Review Article

SALL4 expression is associated with poor outcome in hepatocellular carcinoma

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Abstract: Spalt-like transcriptional factor 4 (SALL4), which is located on chromosome 20q13.12-13.31 and encodes a C2H2 zinc-finger transcription factor, has been identified as a putative stem cell marker. In published studies, SALL4 has been proved to contribute to the development of hepatocellular carcinoma (HCC). To systematically evaluate the clinical and prognostic role of SALL4 in HCC, we performed this meta-analysis. A total of 8 studies containing 290 positive and 1063 negative cases were included in our meta-analysis. The quality assessment of included studies was performed by the Newcastle-Ottawa scale (NOS), with an average NOS score of 7.25. Our data indicated that SALL4 expressing level was not associated with the gender (pooled OR = 0.764, 95% CI = 0.506-1.153, P = 0.200, fixed effect), histological differentiation (pooled OR = 0.420, 95% CI = 0.132-1.337, P = 0.142, random effect) and Child-Pugh score (pooled OR = 2.014, 95% CI = 0.848-4.784, P = 0.113, fixed effect) in HCC patients. However, high SALL4 statistically related to hepatitis B virus infection (pooled OR = 2.083, 95% CI = 1.300-3.338, P = 0.002, fixed effect) and vascular invasion (pooled OR = 1.685, 95% CI = 1.219-2.328, P = 0.002, fixed effect), which led to a lower 1-year disease free survival (1-year DFS, RR = 1.715, 95% CI = 1.078-2.728, P = 0.023) and 1-year overall survival (1-year OS, RR = 2.495, 95% CI = 1.636-3.805, P = 0.000). These findings suggested that SALL4-immunopositive expression might lead to a poorer patient survival in HCC. SALL4 could be served as an efficient marker for prognostic indicator in HCC, and might be a promising target for future HCC therapies.

Keywords: SALL4, clinicopathological characteristics, prognosis, hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second leading cause of cancer mortality worldwide [1, 2]. Multiple risk factors for HCC are the most frequent chronic viral hepatitis (B and C), alcohol abuse, serum alpha-fetoprotein (AFP) concentration, obesity, diabetes and exposure to aflatoxins [3, 4]. To the best of our knowledge, HCC staging affects treatment decisions and patient prognosis largely. Due to lack of distinctive symptoms in early stages, less than 30-40% of HCC patients are eligible for potentially curative therapies including surgical resections and transplantations [5, 6]. As to the patients who lost the surgery opportunity, palliative treatments such as transarterial chemoembolization, sorafenib, small molecular target agents, monoclonal antibodies and Chinese herbal medicine, offer a relative survival benefit [7-10]. Because the molecular mechanisms underlying hepatocarcinogenesis are not well characterized, all the combined treatments do not reach an ideal objective. Hence, it is urgent to elucidate pathogenic mechanisms and find high specificity and sensitivity biomarkers for early detection of HCC, which may benefit the therapy and prognosis of HCC patients.

Stem cells are generally defined as clonogenic cells which are capable of both self-renewal and multilineage differentiation [11]. Given their properties, stem cells are unit of biological organization, product of new and replacement cells for tissues during development and homeostasis [12]. Cancer stem cells (CSCs), a subpopulation of cancer cells which have the
As a marker of stem cells, Spalt-like transcriptional factor 4 (SALL4) is located on chromosome 20q13.12-13.31 and encodes a C2H2 zinc-finger transcription factor [22]. SALL4 protein is expressed in fetal, but it is silenced in normal adult. However, in 2006, Ma et al. firstly reported that SALL4 re-expressed in leukemia and was associated with the poor prognosis of AML patients [23]. Afterwards, SALL4 were gradually unveiled in some solid tumors, including breast cancer, lung cancer, colorectal cancer and hepatocellular carcinoma [24-27]. In 2013, Nilda et al. pointed that these were no correlations between SALL4 expression and clinicopathologic characteristics in HCC, such as histologic grade, vascular invasion and pathologic T stage [28]. However, Yin et al. found that the expression of SALL4 was relevant to the prognosis of HCC patients, and patients with higher expression levels of SALL4 and AFP have worse prognosis [29]. Other similar studies also referred to the expressions of SALL4 in HCC [28-35], but its clinical significance and potential related tumor characteristics were not fully elucidated. Therefore, we investigated this meta-analysis to assess the clinical roles of SALL4 in HCC.

