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Original Article

Effects of alendronate combined with local radiotherapy on serum Akt/GSK3β and bone metabolism levels in patients with bone metastases from primary liver cancer

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Abstract: Objective: To investigate the effects of alendronate combined with local radiotherapy on the level of serum Akt/GSK3β and bone metabolism in patients of primary liver cancer with bone metastases. Methods: Clinical data of 68 patients of primary liver cancer with bone metastases and treated in Shanghai General Hospital, a hospital affiliated to Shanghai Jiao Tong University School of Medicine, were retrospectively analyzed. According to the different surgical methods, the patients were divided into a control group, with 33 cases treated with local radiotherapy plus Oxycodone hydrochloride extended-release tablets, and a study group, with 35 cases treated with alendronate combined with local radiotherapy. The remission rate and adverse reaction rate were compared between the two groups. In addition, we observed and compared the liver function indexes (total bilirubin (TBIL), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)), serum Akt/GSK3β level, bone metabolism levels (bone alkaline phosphatase (BAP) and levels of osteocalcin (OST)), α-fetoprotein (AFP), vascular endothelial growth factor (VEGF), osteopontin (OPN), matrix metallopeptidase 9 (MMP-9), and quality of life of the patients in two groups before and after treatment. Results: A higher remission rate was observed in the study group (94.29%) than that in the control group (75.76%) ($P<0.05$). There was no significant difference in the adverse reaction rate between the study group (20.00%) and the control group (12.12%) ($P>0.05$). In both groups, the post-treatment serum levels of TBIL, ALT, ALP, Akt, GSK3β, AFP, VEGF, OPN, MMP-9, hardship and nausea due to cancer were all decreased, while serum levels of BAP and OST, and psychological, physical and social functions were all increased (all $P<0.05$). The improvement of the above indicators in the study group were better than those in the control group (all $P<0.05$). Conclusion: The use of alendronate combined with local radiotherapy received good response in patients of primary liver cancer with bone metastasis. In addition, their liver function, bone metabolism levels and quality of life all improved without increasing adverse reactions. The underlying mechanism may be related to the regulation of Akt and GSK3β levels.

Keywords: Alendronate, local radiotherapy, primary liver cancer, bone metastases, bone metabolism, quality of life

Introduction

Primary liver cancer has the second highest incidence and mortality among all kinds of cancers in the world [1]. The causes of primary liver cancer are complex. Patients have no obvious symptoms in the early stage. With the progression of the disease, symptoms such as pain, emaciation and ascites in the liver may occur. Most patients are in the middle or advanced stages at diagnosis, missing the best timing for surgical treatment [2]. Bone metastases are one of the common complications in patients with primary liver cancer. According to epidemiological data, about 3% to 28% of patients with primary liver cancer developed bone metastases at the time of diagnosis [3]. Metastatic bone pain is progressive and easy to compress the spinal nerves, leading to limb dysfunction and pathological fractures, which seriously

affects the quality of life of patients [4]. At present, local radiotherapy, which effectively inhibits the development of metastatic lesions, is applied to treat bone metastases from primary liver cancer. However, it fails to relieve severe pain and improve bone metabolism level, which is not conducive to the prognosis [5]. Therefore, how to effectively alleviate the pain and improve bone metabolism needs to be solved urgently in clinical practice. Alendronate is a bisphosphonate drug that effectively promotes bone resorption and reduces bone destruction. Good results have been shown to achieve when alendronate is applied in patients with malignant tumors such as breast cancer complicated with bone metastases. Alendronate could also improve the bone metabolism level of patients [6]. However, there are few clinical reports of the use of alendronate combined with local radiotherapy in patients with bone metastases from primary liver cancer. In recent years, it has been reported that the Akt/GSK3 pathway plays an important role in the proliferation and metastasis of tumor cells [7]. However, there still lacks reports about the mechanism of the Akt/GSK3 pathway in bone metastases from primary liver cancer. In this study, we retrospectively analyzed the effects of alendronate combined with local radiotherapy on bone metabolism level in patients of primary liver cancer with bone metastases.

