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Original Article

P16^{INK4a} overexpression and survival in osteosarcoma patients: a meta analysis

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Abstract: Osteosarcoma is one of the most common primary bone malignancies. Although there is a significant improvement of survival on osteosarcoma patients in the past decades, treatment of osteosarcoma is still unsatisfactory for the development of pulmonary metastasis. The potential prognostic value of p16^{INK4a} in osteosarcoma has been investigated, however, the results from different studies were somewhat controversial. To elucidate whether p16^{INK4a} is indeed a prognostic factor of osteosarcoma, we conducted a meta-analysis of the published literatures to provide a comprehensive evaluation of the significance of p16^{INK4a} expression in patients with osteosarcoma. Eight studies with a total of 354 patients with osteosarcoma were examined. The pooled odds ratio (OR) with corresponding 95% confidence interval (95% CI) was calculated to evaluate the effect of p16^{INK4a} expression on overall survival. Meta-analysis showed that patients with high p16^{INK4a} expression were significantly associated with favourable overall survival when compared to their counterparts with low or undetectable p16^{INK4a} expression (OR = 0.270, 95% CI 0.162-0.451, $P < 0.001$). Sensitivity analysis suggested the pooled OR was stable and not significantly changed when a single study was removed. In conclusion, the results from this meta-analysis highlight that p16^{INK4a} is an effective biomarker of survival in patients with osteosarcoma.

Keywords: Osteosarcoma P16^{INK4a} expression, survival, meta-analysis

Introduction

Osteosarcoma (OS), which is one of the most common primary bone malignancies, occurs most frequently in adolescents [1]. Although the neoadjuvant therapy with aggressive surgical resection has improved the prognosis, the treatment of osteosarcoma is still unsatisfactory for the risk of local relapse and the development of pulmonary metastasis [2, 3]. Moreover, rare definitive prognostic markers have been identified in patients with osteosarcoma [4]. Therefore, a specific prognostic biomarkers which can identify patients with prognosis is critical to the effective treatment of osteosarcoma.

The p16^{INK4a} tumor suppressor protein, encoded by the CDKN2A gene and functioned as an inhibitor of CDK4 and CDK6, is a cell-cycle inhibitor and an important factor in carcinogenesis [5, 6]. P16^{INK4a} was documented as an important predictor of biological behavior in some malignant tumors, such as ewing sarcoma, gastrointestinal stromal tumor, non-small cell lung, colorectal cancer [7-10]. The possible prognostic value of p16^{INK4a} in osteosarcoma has also been investigated, however, the results from different studies were somewhat controversial [11-18]. To elucidate whether p16^{INK4a} is indeed a prognostic factor of osteosarcoma, we conducted a meta-analysis of the published literatures to provide a comprehensive evaluation of the significance of p16^{INK4a} expression in patients with osteosarcoma.

Material and methods

Search strategy and study selection

The Pubmed, Embase, web of Science, and CNKI (China National Knowledge Infrastructure) were searched for relevant articles published before July 7, 2014 without any language restrictions. The search strategy included the following terms: “osteosarcoma” or “osteosarcomas” and “p16” or “p16^{INK4a}”. We also performed a manual search in order to identify other potentially eligible studies.

