Review Article

Study on the relationship between livin expression and osteosarcoma

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ABSTRACT

Objective: The aim of this meta-analysis was to analysis the expression of livin in human osteosarcoma.
Methods: We searched the Pubmed, Science Direct, Embase and Web of Science, CNKI, Wanfang and VIP for relevant original studies. Statistical analysis was performed by Stata 11.0 software.
Results: Our study indicated that livin expressed in the osteosarcoma tissue was significantly higher than the control group (OR = 18.814, P c = 0.000, 95% CI: 10.973–32.257), and the positive expression of livin was correlated with the size of osteosarcoma tumor and Enncking staging (OR = 4.832, 95% CI: 2.198–10.621; OR = 4.851, 95% CI: 3.053–7.709, respectively).
Conclusion: Livin was highly expressed in osteosarcoma, and osteosarcoma Enncking staging and tumor size were positively correlated, both may be involved in the occurrence and development of osteosarcoma, and be closely related to the prognosis of osteosarcoma patients.

1. Introduction

Osteosarcoma is an original malignant bone tumor and that is common in bone tumors, which accounts for 4–5 cases per million people per year [1]. The incidence of osteosarcoma is mainly for children, adolescents and young people [2,3]. In recent years, the incidence has been increasing year by year [4], which has a serious impact on social stability and the health of children, adolescents and young people [5].

The study found that the occurrence and development of osteosarcoma was closed with the abnormal apoptosis of cells [6], while the apoptosis of cells was mainly associated with the inhibitor of apoptosis protein (IAP). IAP is a class of important anti-apoptotic factors. A common feature of this family structure is that N-terminal contains one or more (currently found up to 3) tandem baculovirus IAP repeated sequence and C-terminal with or without a ring finger structure. Apoptosis is a common feature of tumor cells, so IAP family as a regulator of apoptosis is getting more and more attention. The human IAP family has now been found to have eight members, livin is a new member of the IAP family that contains a BIR domain and a RING finger domain. Livin blocks many pathways-induced apoptosis by inhibiting Caspase 3, 7 and 9. Livin is not expressed or lowly expressed in most normal adult tissues, but highly expressed in many human malignant tumor cell lines and most melanoma cell lines, suggesting that this factor may play an important role in tumorigenesis and development

[7]. Recent studies also found that livin was overexpressed in gastrointestinal tumors such as gastric cancer, intestinal cancer, pancreatic cancer and liver cancer. Yagihash et al. showed that high expression of livin in patients with gastrointestinal cancer, and with the occurrence of human gastrointestinal cancer had a certain relationship [8]. Hofmann et al. displayed livin played an anti-apoptotic role in the pathogenesis of non-small cell lung cancer [9].

It had been reported that livin was overexpressed in various human malignancies, including osteosarcoma, and could inhibit apoptosis induced by many apoptotic stimulators [6]. Many studies had shown that livin protein level in osteosarcoma tissue was significantly higher than those in the control group [6,7,10–17]. Li et al. also showed that livin was involved in the tumorigenesis of human osteosarcoma [18].

The above results suggested that the high expression of livin may be closely related to the formation of certain types of human tumors. Further study of livin is of great significance for exploring the occurrence, progression, treatment and prevention of tumors. Osteosarcoma is a malignant tumor that seriously threats the human and its treatment is also a big problem. At present, the study of livin expression in osteosarcoma has rarely been reported in China and abroad. Our research may provide a basis for further understanding of livin, revealing the possible biological mechanism of osteosarcoma and finding effective targets for clinical biomolecular drugs, which will bring new directions for the prevention and treatment of osteosarcoma.

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2. Materials and methods

2.1. Search strategy

We searched Pubmed, Science Direct, Embase and Web of Science together with three Chinese databases CNKI, Wanfang Data and VIP for relevant papers published before December 1, 2017. The search terms were used as follows: 'livin [MeSH]' and 'osteosarcoma [MeSH]'. References cited in all selected articles were manually searched for additional articles. Except for this, we sent e-mails to the authors to obtain data that did not show the results of the control group.

2.2. Selection criteria

All the included studies with the following characteristics: (1) Original research; (2) designed as case-control studies which evaluated the association between livin and osteosarcoma; (3) provided sufficient information to estimate odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs); (4) all cases should meet the diagnostic criteria of osteosarcoma (The patient was diagnosed by pathology); (5) the study was published in English or Chinese. In addition, exclusion criteria included: (1) drug intervention studies; (2) no control group study results data; (3) repeated studies; (4) studies about livin gene.