Materials and methods

General data

A total of 68 patients of primary liver cancer with bone metastases treated in Shanghai General Hospital, an affiliated hospital to Shanghai Jiao Tong University School of Medicine, from March 2019 and February 2021 were enrolled in this retrospective study. Among these patients, 41 were males and 27 were females, aging from 48 to 78 years old, with an average age of (58.02±5.19) years old. The patients were divided into a control group (n=33) and a study group (n=35) according to the different surgical treatment methods. Patients in control group were treated with local radiotherapy, while those in study group were given local radiotherapy combined with alendronate. This study has been approved by the Ethics Committee of Shanghai General Hospital, affiliated to Shanghai Jiao Tong University School of Medicine.

Inclusion criteria: (1) Patients' diagnostic results met the diagnostic criteria for primary liver cancer [8], and confirmed by CT and MRI as a lesion of bone metastasis. (2) Patients aged from 18 to 80 years old. (3) Patients had more than 60 points of Kach score and complete clinical data [9].

Exclusion criteria: (1) Patients were allergic to the drug used in this study. (2) Patients had decompensated liver function. (3) Patients suffered from immune system diseases or other-cause bone metabolism diseases. (4) Patients failed to tolerate local radiotherapy. (5) Patients had multiple metastases other than bone metastases. (6) Patients had severe cardiac and renal dysfunction. (7) Patients had poor compliance, or died during the treatment.

Treatments

All the patients were given nutritional support and general symptomatic treatment after admission. Patients in the control group were treated with local radiotherapy with Siemens Linear Accelerator 6MV-X. It was confirmed by MRI. All flat bones, spinal metastases, vertebral metastases, long bone metastases including full length, and 2-3 cm around pelvic metastases were selected as the range of irradiance. The radiation dose was 2-3 Gy/time, 4-5 times/week, with the total dose of 50-75 Gy. Besides, the patients were given oral Oxycodone hydrochloride extended-release tablets (H2012-0518, Bard Pharmaceuticals Ltd.) once a day, 10 mg per time. Patients in the study group received local radiotherapy combined with alendronate (H20057226, Shaanxi Hanwang Pharmaceutical Co., Ltd.). Alendronate (70 mg) was taken orally with warm water once daily. Patients in both groups were followed up for three months.

Outcome measures

The baseline data, including sex, age, Child-Pugh classification, site for bone metastases and liver cancer course were observed and compared between the two groups. Child-Pugh classification was used for the assessing liver function. Variables measured by this system include bilirubin, serum albumin, prothrombin time, ascites and encephalopathy. Class A: 5 or 6 points, indicating good liver function; class B: 7-9 points, indicating moderate liver function;

Table 1. Comparison of general data n/($\bar{x}$ ± sd)

Group	Control group (n=33)	Study group (n=35)	χ^2/t	P
Sex			0.198	0.656
Male	19	22		
Female	14	13		
Age (years old)	58.1±5.2	57.8±5.4	0.233	0.816
Child-Pugh classification			0.182	0.670
A	7	6		
B	23	25		
C	3	4		
Site for bone metastases			0.157	0.692
Limbs	2	3		
Pelvis	5	5		
Spine	26	27		
Liver cancer course (month)	6.28±2.06	6.09±2.21	0.366	0.716

Note: χ^2, data from Pearson's chi-square test; t, data from independent sample t test.

class C: over 10 points, indicating poor liver function.

After treatment, Visual Analogue Scale (VAS) was used to determine the remission rate for patients. VAS is a 10-point scale [10]. Higher VAS scores indicate more intense pain. A reduction of more than 2 points in the VAS scores after treatment was defined as markedly effective. That of more than 1 point but less than 2 points was regarded as effective. No change was considered as ineffective. Remission rate = markedly effective rate + effective rate.

The adverse reactions, such as rash, dry mouth, constipation and thrombocytopenia were recorded during treatment in the two groups. Adverse reaction rate = (cases with adverse reaction/total cases) *100%.

Before and 1 day after treatment, fasting venous blood was collected in the early morning. The blood was centrifugated at 3000 r/min for 10 min, then the supernatant was collected. The automatic biochemical analyzer AU5800 was applied to determine the levels of total bilirubin (TBIL), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

Akt (EK-H12357) and GSK3β (30176062) levels were measured by enzyme-linked immunosorbent assay (ELISA) before and 1 day after treatment.

