC patients, which could be due to the shorter follow-up time. Moreover, multivariate analysis had not proved that these factors were risk factors for OS, PFS, LRRFS and DMFS in patients with NPC. However, our results indicated that PSPC1 expression (high vs low) is the Independent prognosis factors associated with OS, PFS and DMFS of NPC patients. More research is needed to further clarify the correlation between these factors and patient's survival with extending the follow-up time and increasing the patient's cases.

## Conclusion

In conclusion, our data showed that PSPC1 overexpression was correlated with NPC poor prognosis including shorter overall survival time, disease progression and distant metastasis. PSPC1 overexpression in tumors could be a potential biomarker for prognosis and a potential target for therapy. Future investigations, including prospective studies, are needed to further validate the value of PSPC1 overexpression in NPC prognosis. Cell culture studies and animal experiments are also desired to provide underlying mechanisms for understanding of the values of PSPC1 in cancer resistance, metastasis, and progression to radiation therapy.

**Table 3** Univariate Log Rank Analyses of Prognostic Parameters of NPC

| Parameters                | Category            | No. of Cases | OS     |        | PFS    |        | LRRFS  |       | DMFS   |        |
|---------------------------|---------------------|--------------|--------|--------|--------|--------|--------|-------|--------|--------|
|                           |                     |              | Events | P      | Events | P      | Events | P     | Events | P      |
| PSPC1 expression          | High                | 64           | 12     | 0.034* | 19     | 0.012* | 7      | 0.085 | 12     | 0.029* |
|                           | Low                 | 45           | 2      |        | 4      |        | 1      |       | 2      |        |
| Gender                    | Male                | 92           | 12     | 0.866  | 21     | 0.325  | 7      | 0.789 | 13     | 0.366  |
|                           | Female              | 17           | 2      |        | 2      |        | 1      |       | 1      |        |
| Age (years)               | ≤ 50                | 72           | 7      | 0.156  | 12     | 0.117  | 3      | 0.072 | 8      | 0.397  |
|                           | > 50                | 37           | 7      |        | 11     |        | 5      |       | 6      |        |
| Pathohistological pattern | WHO <sup>#</sup> II | 4            | 0      | 0.442  | 1      | 0.817  | 0      | 0.561 | 1      | 0.450  |
|                           | WHO III             | 105          | 14     |        | 22     |        | 8      |       | 13     |        |
| Clinical stage            | I                   | 5            | 0      | 0.547  | 0      | 0.246  | 0      | 0.614 | 0      | 0.480  |
|                           | II                  | 8            | 0      |        | 0      |        | 0      |       | 0      |        |
|                           | III                 | 49           | 7      |        | 10     |        | 5      |       | 6      |        |
|                           | IV                  | 47           | 7      |        | 13     |        | 3      |       | 8      |        |
| T classification          | T1–T2               | 39           | 4      | 0.557  | 6      | 0.289  | 2      | 0.503 | 4      | 0.549  |
|                           | T3–T4               | 70           | 10     |        | 17     |        | 6      |       | 10     |        |
| N classification          | N0                  | 9            | 0      | 0.236  | 1      | 0.442  | 1      | 0.691 | 0      | 0.235  |
|                           | N1–N3               | 100          | 14     |        | 22     |        | 7      |       | 14     |        |

Notes: \*P < 0.05. Events: numbers of cases in OS, PFS, LRRFS or DMFS.

**Abbreviations:** WHO, World Health Organization.



Table 4 Multivariate Survival Analyses of Prognostic Parameters of NPC

| Parameters                            | OS    |              |        | PFS   |              |        | LRRFS |              |       | DMFS  |              |        |
|---------------------------------------|-------|--------------|--------|-------|--------------|--------|-------|--------------|-------|-------|--------------|--------|
|                                       | HR    | 95% CI       | P      | HR    | 95% CI       | P      | HR    | 95% CI       | P     | HR    | 95% CI       | P      |
| PSPC1 expression (high vs low)        | 5.357 | 1.154–24.872 | 0.032* | 4.135 | 1.367–12.509 | 0.012* | 4.646 | 0.540–40.003 | 0.162 | 5.695 | 1.231–26.340 | 0.026* |
| Gender (male vs female)               | 1.433 | 0.303–6.770  | 0.650  | 0.606 | 0.128–2.856  | 0.526  | 0.973 | 0.106–8.891  | 0.980 | 0.662 | 0.084–5.232  | 0.696  |
| Age (years) ( $\leq 50$ vs $> 50$ )   | 1.638 | 0.560–4.790  | 0.368  | 1.499 | 0.647–3.470  | 0.345  | 2.890 | 0.661–12.638 | 0.159 | 1.197 | 0.408–3.515  | 0.743  |
| Clinical stage (I vs II vs III vs IV) | 1.884 | 0.543–6.534  | 0.318  | 2.610 | 0.988–6.897  | 0.053  | 1.293 | 0.327–5.125  | 0.713 | 2.736 | 0.701–10.675 | 0.147  |
| T classification (T1–T2 vs T3–T4)     | 0.745 | 0.149–3.733  | 0.720  | 0.618 | 0.167–2.291  | 0.471  | 1.245 | 0.130–11.888 | 0.849 | 0.533 | 0.099–2.870  | 0.464  |
| N classification (N0 vs N1–N3)        | 2.698 | 0.960–7.581  | 0.983  | 1.215 | 0.136–10.899 | 0.862  | 0.596 | 0.053–6.713  | 0.675 | 5.075 | 1.581–16.297 | 0.983  |

Note: \*P < 0.05.

Abbreviations: HR, hazard ratio; 95% CI, 95% confidence interval.

## Abbreviations

PSPC1, Paraspeckle component 1; NPC, Nasopharyngeal carcinomas; OS, Overall survival; DMFS, Distant metastasis-free survival; PFS, Progression-free survival; LRRFS, Locoregional recurrence-free survival; IMRT, Intensity-modulated radiotherapy; MRI, Magnetic resonance imaging; ECT, Emission computed tomography; PET, Positron emission tomography; AJCC, American joint committee on cancer; IHC, Immunohistochemical; Low PSPC1, low level of PSPC1 expression; High PSPC1, high level of PSPC1 expression; EDTA, Ethylenediaminetetraacetic acid; HRP, Horseradish Peroxidase; CTV, Clinical tumor volume; ESC, Esophageal squamous cell carcinoma; GTV, Gross tumor volumes; PTV, Planning tumor volume; Normal, Nasopharyngeal epithelial tissues; RS-NPC, Radiosensitive NPC; RR-NPC, Radioresistant NPC; RNA, Ribonucleic acid; DNA, Deoxyribonucleic acid.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Disclosure

The authors declare that they have no conflicts of interest.

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