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Review

Anticancer Effects of Constituents of Herbs Targeting Osteosarcoma*

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ABSTRACT Osteosarcoma is a rare primary malignancy of bone that is prone to early metastasis. Resection surgery and chemotherapeutic regimens are current standard treatments for osteosarcoma. However, the long-term survival rate of patients with osteosarcoma is low due to a high risk of metastasis. Hence, a new approach is urgently needed to improve the treatment of osteosarcoma. Compared with chemotherapy, natural active constituents isolated from herbs exhibit less adverse effects and better anti-tumor effects. This study aimed to summarize the anticancer effects of constituents of herbs on the progression and metastasis of osteosarcoma cells. It showed that many constituents of herbs inhibited osteosarcoma by targeting proliferation, matrix metalloproteinases, integrin and cadherin, and angiogenesis. The findings might be beneficial for the development of new drugs and treatment strategies.

KEYWORDS constituents of herbs, osteosarcoma, metastasis, review

Osteosarcoma is an aggressive bone cancer commonly found in children and adolescents. Pediatric patients between 10 and 20 years of age contribute to approximately 6% of cases.[1] The important clinical symptoms of osteosarcoma include local tissue enlargement induced by abnormal bone hyperplasia, consequent pain, and rapid deterioration. For the majority of patients, osteosarcoma has become a high-grade malignant tumor with metastasis at the time of diagnosis.[2] As osteosarcoma is an intractable disease, the early exploration stage of treatment is resection. However, the outcome is usually poor. Patients with osteosarcoma undergoing amputation are prone to metastasis within 1 year, mainly in the lungs and less common in the brain, kidney, and abdomen. Hence, lung cancer and respiratory failure become the main cause of death.[3] With the advent of multiagent chemotherapeutic regimens, the 5-year survival rate of patients with nonmetastatic osteosarcoma steadily improved from less than 20% to 70% in the 1970s.[4] However, no significant improvements were achieved using the combination of surgery and chemotherapy in the last decades, especially in patients with metastatic or recurrent osteosarcoma having a 5-year event-free survival less than 30%.[5] Hence, a new therapeutic approach is urgently needed to improve the outcomes for these patients.

Natural constituents from a variety of natural resources (mostly herbs) are good sources for developing new drugs for cancer prevention and treatment.[6-8] Herbal active constituents contain a variety of chemical components, contributing to their medical benefits. They are effective at inhibiting or killing the cancer cells by targeting multiple signaling pathways.[6,9,10] These natural constituents have fewer side effects and no chemotherapy resistance. Therefore, they are being recognized and accepted as an alternative treatment for combating the side effects of cancer treatments, such as pain, fatigue, and sleep problems.[11,12]

Pathogenesis of Osteosarcoma Metastasis

Osteosarcoma originates from mesenchymal stem cells or mutated osteoblasts.[13] Histologically, although tumors with osteoblast phenotype

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accounted for the majority (60%–70%), no evidence shows that the metastasis is dependent on the cell types.[14] Unfortunately, the genome-wide microarray analysis of osteosarcoma cells and tumor tissues revealed an unstable genomic background[15] and profound tumor heterogeneity.[16] For example, 33% of primary osteosarcoma is associated with chromosomal breakage, chromosomal ablation, or chromothripsis,[17,18] which may induce tumorigenesis and promote cancer cell metastasis.[19,20] However, no specific genetic mutations or biomarkers related to osteosarcoma metastasis were identified in genetic distortion analysis or genome-wide studies on osteosarcoma tissues.[19,21] Most patients with osteosarcoma do not have a family history of this disease.[22] Therefore, the mechanism driving osteosarcoma metastasis is still ambiguous.

The major concern is a high mortality in these patients. Therefore, a new therapeutic approach needs to be developed to target cancer metastasis. In general, the genetic instability of cancer cells is due to the original motility of osteosarcoma cells undergoing metastasis. The metastasis usually begins with invading the surrounding tissues and then leaving the primary tumor site. After the tumor cells enter the bloodstream, they circulate via the lymphatic system and seed in distant organs. Metastasis finally results in measurable metastatic lesions.[23]

Although the mechanism driving osteosarcoma metastasis has not been identified, many potential targetable genes have been assessed in preventing osteosarcoma metastasis and exhibited promising therapeutic effects.[24] These molecular targets include oncogenes and tumor suppressor genes that affect gene stability, relevant molecules contributing to the escape of the tumor from the original site, and angiogenic molecules. Many constituents of herbs have been demonstrated to exert an anticancer effect on osteosarcoma, and some have remarkable capacities to block cell metastasis. Recent studies using constituents of herbs to prevent the progression and metastasis of osteosarcoma are shown in Table 1.