ELISA was used to measure osteocalcin (OST, YS-2128R) and bone alkaline phosphatase (BAP, P0200) levels before and 1 day after treatment.

Before and 1 day after treatment, Chemiluminescence was adopted for measuring α-fetoprotein (AFP) level. ELISA was applied to determine vascular endothelial growth factor (VEGF, PL0305641), osteopontin (OPN, YLK-E1857D), and matrix metallopeptidase 9 (MMP-9, EK-M26587) levels. The mentioned kits were provided by Beijing Kaishiyuan Biotechnology Co., Ltd. All steps were strictly following the instructions.

Before and 1 day after treatment, Life Change Index Scale was adopted to access psychological function, physical function, social function, hardship and nausea caused by cancer [11]. Among them, higher scores of psychological, physical, and social functions indicate higher quality of life. But as for the scores of hardship and nausea caused by cancer, the reverse was true.

Statistical analysis

SPSS 23.0 software was adopted for statistical analysis. Measurement data were expressed as mean ± standard deviation ($\bar{x}$ ± sd). Independent sample t test and paired sample t test were adopted to compare differences between groups and between before and after treatment within group, respectively. The count data were tested by Pearson's chi-square test and expressed as percentage (%). A *P*-value less than 0.05 is statistically significant.

Results

Comparison of general data

No significant difference was found in sex, age, Child-Pugh classification, site for bone metastasis and liver cancer course between the two groups (all P>0.05). See **Table 1**.

Comparison of remission rate

A higher remission rate was observed in the study group (94.29%) than that of the control group (75.76%) (P<0.05). See **Table 2**.

Table 2. Comparison of remission rate (n, %)

Group	Markedly effective rate	Effective rate	Ineffective rate	Remission rate
Control group (n=33)	14 (42.42)	11 (33.33)	8 (24.24)	25 (75.76)
Study group (n=35)	20 (57.14)	13 (37.14)	2 (5.71)	33 (94.29)
χ^2				4.649
P				0.031

Note: χ^2, data from Pearson's chi-square test.

Comparison of adverse reaction rate

There was no significant difference in terms of adverse reaction rate between the study group (20.00%) and the control group (12.12%) (P>0.05). See **Table 3**.

Comparison of liver function indexes

Before treatment, no significant difference was found in liver function indexes (P<0.05). After treatment, the serum levels of TBIL, ALT and ALP were decreased in both groups, and the study group had lower indexes than the control group (all P<0.05). See **Table 4**.

Comparison of serum Akt/GSK3β levels

There was no significant difference in serum Akt/GSK3β levels between the groups before treatment (P>0.05). After treatment, the serum Akt/GSK3β levels of the two groups were decreased (P<0.001). The levels in the study group were lower than those in the control group (P<0.001). See **Table 5**.

Comparison of bone metabolism levels

Before treatment, no significant differences in bone metabolism levels were observed between the two groups (P>0.05). After treatment, the serum BAP and OST levels in the two groups were increased, and the study group had higher levels than the control group (P<0.001). See **Figure 1**.

Comparison of AFP, VEGF, OPN and MMP-9 levels

There were no significant differences in serum AFP, VEGF, OPN and MMP-9 levels between the two groups before treatment (all P>0.05). After treatment, the above indicators were reduced in both groups, and the levels in the study group were lower than those in the control group (P<0.001). See **Figure 2**.

Comparison of quality of life

No significant difference in survival rate was observed between the two groups (P>0.05). The scores of psychological function, physical function and social function were increased and the scores of hardship and nausea caused by cancer were decreased significantly in both groups. The study group had better psychological function, physical function, social function, and less hardship and nausea caused by cancer than the control group (all P<0.05). See **Table 6**.

Discussion

In recent years, the survival time and quality of life of patients with primary liver cancer have been significantly improved due to the rapid progress in medical science and technology. However, the incidence of bone metastases is also rising. Patients with bone metastases from primary liver cancer are often accompanied by severe bone pain. The behind mechanisms of the pathogenesis are as the following: (1) In the process of tumor cell division and reproduction, inflammatory cytokines proliferate rapidly. It causes a surge in pain-causing mediators such as tumor necrosis factor α and prostaglandins, which promote the sensitivity of peripheral nerves, resulting in severe pain. (2) Proliferation and invasion of tumor cells penetrate into the bones and then cause abnormal damage and dissolve the bone to induce pain. (3) In addition, tumor cells can even penetrate into the soft tissues and periosteum around the bone, leading to severe pain [12-14].