2.3. Data extraction

Two review authors (Du and Wang) extracted data from eligible studies independently. Discontents between reviewers were resolved by a third investigator (Zhao) to reach consensus. And the following items were recorded from each included studies: first author (year), sample size, detection method, source of controls, case collection time, average patient age, ethnicity.

2.4. Statistical analysis

All statistical data were performed with Stata11.0 software. The pooled OR with corresponding 95% confidence interval (CI) was designed to measure the strength of association between livin and osteosarcoma patients. We used the Higgins's (I^2) test to calculate the heterogeneity. If the heterogeneity was statistically significant ($I^2 > 50\%$ or the p-value of heterogeneity < 0.05), the random effect-model was employed to combine the data. If not, the fixed-model was used. Publication bias was detected by the method of Bgger's test. And sensitivity analysis was used to evaluate the stability of the result by excluding one study at a time.

3. Results

3.1. Search results

In Fig. 1, 107 articles were identified for initial review based on search criteria. 51 manuscripts were excluded because of irrelevant studies. In the remaining 56 articles, of which 22 were not clinical studies and eight were not case-control studies. After reading the full article, 16 studies were excluded. Finally, we adopted ten manuscripts published from 2008 to 2016 in our meta-analysis.

3.2. Study characteristics

In Table 1, there were ten researches containing 482 osteosarcoma patients and 211 controls. All of the eligible articles, the ethnicity involved was Asian only. Four immunohistochemistry detection methods were employed in the included studies such as Max Vision TM/AP, streptavidin peroxidase (SP), Western Blot and strept avidin-biotin complex (SABC). The control group included two categories: osteochondroma tissue and normal bone tissue. Among the articles, one

study lacked the case collection time and another manuscript was not provided the average patient age.

3.3. Association between livin and osteosarcoma

Our meta-analysis showed that livin expressed in the osteosarcoma tissue was significantly higher than the control group (OR = 18.814, $P_c = 0.000$, 95% CI: 10.973–32.257). Furthermore, the heterogeneity was very small ($I^2 = 0.0\%$, $p_h = 0.800$) (Fig. 2).

3.4. Correlation between livin expression and tumor clinicopathological features

In Table 2, eight studies and three studies were respectively assessed the correlation of livin expression with Enncking staging and tumor diameter in osteosarcoma patients. As for Enncking staging, the pooled OR of our study was 4.851 (95% CI: 3.053–7.709), indicating that livin expression was significantly associated with higher Enncking staging (IIB ~ III) (Fig. 4). For the tumor diameter, livin expression was significantly associated with larger tumor diameter (OR = 4.832, 95% CI: 2.198–10.621) (Fig. 5).

However, no statistically significant association was found in the analysis of gender, age, tumor site and pathological classification of the patients ($P > 0.05$).

3.5. Sensitivity analysis

We performed a sensitivity analysis to examine the effect of each study on the pooled OR value and the result showed that none of the articles affected the result of our study.

3.6. Publication bias

Since the funnel plot was not symmetrical, it demonstrated that publication bias existed (Fig. 3).

4. Discussion

Our study demonstrated that livin significantly increased in osteosarcoma tissue, and osteosarcoma Enncking clinical stage and tumor size were positively correlated with the expression of livin. The heterogeneity was very low ($I^2 = 0.0\%$, $P_h = 0.800$), which displayed the differences between the studies we adopted were small. In the sensitivity analysis, the pooled OR and 95% CI direction of the study remained stable, indicating the results were consistent. What's more, when we analyzed the result by using the Z test ($z = 10.67$, $P = 0.000$), it remained showed statistical significance.

