Clinicopathological Significance of DLC-1 Expression in Cancer: a Meta-Analysis

Yan Jiang, Jian-Ming Li, Huai-Qing Luo*

Abstract

Background: Recent reports have shown that DLC-1 is widely expressed in normal tissues and is down-regulated in a wide range of human tumors, suggesting it may act as a tumor suppressor gene. We conducted a meta-analysis to determine the correlation between DLC-1 expression and clinicopathological characteristics in cancers. Materials and Methods: A detailed literature search was made for relevant publications from PubMed, EMBASE, Cochrane library databases, Web of Science, CNKI. The methodological quality of the studies was also evaluated. Analyses of pooled data were performed and odds ratios (ORs) were calculated and summarized. Results: Final analysis was performed of 1,815 cancer patients from 19 eligible studies. We observed that DLC-1 expression was significantly lower in cancers than in normal tissues. DLC-1 expression was not found to be associated with tumor differentiation status. However, DLC-1 expression was obviously lower in advance stage than in early-stage cancers and was more down-regulated in metastatic than non-metastatic cancers. Conclusions: The results of our meta-analysis suggested that DLC-1 expression is significantly lower in cancers than in normal tissues. Aberrant DLC-1 expression may play an important role in cancer genesis and metastasis.

Keywords: DLC-1 expression - metastasis - meta-analysis - cancer

Introduction

Distant metastasis is always occurred in advanced cancer and is proved to be the leading cause of cancer mortality, so it is a predominant issue to understand the molecular mechanisms that enables this phenomenon in cancer biology. Metastasis suppressor gene deregulation was certified to play a key role in metastasis and several new metastasis suppressor genes have been fond and studied in cancers. DLC-1, a member of the deleted-in-liver cancer family of proteins which includes DLC-1, DLC2 and DLC3, was first identified as a tumor metastasis suppressor gene in hepatocellular carcinoma (Yuan et al., 1998). The human DLC-1 gene is localized on chromosome 8p21-22 and encodes a 1,091 amino acid protein that is highly homologous to the rat p122-RhoGAP, which is a GTPase-activating protein(GAP) for Rho family proteins (Homma and Emori, 1995). Rho family proteins play essential roles in regulating cytoskeletal organization, cell adhesion, and cell cycle progression (Etienne-Manneville and Hall, 2002; Moon and Zheng, 2003; Tcherkezian and Lamarche-Vane, 2007). It was determined that DLC-1 is widely expressed in normal tissues and is down-regulated in a wide range of tumor tissues. Underexpression of DLC-1 was associated with either heterozygous deletions of the DLC-1 gene or hypermethylation of the gene promoter region (Guan et al., 2006; Seng et al., 2007; Guan et al., 2012). Recent studies have extensively investigated DLC-1 expression and function in many human cancers, including liver, breast, lung, ovarian, kidney, colon, stomach, prostate, nasopharynx, Gallbladder and so on (Guan et al., 2006; Peng et al., 2006; Zhang et al., 2009; Hua et al., 2010; Yufei et al., 2010; Fan and Shi, 2011; Yun et al., 2011; Fang et al., 2012; Quanrui and Zhimei, 2012; Peng et al., 2013; Ren et al., 2013; Yang et al., 2013a; Yang et al., 2013b; Peng et al., 2014; Mingrui et al., 2014; Qin et al., 2014; Yufei et al., 2014; Yujie et al., 2014; Zhefeng et al., 2015). Wang Y reported that DLC-1 is an important regulator of TGF-β responses and DLC-1 overexpression suppressed bone metastasis of breast cancer (Wang et al., 2014). Ren reported that the expression of DLC-1 was closely related with the metastasis and invasion of ovarian carcinoma (Ren et al., 2013). However, controversies still exist due to the limited number of patients in individual studies. In addition, the association between DLC-1 expression and clinical significance has not been thoroughly investigated. In this study, we pooled and analyzed the published clinical investigations regarding the effect of DLC-1 on cancer patients.