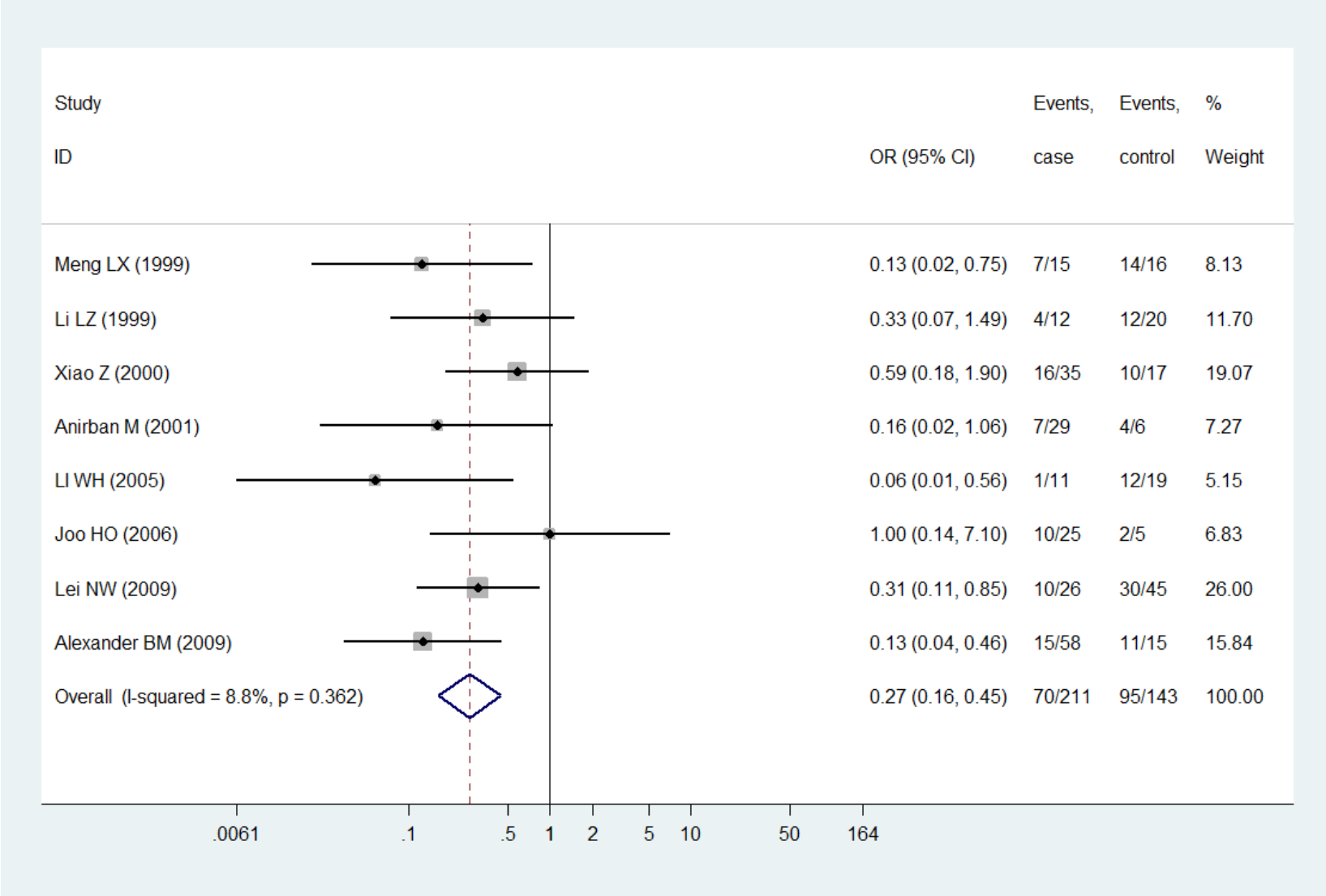

Figure 1. Forest plot showed the meta-analysis of the effect of p16^{INK4a} expression on the overall survival rate.