The diagnosis and treatment of osteosarcoma have always been the world problems [19]. Early diagnosis of osteosarcoma is difficult and its metastasis occurs early, which is easy to miss the best timing of surgery. At present, the main treatment of osteosarcoma is mainly surgical resection, supplemented by radiotherapy and chemotherapy comprehensive treatment. However, it is easy to recur after operation and the five-year survival rate is low. And the radiotherapy and chemotherapy are prone to tolerance and resistance, and the side effects are extremely great. Therefore, in order to study how to completely kill tumor cells and control the proliferation of tumor cells, biological treatments such as tumor molecules and genes are beginning to be emphasized. Immune targeting therapy of specific antigens in osteosarcoma tissue can identify both alien and non-cancerous cells and specifically kill tumor cells throughout the patient's body, thereby avoiding the side effects of radiotherapy and chemotherapy [12]. An important pathogenesis of osteosarcoma is the abnormality of the apoptosis system [10]. Therefore, finding the target of the apoptosis-related gene as the target of future osteosarcoma therapy is the key to defeat and cure osteosarcoma [10].

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│  Records for selection (n=107) │
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              │        ┌──────────────────────────────────────────┐
              │───────▶│ (n=30)Not the relationship between       │
              │        │ Osteosarcoma and livin                   │
              ▼        │ (n=21)Drug intervention studies          │
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│ Studies for Further evaluation│
│ (n=56)                        │
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              │───────▶│ (n=22) Not a clinical study              │
              │        │ (n=8) Not case-control study             │
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│ Case-control literatures for │
│ meta-analysis (n=26)         │
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              │───────▶│ (n=1) No control group study results     │
              │        │ data                                     │
              │        │ (n=12) Study about livin gene            │
              ▼        │ (n=3) Repeat article                     │
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│ Manuscripts adopted in the study│
│ (n=10)                        │
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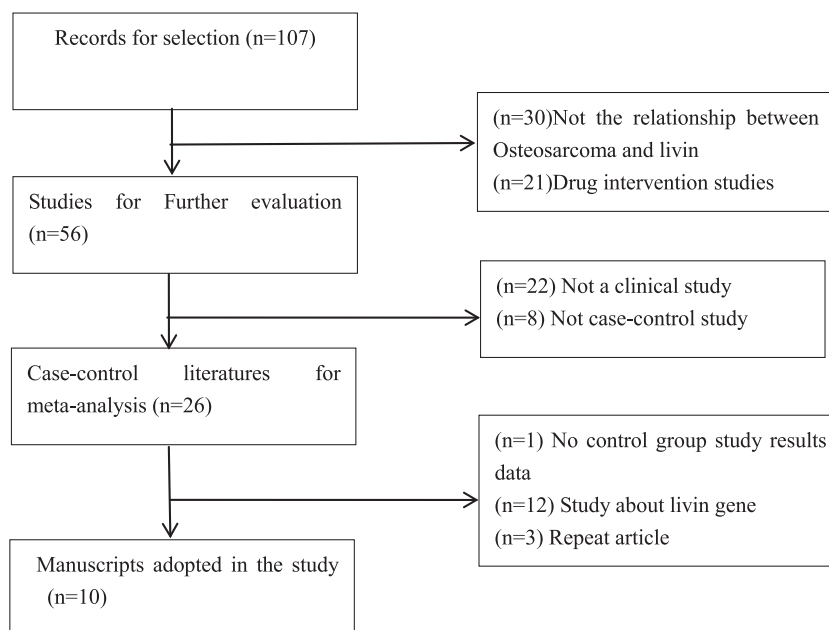
Fig. 1. The flow diagram of literature screening.

However, the mechanism of livin overexpression in osteosarcoma has not been elucidated. Studies suggested that the emergence of osteosarcoma was related to apoptosis system dysfunction [6,17,20–22]. Kasof et al. showed that livin combined with caspases -3,7,9 which mediated cell apoptosis and inhibited its activity, thus preventing the caspases protein from performing protein excision during cell apoptosis [23]. When the process of apoptosis was inhibited, it would lead to unlimited proliferation and metastasis of tumor cells [10]. Another mechanism of cell apoptosis was that livin may stimulate the TAK 1/JNK1 signaling pathway, thus the cell apoptosis induced by TNF and ICE was inhibited by non C pathway [24]. Considering apparent anti-apoptotic effect of livin, recently, one study found that livin kockdnown could significantly decrease cell proliferation and colony formation, at the same time, increase their chemosensitivity to cisplatin [18]. Recently discovered livin is a new member of the human apoptosis protein family, which is highly expressed in various malignant tumor tissues and tumor cell lines and can inhibit the apoptosis of tumor cells [12]. Therefore, livin may be used as a potential therapeutic target for osteosarcoma.