In this study, the study group had higher remission rate, better liver function indexes and quality of life than the control group after treatment, and the adverse reaction rate in the two groups was comparable. It was suggested that alendronate combined with local radiotherapy may safely and effectively alleviate the pain, and improve the liver function and quality of life of patients with primary liver cancer and with bone metastases. The possible reasons are (1) local radiotherapy effectively shrinks the secondary bone site, and reduces the pressure in the bone marrow cavity and periosteum. (2) Local radiotherapy quickly kills inflammatory

Table 3. Comparison of adverse reaction rate (n, %)

Group	Rash	Dry mouth	Constipation	Thrombocytopenia	Total
Control group (n=33)	1 (3.03)	1 (3.03)	1 (3.03)	1 (3.03)	4 (12.12)
Study group (n=35)	1 (2.86)	2 (5.71)	2 (5.71)	2 (5.71)	7 (20.00)
χ^2					0.778
P					0.378

Note: χ^2, data from Pearson's chi-square test.

Table 4. Comparison of liver function indexes ($\bar{x}$ ± sd)

Group	Total bilirubin (μmol/L)		Alanine aminotransferase (U/L)		Alkaline phosphatase (U/L)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group (n=33)	21.25±2.94	17.51±1.71***	82.63±6.28	75.17±5.62***	125.32±13.36	118.36±8.62***
Study group (n=35)	21.53±2.88	14.32±1.43***	82.22±8.17	66.15±5.21***	124.21±13.08	110.29±7.35***
t	0.397	8.363	0.231	6.868	0.346	4.162
P	0.693	<0.001	0.818	<0.001	0.730	<0.001

Note: Compared with the same group before treatment, ***P<0.001. t, data from independent sample t test.

Table 5. Comparison of serum Akt/GSK3β levels ($\bar{x}$ ± sd)

Group	Akt (ng/mL)		GSK3β (μg/L)	
	Before treatment	After treatment	Before treatment	After treatment
Control group (n=33)	278.61±58.23	207.58±45.67***	35.43±3.12	24.17±2.18***
Study group (n=35)	280.39±62.82	152.84±34.65***	35.03±3.03	16.37±2.26***
t	0.121	5.588	0.536	14.470
P	0.904	<0.001	0.594	<0.001

Note: Compared with the same group before treatment, ***P<0.001. t, data from independent sample t test.

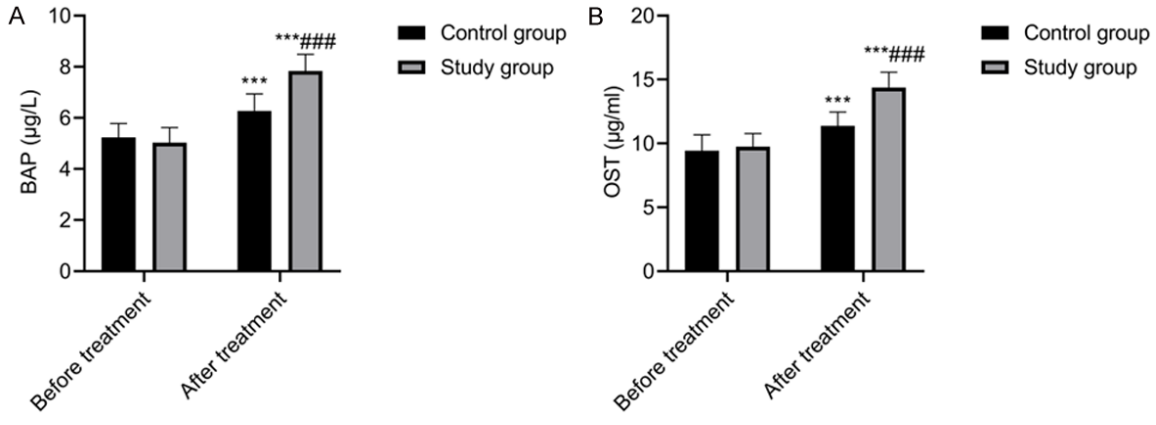

Figure 1. Effects of alendronate combined with local radiotherapy on bone metabolism levels in patients with bone metastases from primary liver cancer. A: BAP (μg/L); B: OST (μg/mL). BAP: bone-specific alkaline phosphatase; OST: osteocalcin. Compared with the same group before treatment, ***P<0.001; Compared with the control group, ###P<0.001. t, data from independent sample t test.