Materials and Methods

Search strategy and selection criteria

The following electronic databases were searched for relevant articles without any language restrictions:

Department of Basic Medical Science, Department of Human Anatomy, Histology and Embryology, Institute of Neuroscience, Changsha Medical University, Changsha, PR China *For correspondence: 894269826@qq.com
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We also using the Begg test and visual inspection of a funnel plot for significant.

The pooled OR was performed using a fixed model (OR=0.441, 95% CI=0.374-0.520, p<0.000), which indicated that DLC-1 expression is significantly lower in cancers than in the normal tissues.

The role of DLC-1 expression in cancer progression

We first determined whether DLC-1 expression is significantly higher in cancers than in normal tissues. The pooled OR from nineteen studies including 1182 cancers and 633 normal tissues is shown in Figure 2. There was no significant heterogeneity (I²=0.0%, p=0.782), and the pooled OR was performed using a fixed model (OR=0.441, 95% CI=0.374-0.520, p=0.000), which indicated that DLC-1 expression is significantly lower in cancers than in the normal tissues.

Increased DLC-1 expression in cancers

We then analyzed 810 cancer patients pooled from thirteen studies to assess whether DLC-1 expression in cancers was associated with advanced stage. As shown in Figure 2, aberrant DLC-1 expression was significantly lower in advanced cancers (stages III and IV) than in early-stage cancers (stages I and II) (OR=0.435, 95% CI=0.332-0.570, p=0.000) by fixed model (I²=0.0%, p=0.993). In addition, as shown in Figure Supplement 1, aberrant DLC-1 expression was not significantly lower in poorly and moderately differentiated cancers than in highly differentiated cancers.
<table>
<thead>
<tr>
<th>Study</th>
<th>Cancer</th>
<th>Year</th>
<th>Country</th>
<th>Patients</th>
<th>Gender</th>
<th>Methods</th>
<th>Tumor stage</th>
<th>Lymph node metastasis</th>
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*IHC, immunohistochemistry; RT-PCR, reverse transcription polymerase chain reaction; NR, not reported*
differentiated cancers (OR=0.916, 95% CI=0.552-1.519, p=0.734). These results suggest that DLC-1 expression may not associate with tumor’s differentiated status, but may play an important role in cancer progression and development.

The role of DLC-1 expression in metastatic cancers

We then analyzed 744 cancer patients pooled from twelve studies to assess whether DLC-1 expression in cancers was associated with lymphoma metastatic status. As shown in Figure 3, there was no significant heterogeneity (I²=0.0%, p=0.910) among studies and the pooled OR was performed using a fixed model. Aberrant DLC-1 expression was significantly lower in metastatic cancers than in nonmetastatic cancers (OR=0.432, 95% CI=0.323-0.579, p=0.000). The result suggests that DLC-1 expression is strongly correlated with metastatic status in cancer patients.

Sensitivity analyses and publication bias

A sensitivity analysis, in which one study was removed at a time, was conducted to assess the stability of the results. The pooled ORs were not significantly changed, indicating the stability of our analyses. Two funnel plots were largely symmetric (Figure 4A-B) suggesting there were no publication biases in the meta-analysis of DLC-1 expression with cancer progression (p=0.161). Visual inspection of the Begg funnel plot revealed asymmetry in the analysis of DLC-1 expression with metastatic status in cancers (p=0.011). The funnel plot with Trim and Fill from seventeen studies determined the relationship between DLC-1 expression and metastatic status in cancers (p=0.011). The Begg test was also statistically significant (p=0.011).

This indicated the possibility of publication bias. Because of this, we undertook a sensitivity analysis using the trim and fill method (Duval and Tweedie, 2000), which
conservatively imputes hypothetical negative unpublished studies to mirror the positive studies that cause funnel plot asymmetry. The imputed studies produce a symmetrical funnel plot (Figure 4D). The pooled analysis incorporating the hypothetical studies continued to show a statistically significant association between DLC-1 expression and cancer metastasis (OR=0.537, 95% CI=0.416-0.693, P=0.000).