Materials and methods

Literature search

For obtaining potentially eligible studies, several databases were retrieved including PubMed, Embase, Cochrane Library and the European Society for Medical Oncology. We used a combination of relevant keywords to construct the search strategy including “SALL4” and “Hepatocellular carcinoma”. A study was considered eligible for inclusion if: (1) articles

<table>
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<th>SALL4 negative (n)</th>
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</table>
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were published before July 2016; (2) articles were written as original research; (3) studies were written in English; (4) the study provided sufficient information which reported the relationships between SALL4 expression and potential prognostic factors (or DFS/OS); (5) articles must be the full-text manuscripts. The following studies were excluded: studies (1) were researched by RT-PCR or other non-immunohistochemistry methods; (2) could not be acquired the relevant original data or could not be used for statistical analyses; (3) were reviews, letters, case reports and expert opinions.

**Data extraction and quality assessment**

With the standard protocol, the eligible articles were assessed independently by two experienced investigators (Yuliang Jiang and Wei Li). General information extracted from the eligible studies include: authors, the year of publications, the methods of measuring SALL4 expression in HCC tissues, tumor stage, the total number of HCC patients in each study, the number of SALL4 positive and negative in HCC patients, the correlations between SALL4 and clinicopathological parameters, the 1-year DFS rate and OS rate.

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**Results**

**Study characteristics**

The process of study selection was shown in Figure 1. According to the criteria for selection, 26 studies were discarded (14 articles lacked correlations between SALL4 expressions and HCC, 11 articles were classified as non-clinical articles, 1 study showed unavailable sufficient data). Finally, 8 studies [28-35] were included in this meta-analysis, and the major characteristics were listed in Table 1. Our 8 included studies contained 1353 patients, ranging from...
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Figure 2. Forrest plot of ORs for the association of SALL4 expression with the (A) gender; (B) Histological differentiation; (C) Child-Pugh score in HCC patients.
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Figure 3. Forrest plot of ORs for the association of SALL4 expression with the (A) Hepatitis B virus infection; (B) Vascular invasion; (C) 1-year DFS; (D) 1-year OS in HCC patients.
38 to 337 patients per study, with 290 SALL4 positive and 1063 SALL4 negative HCC patients. The details of the quality evaluation for eligible studies are shown in Table 2. The average NOS score was 7.25. Immunohistochemistry (IHC) was the only applied method in this meta-analysis.

Correlations of SALL4 expression with clinicopathological parameters in HCC

As shown in Figure 2A-C, our results indicated that SALL4 expression was not associated with the gender (pooled OR = 0.764, 95% CI = 0.506-1.153, P = 0.200, fixed effect), histological differentiation (pooled OR = 0.420, 95% CI = 0.132-1.337, P = 0.142, random effect) and Child-Pugh score (pooled OR = 2.014, 95% CI = 0.848-4.784, P = 0.113, fixed effect) in HCC patients. However, the SALL4-immunopositive expression showed a connection towards Hepatitis B virus infection (pooled OR = 2.083, 95% CI = 1.300-3.338, P = 0.002, fixed effect). Similar positive results were also found in vascular invasion (pooled OR = 1.685, 95% CI = 1.219-2.328, P = 0.002, fixed effect) (Figure 3A, 3B). In the process of evaluation of the related studies, no significant heterogeneity was observed among the studies on gender (I² = 0.0%, P = 0.477), histological differentiation (I² = 23.2%, P = 0.259), Child-Pugh score (I² = 15.0%, P = 0.317), Hepatitis B virus infection (I² = 49.6%, P = 0.138) and vascular invasion (I² = 23.2%, P = 0.259).

Correlations of SALL4 expression with DFS and OS in HCC

Because RRs were not described directly in include studies, we extracted the related date from the Kaplan-Meier cures or calculated from original papers [36, 37]. Based on our calculations, the high tissue SALL4 level in HCC patients was statistically related to the 1-year disease free survival (1-year DFS, RR = 1.715, 95% CI = 1.078-2.728, P = 0.023) and 1-year overall survival (1-year OS, RR = 2.495, 95% CI = 1.636-3.805, P = 0.000) with no significant heterogeneity (1-year DFS: I² = 37.2%, P = 0.203; 1-year OS: I² = 0.0%, P = 0.844) (Figure 3C, 3D). This finding indicated that SALL4-immunopositive expression might lead to a poorer patient survival in HCC.