cells capable of releasing cytokines that cause pain in the tissues surrounding the secondary bone site. (3) Osteoclasts play an important role in bone metastasis from primary liver cancer, and local radiotherapy effectively inhibits osteoclast activity [15, 16]. As a common drug

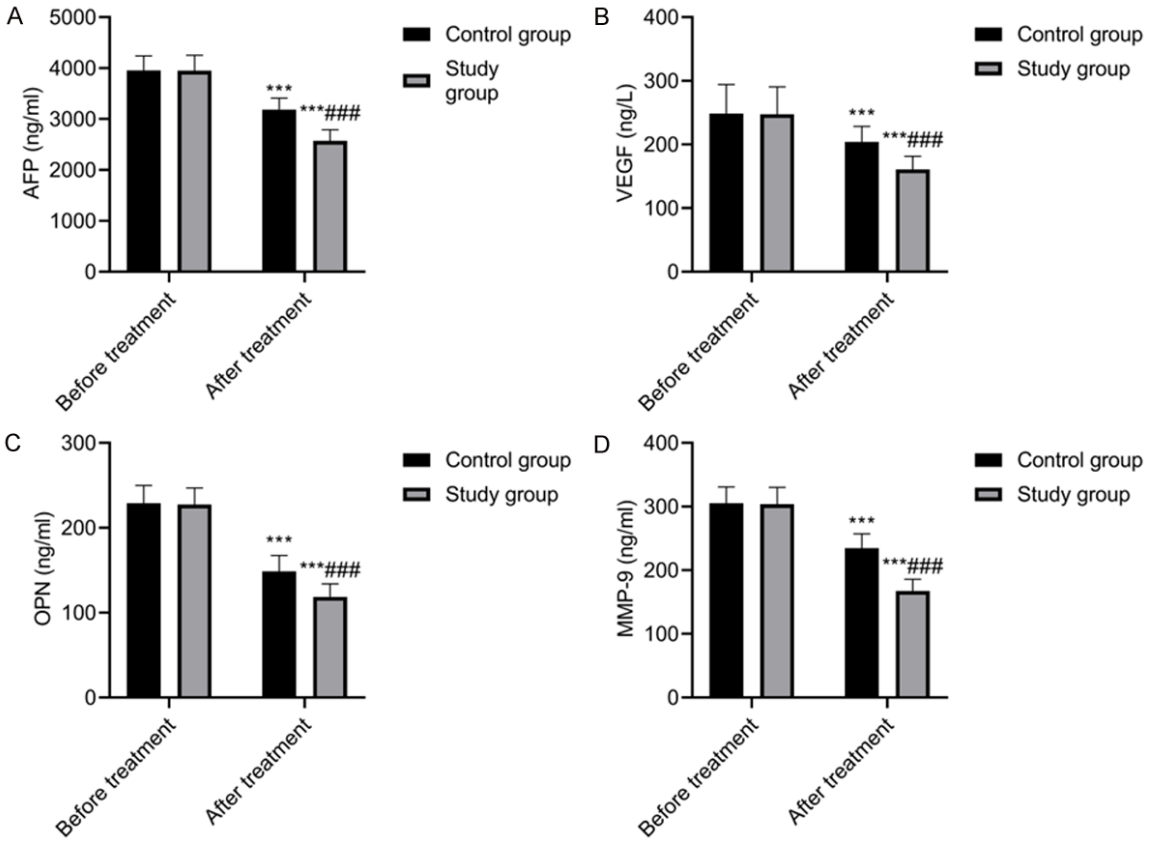

Figure 2. Effects of alendronate combined with local radiotherapy on serum AFP, VEGF, OPN and MMP-9 levels in patients with bone metastases from primary liver cancer. A: AFP (ng/ml); B: VEGF (ng/ml); C: OPN (ng/ml); D: MMP-9 (ng/ml). AFP: α-fetoprotein; VEGF: vascular endothelial growth factor; OPN: osteopontin; MMP-9: matrix metallopeptidase 9. Compared with the same group before treatment, $^{***}P<0.001$; Compared with the control group, $^{###}P<0.001$.

that inhibits bone resorption by osteoclasts, alendronate acts by inhibiting the activity of osteoclasts, resulting in bone resorption disorders [17]. Meanwhile, it shows affinity with calcium phosphate, which plays an important role in promoting bone structure stability and increasing bone density, effectively reducing the pathological fractures [18, 19].