Constituents of Herbs Inhibiting Osteosarcoma

Chinese herbal extracts are prepared in a variety of solvents and methods. The combination of active phytochemicals from multiple herbal plants displays a strong anticancer activity. A meta-analysis revealed that the external application of Chinese medicine

Table 1. Effect of Constituents of Herbs Targeting Osteosarcoma Progression and Metastasis

Herbal extract	Source	Target cell line	Target molecule	Effect	References
Chamaejas-mine	*Stellera chamaejasme* L.	MG63	p53, Bcl-2, P21, Bax, caspase-3	Anti-proliferative, promoting apoptosis	Yang, et al[25]
Berberine	*Rhizoma coptidis*	MG-63		DNA damage, promoting apoptosis	Zhu, et al[26]
		U2OS	PI3K/Akt	Anti-proliferative, promoting apoptosis	Chen[27]
		U2OS, Saos-2, HOS	p53	DNA damage	Liu, et al[28]
		MG63	VE-cadherin, integrin β3	Inhibiting adhesion and angiogenesis	Yu, et al[29]
Artemisinin	*Gaeddongssuk*	HOS, MG-63, U2OS, Saos-2	Bcl-2, Bax, FAS, caspases	Blocking growth, inducing apoptosis (especially p53 wild-type cells)	Ji, et al[30]
WIN-55,212-2	*Cannabis*	MG-63	Notch-1, MMP-2, VEGF	Inhibiting matrix degradation and angiogenesis	Niu, et al[31]
Shikonin	*Lithospermum*	U2OS	MMP-13	Inhibiting matrix degradation	Deng, et al[32]
Deguelin	*Derris trifoliata* Lour. or *Mundulea sericea* (Leguminosae)	U2OS	MMP-2, MMP-9	Anti-proliferative, inhibiting matrix degradation	Shang, et al[33]
Piperine	*Black pepper*	HOS, U2OS	MMP-2, MMP-9, TIMP-1/-2	Anti-proliferative, inhibiting matrix degradation	Zhang, et al[34]
Paris saponin Ⅶ	*Trillium tschonoskii Maxim*	HOS, U2OS	MMP-2, MMP-9, p38 MAPK	Anti-proliferative, inhibiting matrix degradation	Cheng, et al[35]
Nimbolide	*Melia azedarach*	MG63, U2OS, HOS	Integrin αvβ5	Inhibiting adhesion	Liu, et al[36]
Sinomenine	*Sinomenium acutum Rehd. et Wils*	HOS, U2OS	MMP-2, MMP-9, RANKL, VEGF	Inhibiting proliferation and angiogenesis	Xie, et al[37]

Notes: FAS: factor-related apoptosis; MMP: matrix metalloproteinase; RANKL: receptor activator for nuclear factor-κ B ligand; TIMP: tissue inhibitor of metalloproteinase; VEGF: vascular endothelial growth factor

could promote pain relief and improve the quality of life for patients with bone cancer.[38] The constituents of herbs are usually isolated as natural health products, which are further analyzed in the laboratory. The anticancer effects of these constituents have been widely evaluated on animal models and *in vitro* osteosarcoma cell lines. The findings suggested a promising application of these constituents of herbs in anti-osteosarcoma therapy (Figure 1).

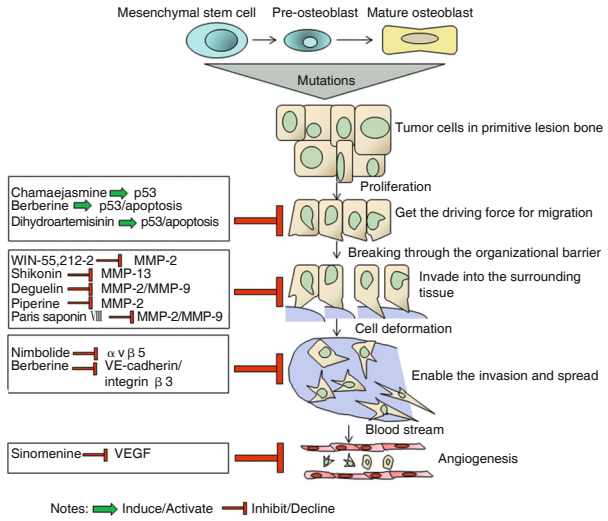

Figure 1. Progression and Metastatic Cascade in Osteosarcoma Cells and Targets of Constituents of Herbs

Blocking of Cell Cycle and Inhibition of Tumor Growth

Tumor metastasis is a complex process. The most critical modification is the conversion of static primary tumors into invasive disseminating metastasis after the loss of control of cell proliferation leading to tumorigenesis.[39] Osteosarcomas are considered to be the most disordered cancers.[40] High-grade osteosarcomas contain as high as or even higher levels of genomic instability compared with any other cancers. Therefore, they are ideally suited for genomic instability analyses.[41]