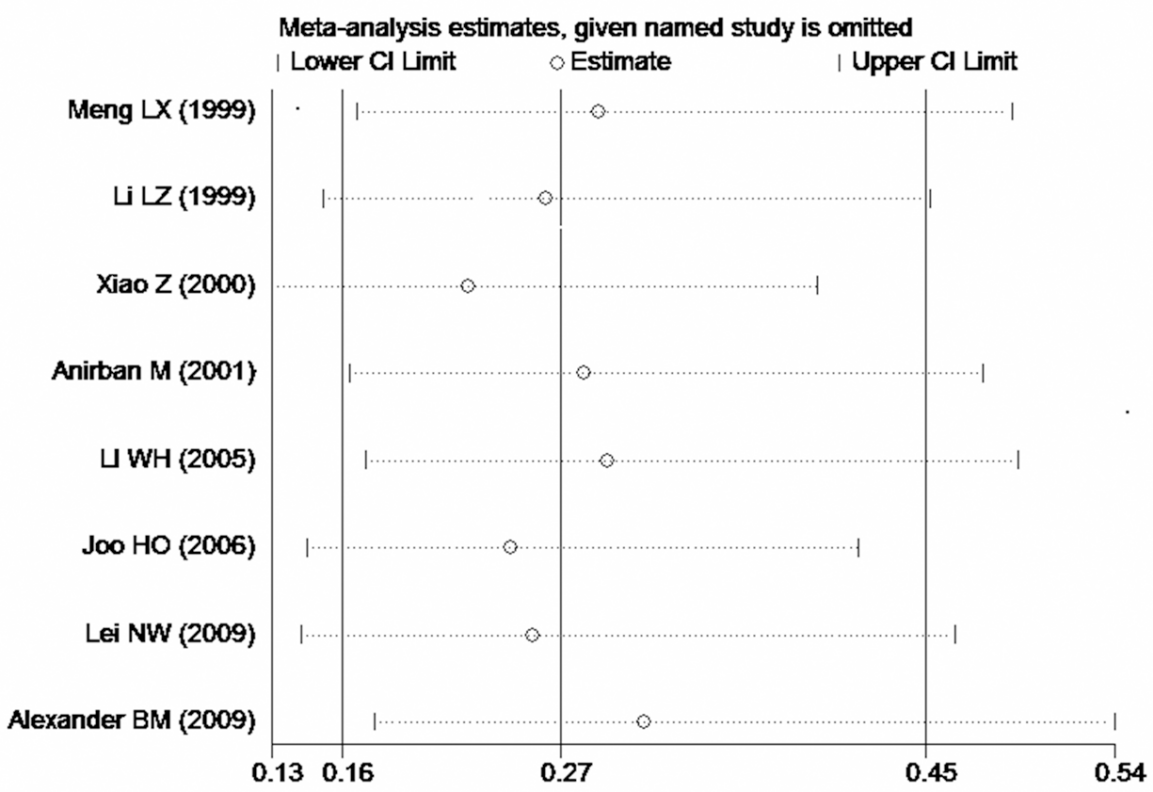

Figure 2. Forest plot for the sensitivity analysis in the meta-analysis.

Inclusion and exclusion criteria

The included studies in this meta-analysis must meet all the following criteria: (1) all patients were diagnosed with osteosarcoma through pathological examination; (2) the 3 or 5-year survival outcomes were reported; (3) the associations between p16INK4a expression and survival outcomes were specifically provided. Case reports and reviews were all excluded. When multiple articles included overlapping data by the same authors or group, we selected the newest or most complete article. Two independent reviewers firstly searched potentially relevant studies by reading the titles and abstracts and then further checked by reading the full texts and assessed for inclusion.

Primary outcome and data extraction

The primary outcome used in this meta-analysis was overall survival rate, defined as the proportion of people within a treatment group who were still alive after 3 or 5 years. Data were extracted from the included studies, and the main information included first author name, the publication year, stages of osteosarcoma, treatment method, number of patients, p16INK4a expression in tissue, and overall survival rate.

Analysis methods

We evaluated the effect of p16INK4a expression on overall survival by calculating the pooled odds ratio (OR) with corresponding 95% confidence interval (95% CI). Statistical heterogeneity between studies in our meta-analyses was assessed by the I^2 -statistic (< 25%, no heterogeneity; 25%-50%, moderate heterogeneity; > 50%, strong heterogeneity) [19]. When a significant heterogeneity existed across the included studies (I^2 > 50%), a random effects model was used for the analysis [20]; otherwise, the fixed effects model was used [21]. Begg's funnel plots and Egger's test were performed to assess a potential publication bias [22]. All statistical analyses were conducted with the software Stata version 13 (Stata Corp LP, College Station, TX, USA). A two-sided *P* value less than 0.05 was considered statistically significant.

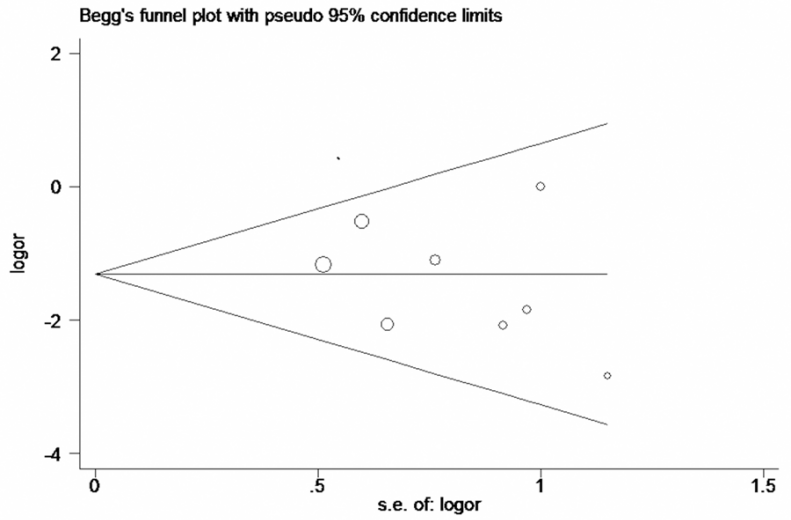

Figure 3. Funnel plot in the meta-analysis of the effect of p16INK4a expression on the overall survival rate.