It had been reported that livin was highly expressed specifically in most malignancies such as lung cancer, colorectal cancer, prostate cancer, bladder cancer, cervical cancer and breast cancer [25–28], and that livin was a potentially valuable tumor marker [29]. In osteosarcoma, Li et al. [18] reported that livin was overexpressed in

osteosarcoma cell lines and considered livin as a gene target for the treatment of osteosarcoma. Therefore, tumor markers have always been the hot spots and new ways of tumor diagnosis. Livin as a diagnostic target, using DNA gene probe technology, RT-PCR technology or enzyme-linked immunosorbent assay to detect the expression of livin gene and its mRNA or livin protein to achieve the early diagnosis of cancer. Lumachi et al. [30] through the use of oligonucleotide probe analysis of p53 gene mutations in breast cancer tissues, confirmed the use of gene probe technology to detect the feasibility of the target gene in tumor tissue. Williams et al. [31] using RT-PCR detection of bone and soft tissue tumor paraffin sections of ectopic gene, preliminary confirmed that RT-PCR technology could help early diagnosis of cancer, but the feasibility of the technology to be further. Kitamura et al. [32] showed that by immunohistochemical staining of renal cell carcinoma, livin expression was found to be as high as 57.8%, and livin autoantibodies detected in patients were significantly higher than normal. Yagihashi et al. [8] further confirmed that the detection of livin autoantibodies was valuable for the early detection of tumors by measuring livin autoimmune antibodies in sera from patients with lung cancer.

The development of malignant tumors is that tumor cells can evade cell apoptosis through a variety of mechanisms. Therefore, it has been a hotspot and a new way of tumor targeted therapy to guide tumor cells to increase apoptosis and inhibit their proliferation. Livin as a therapeutic target, using gene recombination technology or miRNA

Table 1
Main characteristics of the included articles.

First author (year)	Sample size		Detection method (immunohistochemistry)	Source of controls age	Time	Average patient age	Ethnicity
	Cases	Controls					
Fu et al. [10]	64	64	Max Vision TM/AP	Normal bone tissue	2008–2015	19.8 ± 2.6	Asian
Zhang [11]	58	35	SP	Osteochondroma tissue	2011–2013	19.4	Asian
Ji [12]	61	10	Max Vision TM/AP	Normal bone tissue	2001–2013	20.5	Asian
Li et al. [18]	51	12	Max Vision TM/AP	Normal bone tissue	2001–2012	N	Asian
Sun et al. [14]	57	10	SP	Normal bone tissue	2003–2008	22.6	Asian
Ni & Lu [15]	40	10	SP	Osteochondroma tissue	Before 2000	23.8	Asian
Zhao [16]	31	5	SP	Normal bone tissue	N	22	Asian
Wang [7]	30	20	Western Blot	Normal bone tissue	2002–2007	18	Asian
An et al. [17]	45	30	SABC	Osteochondroma tissue	1995–2007	36.5	Asian
Liu [6]	45	15	SP	Osteochondroma tissue	1999–2005	14.5	Asian

SP: streptavidin peroxidase; N: not mentioned in the original text; SABC: strept avidin-biotin complex.
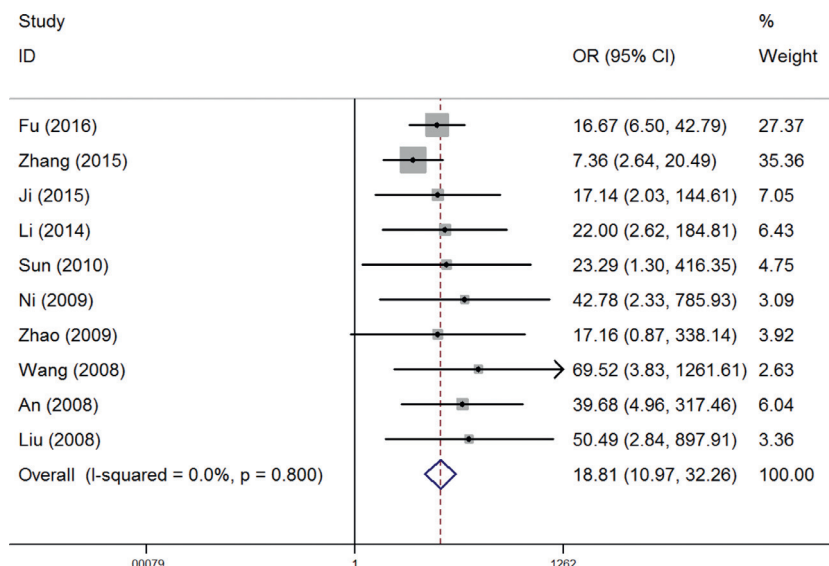
Fig. 2. Forest plot showing the association between livin expression and osteosarcoma.