Cell cycle abnormalities and DNA repair inactivation can undermine DNA integrity and cause chromosomal gains, losses, and rearrangements, leading to genomic instability and hence increasing the metastasis frequency.[42] Almost all of the osteosarcoma samples showed p53 pathway damage.[43,44] The primary function of p53 is to prevent cells from passing through the cell cycle when they undergo stress, damage, or errors that interfere with the preprogrammed cell cycle progression.[45] Loss of p53 allows the accumulation of cells with DNA damage.[46]

Detection of primary osteosarcoma samples revealed that p53 mutations were significantly associated with total genomic DNA instability, suggesting that p53 was an effective biomarker of osteosarcoma survival.[47] p53 deficiency can increase the ability of cells to migrate, including cell spread, establishing cell polarization and causing protrusions.[39,48] Activated p53 induces the expression of many target genes that mediate several tumor-suppressing mechanisms, such as cell cycle arrest, programmed cell death, aging, and angiogenesis inhibition.[49] Several herbal extracts exhibit regulatory effects on the activation of p53 and promote DNA repair in osteosarcoma.

As the major ingredient in *Stellera chamaejasme* L., chamaejasmine (Figure 2A) had a profound anti-proliferative effect on human osteosarcoma cells in a concentration- and time-dependent manner. The major anticancer effect of this herbal extract was mediated through the induction of p53 activation. Chamaejasmine significantly elevated the expression of p53, increased the activity of P21, Bax, and caspase-3, and reduced the expression of Bcl-2 in cells.[25]

Berberine (Figure 2B), an alkaloid isolated from *Rhizoma coptidis*, is widely used for treating inflammation and cancer. Berberine has a strong anti-osteosarcoma effect, but the toxic effect on normal cells is quite low.[29] Berberine displayed a strong toxic effect on MG-63 cells, one of human osteosarcoma cell lines, resulting in DNA damage and cell apoptosis in MG-63 cells.[26] Other experimental results suggested that berberine could inhibit proliferation and induce the apoptosis of U2OS cells by inhibiting the activation of PI3K/Akt signaling pathway,[27] or alleviate the symptoms of osteosarcoma and inhibit the growth of Saos-2 and MG-63 cell lines by the downregulation of caspase-1/IL-1β signaling axis.[50] In addition, a previous study concluded that the toxic effect of berberine on U2OS, Saos-2, and HOS cell lines was partly dependent on p53-mediated G_1 cell cycle arrest and DNA double-strand breaks, which in turn triggered p53-dependent cell apoptosis.[28] In short, berberine inhibited the growth and metastasis of osteosarcoma cells through multiple pathways and hence deserved further evaluation in animal models and clinical trials.

Artemisinin, a natural product isolated from Chinese medicinal herb Gaeddongssuk (*Artemisia annua* L.),

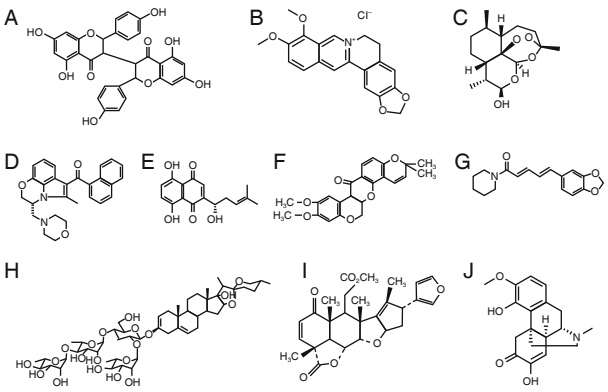

Figure 2. Natural Constituents of Herbs Targeting Osteosarcoma

Notes: A: chamaejasmine (MW 542); B: berberine (MW 371.81); C: dihydroartemisinin (MW 284.35); D: WIN 55,212-2 (MW 430.52); E: shikonin (MW 288.3); F: deguelin (MW 394.42); G: piperine (MW 285.34); H: paris saponin Ⅶ (MW 1031.2); I: nimbolide (MW 466.52); J: sinomenine (MW 329.38) MW: molecular weight.

its main active derivative dihydroartemisinin (DHA, Figure 2C) has been used to treat malaria and fever. Recent studies reported that artemisinin and its derivatives could inhibit the growth and metastasis of cells associated with melanoma, lung tumor, and liver cancer.[51-53] The four cell lines HOS, MG-63, U2OS, and Saos-2 with different p53 statuses were treated with DHA. The results showed that DHA induced G_2/M cycle arrest of all types of cells and led to cell apoptosis by increasing Bax/bal-2 ratio and FAS-dependent caspase activation.[30] DHA-induced apoptosis and cell cycle arrest were more pronounced in U2OS (p53 wild-type) cells compared with p53-inactivated osteosarcoma cells,[30] indicating that functional p53 activation might play an important role in the inhibition effect of DHA on osteosarcoma cells.