Results

Study characteristics

After including irrelevant studies in Pubmed, Embase, web of Science, and CNKI and then checking the full texts, eight studies that met the inclusion criteria were finally identified in this meta-analysis. Among those eight included studies, three were published in English and the others were published in Chinese. The total number of included patients in these eight studies was 354 ranging from 30 to 73 with a mean of 51.5. In addition, there were 211 osteosarcoma patients with high p16INK4a expression and 143 patients with low or undetectable p16INK4a expression. And there were 7 studies reporting data on the 3-year overall survival [11-16, 18] and 1 study reporting data on the 5-year overall survival [17].

Meta-analysis results

In this meta-analysis, there was no obvious heterogeneity among those studies (I^2 = 8.8%). Thus, a fixed effects model was used [11-18]. Meta-analysis of those eight studies showed that patients with high p16INK4a expression were significantly associated with favorable overall survival compared with their counterparts with low or undetectable p16INK4a expression (OR = 0.270, 95% CI 0.162-0.451, *P* < 0.001) (**Figure 1**). Sensitivity analysis suggested that the pooled OR was stable and not significantly changed when a single study was removed (**Figure 2**).

Publication bias

Publication bias statistics in this meta-analysis were determined by using the methods of Begg's funnel plot and Egger's test. There was no obvious evidence of funnel plot asymmetry among those eight studies (**Figure 3**). In addition, no evidence of publication bias was observed from Egger's test (*P* = 0.404).

Discussion

Osteosarcoma, the most frequent malignant primary bone tumor, has become a health threat for children and adolescents. With the introduction of neoadjuvant therapy, the 5-year survival rate has increased from about 20% to 65-70% [23]. However, even remain almost 15% to 20% of all patients with osteosarcoma were detected metastatic disease at the time of initial diagnosis [24]. Therefore, there is urgent need for the identification of novel prognostic and predictive biomarkers to improve diagnosis and treatment of patients with osteosarcoma [25].

P16INK4a, a classic tumour suppressor, is an inhibitor of cyclin-dependent kinase and a key component of the G1/S checkpoint [26]. A number of studies have evaluated the role of p16INK4a expression in patients with osteosarcoma, while there are no consistent and conclusive results. In order to evaluate the prognostic role of p16INK4a expression in patients with osteosarcoma, we systematically reviewed the published studies and performed a meta-analysis.

Meta-analysis, acts as a quantitative approach which integrates all possible studies of the same topic, has been conducted to assess several cancer prognostic markers [27]. In our study, high expression of p16INK4a was associated with favorable overall survival in patients with osteosarcoma (OR = 0.270, 95% CI 0.162-0.451, *P* < 0.001) (**Figure 1**). Sensitivity analy-

sis suggested that the pooled OR was stable and not significantly changed when a single study was removed (**Figure 2**). Overall, finding from this meta-analysis indicate that p16INK4a is a valuable prognostic biomarker in osteosarcoma patients.

In this meta-analysis, it is clear that some limitations need to be discussed. First, the main limitation was the small sample size of the total patients, there were only eight documents with a total of 354 osteosarcoma patients in our meta-analysis. The limited sample size might inevitably increase the risk of bias in this meta-analysis. Second, various thresholds in defining high expression in assessment of p16INK4a were used in different studies, so publication bias might exist in this meta-analysis. Third, different treatments of the patients might affect the survival time which should be taken into account. Fourth, there were no consistent survival rates among the eligible studies which may contribute to between-study heterogeneity. Seven studies used a 3-year survival rate, while a study used a 5-year survival rate [17]. Therefore, further studies with larger sample sizes are needed to evaluate the prognostic value of p16INK4a in osteosarcoma.

In conclusion, the results from this meta-analysis highlight that p16INK4a is an effective biomarker of survival in patients with osteosarcoma. In addition, the large prospective clinical studies are needed to further identify the effect of p16INK4a expression in osteosarcoma patients.

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Disclosure of conflict of interest

None.

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