Sensitivity analysis and publication bias

A sensitivity analysis was performed to validate the stability of the pooled results. The corre-
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Figure 4. Sensitivity analyses in our meta-analysis. A. Gender; B. Histological differentiation; C. Child-Pugh-score; D. Hepatitis B virus infection; E. Vascular invasion; F. 1-year DFS; G. 1-year OS in HCC patients.

Figure 5. Funnel plots for publication bias. All the graphical funnel plots appeared to be symmetrical. A. Gender; B. Histological differentiation; C. Child-Pugh-score; D. Hepatitis B virus infection; E. Vascular invasion; F. 1-year DFS; G. 1-year OS in HCC patients.
entiation, which was consistent with the results of some similar studies [28, 29, 31]. Emerging evidence showed that some CSCs markers, such as K19 and EpCAM, had been proved to be significantly higher in cirrhotic patients of Child-Pugh Class B or C than those of Child-Pugh Class A, which might promote the cancer recrudescence in HCC [46]. However, no reports specifically discussed the potential relationships or mechanisms between SALL4 and Child-Pugh score until now. Our results also confirmed no relevance between them.

It is known that the antecedent HBV-related chronic hepatitis is a common precursor condition for HCC. Recently, Minuk et al. addressed a possible association between HBV and CSCs in HCC [47]. As a proved biomarker of CSCs in HCC, we speculated that SALL4-positivity might be more frequently associated with hepatitis B virus infection. Though the potential role or exact mechanisms of SALL4 in HBV infection need further explored, our data firstly confirmed their correlations in HCC. As we know, a tumor cell or tumor cell colonies that initiates a metastatic colony at the distant organ must firstly (a) detach from the primary mass, (b) invade the local host tissue stroma, (c) penetrate local lymphatic and blood vessels. Afterwards, these survived tumor cells within the circulation become transported until they arrest in the capillaries of a distant organ [48, 49]. Experimental studies have shown that these CSCs or stem-like cells are more capable of escaping from the primary tumor (intravasation) and disseminating to the blood or lymphatic system [49-51]. Fortunately, our data also confirmed that SALL4 up-regulation worsened the vascular invasion in our study. Similarly, because of its related characteristics of CSCs, we hypothesized that SALL4 immuno-positive cells might lead to more tumor invasion and metastasis. Similar results were also confirmed by Shibahara or Yin, in which high SALL4 expressions related with more intrahepatic metastasis or advanced clinical stages respectively [29, 33]. Furthermore, our data also demonstrated that SALL4-positive expression was statistically associated with the disease free survival and overall survival in HCC. This finding strongly supported that high SALL4 might be served as an independent prognostic predictor for HCC patients.

In interpreting our results from our meta-analysis, there were still some limitations should be addressed. Firstly, IHC was the only applied method in our study and the cutoff values were defined differently. Secondly, the small amount of studies and case samples might relatively lead to higher heterogeneity. Thirdly, lacking the available data from our included studies, the potential relationships between SALL4 and some other clinicopathological features shall be further researched, such as tumor size, liver cirrhosis, EpCAM and serum AFP. These key factors may help us better understand the molecular mechanisms of SALL4 in HCC. Finally, lack of directly obtained disease free survival data or survival data, we calculated DFS/OS from the available data or Kaplan-Meier curves, which might be less reliable. What’s more, a longer period of DFS/OS rather than only 1 year might be more statistically significant. All these factors could potentially affect the results of our meta-analysis. Therefore, more well-designed studies or researches, which refer to the expression or mechanisms of SALL4, are needed for our deep understanding of CSCs in HCC.

Our meta-analysis is the preliminary one to explore the relationships between SALL4 expression and clinicopathological characteristics (or DFS/OS) in HCC. Our data indicated that SALL4 expressing level was not associated with the gender, histological differentiation and Child-Pugh score in HCC patients. However, high SALL4 statistically related to hepatitis B virus infection and vascular invasion, which led to a poorer patient survival in HCC. These findings suggested that SALL4 could be served as an efficient marker for prognostic indicator in HCC, and might be a promising target for future HCC therapies.

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Disclosure of conflict of interest

None.

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References

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