Table 2
Meta-analysis of the correlation between livin expression and clinicopathological features.

Projects	Number of studies	Patients	I^2	OR	95%CI	P_h	P_c
Gender(male vs. female)	8	387	0.0	0.917	0.604,1.393	0.585	0.685
Age(≤20 vs. >20)	4	214	0.0	1.036	0.594,1.807	0.606	0.901
Enneking staging(IIB-IIIvs.IB~IIA)	8	392	0.0	4.851	3.053,7.709	0.709	0.000
Tumor diameter(>5 cm vs. ≤5 cm)	3	169	0.0	4.832	2.198,10.621	0.866	0.000
Pathological classification (fibroblast/chondroblast/osteoblast vs. others)	6	321	0.0	0.734	0.401,1.345	0.692	0.317
tumor location (humerus/femur/tibiofibula vs. other)	3	172	42.7	1.191	0.530,2.678	0.175	0.672

OR: odds ratio; P_h: p-value for heterogeneity; P_c: p-value for comparability; CI: confidence interval; I^2: the Higgins's (I^2) test for heterogeneity.
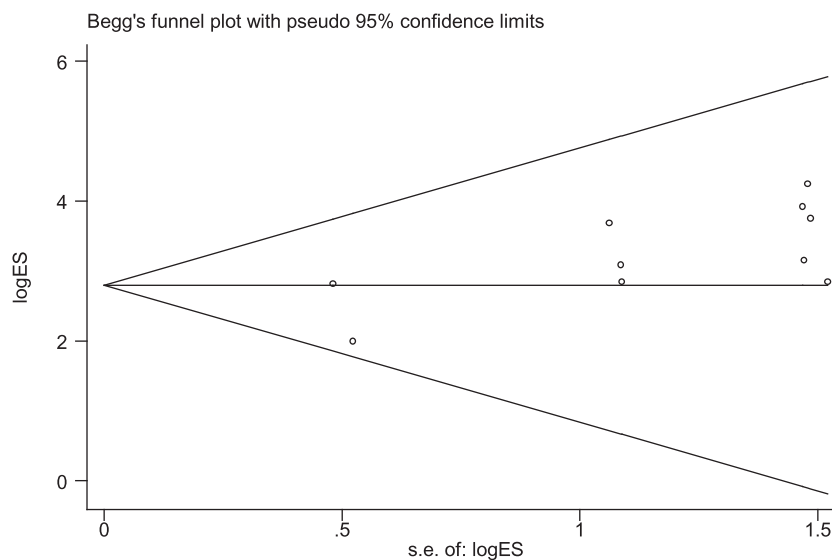

Fig. 3. Funnel plots assessing possible publication bias for the relation between livin and osteosarcoma.

silencing technology, specifically to identify tumor cells or inhibit the expression of livin, is currently a new target for the study of malignant tumor targeted therapy. Chen et al. [33] demonstrated that cytotoxic T lymphocytes (CTLs) were activated by transfection of cord blood (UCB) -derived dendritic cells (DCs) with livin recombinant adenoviruses as vaccines that specifically recognized and killed livin expressing tumor cells. Recent research shows that miRNA can be used for the treatment of tumors by inhibiting the expression of livin. Ye et al. [34] found that the expression of mi R-198 in prostate cancer was negatively correlated

with the expression of livin, further confirming mi R-198 inhibited livin expression in tumor cells. Wang et al. [35] found that high expression of livin in tumor tissue was positively correlated with chemoresistance of tumor. Targeting silencing livin gene in tumor tissue can greatly increase chemosensitivity to malignant tumor and improve drug sensitivity to improve the efficacy of chemotherapy on malignant tumors.