Clinically, Akt and GSK3β are closely related to bone metastases from primary liver cancer [20]. As a serine/threonine protein kinase, Akt is involved in protein degradation, as well as cell proliferation and apoptosis. It is a pivotal initiator of the PI3K/Akt signaling pathway. The function of osteoblasts and osteoclasts is regulated by activating the PI3K/AKT signaling pathway to maintain the dynamic balance of bone tissue. Activation of the Akt signaling pathway effectively promotes the proliferation and metastasis of tumor cells in the course of malignant tumors [21]. GSK3β belongs to the glucan synthase family and regulates bone homeostasis through Wnt signaling pathway. GSK3β inhibits the expression of MMP-3 and lymphocytoma-2 by accelerating the degradation of β-catenin, thereby inhibiting tumor cell proliferation, invasion and metastases [22]. If a balance between osteoclasts and osteoblasts in patients with bone metastases from primary liver cancer is broken, the rate of femoral conversion is increased, and bone lysis and resorption are accelerated, resulting in pathological fractures [23]. BAP and OST have the effects of regulating bone calcium metabolism and reflecting bone metabolism. AFP, a glycogen protein, is an important tumor marker to help detect and diagnose liver cancer and a vital indicator to determine tumor recurrence and metastases [24]. Cytokines such as VEGF play an important role in the process, where the proliferation of tumor neovascularization is

Table 6. Comparison of quality of life ($\bar{x} \pm sd$, point)

Group	Psychological function		Physical function		Social function		Hardship caused by cancer		Nausea caused by cancer	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group (n=33)	25.38±2.19	31.58±2.77***	35.51±3.41	41.21±3.18***	6.80±1.37	9.68±1.64***	15.28±1.61	10.52±1.76***	13.28±1.39	10.55±1.50***
Study group (n=35)	25.73±2.37	35.67±3.11***	35.89±2.96	47.52±3.66***	6.52±1.43	13.53±1.82***	15.61±1.72	7.83±1.56***	13.53±1.46	7.65±1.60***
t	0.631	5.714	0.492	7.569	0.824	9.145	0.816	7.128	0.722	8.230
P	0.530	<0.001	0.624	<0.001	0.413	<0.001	0.417	<0.001	0.473	<0.001

Note: Compared with the same group before treatment, ***P<0.001. t, data from independent sample t test.

inseparable in bone metastases [25]. OPN is a specific link to its receptor-binding protein (alphavβ3) that induces bone metastases of tumors by activating downstream signaling pathways. At the same time, the activity of osteoclasts can also be controlled by the secretion of MMP-9, which triggers the bone transmission of tumors, and causes intense pain [26]. MMP-9, a proteolytic enzyme that promotes tumor cell adhesion and neovascularization, is involved in the metastasis and infiltration of tumor cells. In this study, after treatment, the study group had higher Akt, GSK3β, BAP, OST, AFP, VEGF, OPN and MMP-9 levels than the control group. Alendronate combined with local radiotherapy may make a difference in the treatment of bone metastases from primary liver cancer by inhibiting serum Akt, GSK3β levels, regulating bone metabolism and inhibiting tumor angiogenesis.

However, this was a small sample size and single-center retrospective analysis, which did not assess long-term effects of alendronate combined with local radiotherapy on the prognosis of patients with primary liver cancer and with bone metastasis. In the future, large-size, multi-center research should be carried out.

In short, the combination of alendronate and local radiotherapy can reduce pain, improve liver function, regulate bone metabolism levels, and improve quality of life of patients with primary liver cancer and with bone metastasis. It has a good safety, and the mechanism may be related to regulation of the levels of Akt and GSK3β.

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Disclosure of conflict of interest

None.

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