Discussion

To date, there have been some studies describing the precise expression and prognostic impact of DLC-1 in cancers. However, the roles and clinical significance of DLC-1 expression in cancers have not been thoroughly investigated. We conducted the meta-analysis to determine the correlation between DLC-1 expression and clinicopathological characteristics in cancers. Analyses of the pooled data showed that cancers had a significantly lower DLC-1 expression than normal tissues (OR=0.432, 95% CI=0.323-0.579, P=0.000). Recent studies have shown that down-regulation or inactivation of the DLC-1 gene during tumor development appears to be primarily due to aberrant methylation at the gene promoter. In an early study, the promoter methylation was proved to major responsible for down-regulation of DLC-1 by screening several cell lines derived from HCC, prostate tumors and colon (Yuan et al., 2003). Then the analysis of 73 surgical samples of HCC showed that the level of DLC-1 methylation in highly invasive HCC was significantly higher than in low invasion cases (Liu, 2008). A recent methylation analysis of 68 patients with pancreatic ductal adenocarcinoma showed that DLC-1 hypermethylation strongly correlated with lymph node metastasis (Xue et al., 2013). Promoter methylation and LOH result in DLC-1 inactivation in nasopharyngeal tumors (Feng et al., 2014). Still, more studies are needed to reveal the relationship between DLC-1 expression and promoter methylation, and the affect of promoter methylation on clinicopathological features.

DLC-1 also played an important role in cancer progress and metastasis. This meta-analysis pooled the data and showed that aberrant DLC-1 expression was significantly lower in metastatic cancers than in nonmetastatic cancers (OR=0.432, 95% CI=0.323-0.579, P=0.000) and in advanced cancers (stages III and IV) than in early-stage cancers (stages I and II) (OR=0.435, 95% CI=0.332 - 0.570, P=0.000). While aberrant DLC-1 expression was not significantly lower in poorly and moderately differentiated cancers than in highly differentiated cancers (OR=0.916, 95% CI=0.552-1.519, P=0.734). The results suggest that DLC-1 expression play an important role in cancer progression and metastasis even not in cancer differentiation. Because of limited studies involve in cancer differentiation analysis, the differentiation result is not certain and need more new evidence to prove it. The mechanism that DLC-1 expression inhibits cancer progression was focused on DLC-1 interaction proteins. Members of the tensin family of focal adhesion proteins were identified the first DLC-1 binding partners and the impact of this interaction has been examined in HCC, NSCLC and breast carcinoma cells (Yam et al., 2006; Qian et al., 2007). Other proteins, like phosphatase and tensin homolog (PTEN), p120Ras-GAP (RASA1) and S100A10 are also studied in several cancers and targeting prooncogenic proteins activated by DLC-1 down-regulation was thought to be therapeutically effective for the suppression of cancer progression and metastasis (Heering et al., 2009; Yang et al., 2009; Yang et al., 2011).

Consistent results were shown in sensitivity analyses, and no evidence of heterogeneity was found. This meta-analysis met publication bias issues when analyze the association between DLC-1 expression and cancer metastasis. We undertook a sensitivity analysis using the trim and fill method to remove publication bias and the pooled analysis after adjusted continued to show a statistically significant association between DLC-1 expression and cancer metastasis. This study has several potential limitations. First, the possibility of information and selection biases as well as unidentified confounders could not be completely excluded because all of the included studies were observational. Second, the searching strategy was restricted to articles published in English and Chinese. Articles with potentially high-quality data that were published in other languages were not included. Third, the samples and studies were limited by a presence of heterogeneity between other studies.

In conclusion, our meta-analysis showed that DLC-1 expression was significantly lower in cancers than in normal tissues. The aberrant DLC-1 expression plays an important role in cancer carcinogenesis and metastasis. Thus, it is safe to say that the remarkable potential of DLC-1 could serve as a prognostic biomarker for cancer patients. Further large-scale studies, especially multicenter, could serve well-matched cohort will provide more insight into the role of DLC-1 in the prognosis and clinical implementation of cancer patients.

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