We found that livin expression was significantly associated with higher Enncking stage (IIB~III) and larger tumor diameter, which was consistent with the results of four original studies [10,12–14].
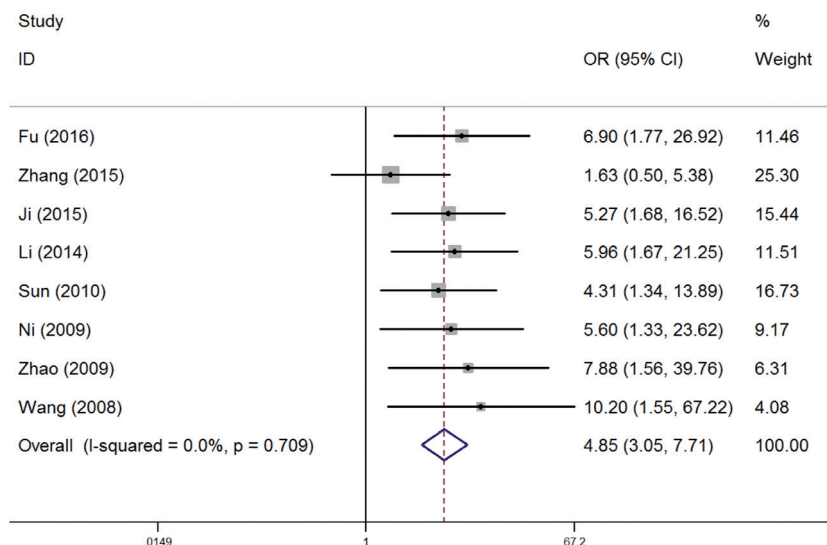
Fig. 4. Forest plot showing the association between livin expression and Enncking staging in osteosarcoma patients.
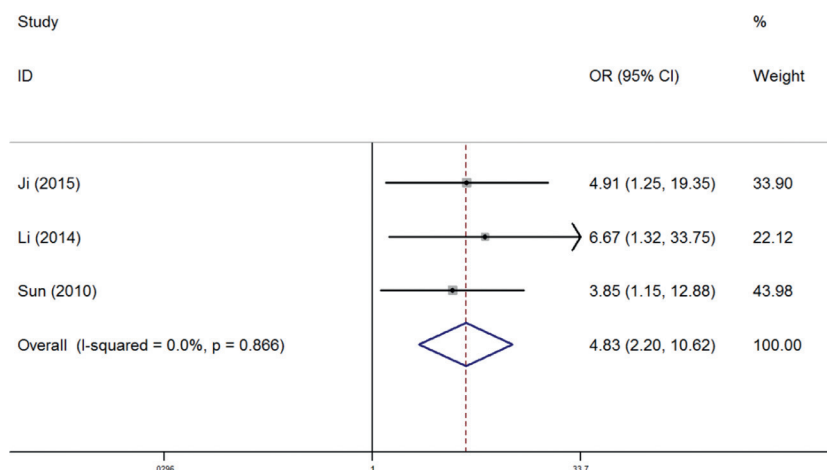

Fig. 5. Forest plot showing the association between livin expression and Tumor diameter in osteosarcoma patients.

Moreover, the expression of livin mentioned in three articles was related to the survival time [10,12,13]. What is more, livin expression was associated with the tumor invasion and metastasis [6,11,17]. Ni et al. [15] suggested that a link between pathological grading and livin. However, our study did not investigate the association between livin expression and survival time, tumor invasion, metastasis, and pathological grade, because the original literature under the same standard data was less than three. However, more authoritative researches are needed to prove the relation between livin expression and osteosarcoma patient's gender, age, pathological stage, tumor diameter et al.

Limitations of the meta-analysis needed to be considered. First, some studies had only given data on the outcome of osteosarcoma patients and did not give data on the results of control studies. Second, although we included all available data, the sample size was still small. Third, the articles we could include in our study were all from China, and the studies in other countries on osteosarcoma and livin were very few. Fourth, although all of the patients we included were pathologically diagnosed, but not all patients were in the same pathological stage, which may have an impact on our result. Fifth, the specimens we included in the articles were all detected by immunohistochemistry for livin, but not all by the same immunohistochemical method.

In conclusion, our study shows that livin is significantly elevated in osteosarcoma tissue, which is positively correlated with the Enncking staging and tumor diameter. However, more researches are needed to confirm this conclusion.

Conflict of Interest

The authors declare that they have no conflict of interest.

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