Inhibition of Expression of Matrix Metalloproteinases

The osteosarcoma cells invade the surrounding cells and interrupt tissue barriers, such as the extracellular matrix (ECM) and the basement membrane before they travel to the surrounding blood vessels and tissues along the degraded ECM. The metastatic potential of various cancers is associated with the ability of the basement membrane to be degraded. Therefore, matrix metalloproteinases (MMPs) have been considered as the potential diagnostic and prognostic biomarkers in many cancers.[54] MMPs are important proteolytic enzymes that hydrolyze ECM. MMPs not only participate in the invasion of osteosarcoma cells,[55,56] but also are involved in the cytokine-mediated immune response to metastatic osteosarcoma cells.[57] Interestingly, the high expression levels of MMP-2, MMP-9, and MMP-13 have been found to be associated with the high metastatic ability of osteosarcoma tissue.[56,58-60]

Cannabis is another natural active ingredient from cannabis plants, and WIN-55, 212-2 (Figure 2D) is a synthetic cannabinoid. An *in vitro* study showed that the expression levels of Notch-1, MMP-2, and VEGF in MG-63 cells were downregulated following the treatments with cannabinoid alone or its combination with adriamycin, suggesting that cannabinoid could inhibit the migration, invasion, and angiogenesis of osteosarcoma cells.[31]

Shikonin (Figure 2E), one of the active ingredients of *Lithospermum*, has anti-inflammatory, anti-cancerous, anti-microbial, and wound-healing effects.[61] A high dose (> 5 μmol/L) of shikonin directly inhibited the proliferation of osteosarcoma cells by inducing receptor-interacting protein 1 (RIP) 1- and RIP3-dependent necroptosis.[62] Moreover, low doses of shikonin (0.1 and 1 μmol/L) reduced osteosarcoma cell migration in wound healing and transwell cell migration assays. This anti-metastatic effect was mediated through the reduction of MMP-13 but had no effect on the expression of MMP-7 and MMP-9.[32]

Deguelin (Figure 2F) is a common rotenone compound in plants. It has been reported to affect different types of human cancer cells.[63-65] Shang, et al[33] found that deguelin slowed down the proliferation of osteosarcoma cells U2OS and suppressed the migration of osteosarcoma cells by inhibiting the activity of MMP-2 and MMP-9.

To date, many constituents of herbs have demonstrated an anti-metastatic effect on osteosarcoma cells by manipulating the activity of MMP-2 and MMP-9. Piperine (Figure 2G) is the main component from black pepper that has shown an inhibitory effect on MMP-2/-9 activity through the increased expression of tissue metalloproteinase inhibitor (TIMP)-1/-2.[34] Paris saponin Ⅶ (Figure 2H), another herbal extract, can downregulate the phosphorylation of p38MAPK pathway in osteosarcoma cells in a dose- and time-dependent manner to reduce the expression of MMP-2/-9, thereby inhibiting the migration and invasion of osteosarcoma cells.[35]

Reduction of Expression of Integrin and Cadherin

The integrin and cadherin are important adhesion molecules associated with tumor cell invasion and

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metastasis. The transmembrane complex is formed by the combination of integrin and extracellular matrix proteins, which not only induce cytoskeletal rearrangement, but also serve as a bridge to transmit cell signals bidirectionally. The expression of integrins can affect the biological behavior of cell invasion and metastasis.[66] Av β 5, an integrin, is mainly expressed on tumor cells, and has been correlated with the metastasis of osteosarcoma.[67] The identification of phenotypes of primary osteosarcoma cells in *in vitro* and *in vivo* studies found that α v β 5 was highly expressed in most of primary osteosarcoma cells.[68]

Nimbolide is a natural compound isolated from the leaves of the genus *Melia azedarach* (Figure 2I). It can inhibit the growth of cancer cells by inducing apoptosis and anti-angiogenesis and also suppress metastasis through multiple pathways.[69] The study regarding the regulation of osteosarcoma metastasis by nimbolide is still under investigation. A study suggested that nimbolide hindered the migration of osteosarcoma cells by the reduction of the expression of integrin α v β 5 in osteosarcoma cell lines, which was mediated through PI3K/Akt and NF-κ B signaling pathways.[36]

Homotypic cadherin molecules are ligands and receptors involved in establishing adherent junctions among tumor cells. The microarray analysis of osteosarcoma samples revealed that one of the significant enrichment routes of the differentially expressed genes was lesion adhesion.[70] Of these, vascular endothelial-cadherin is the most important adhesion molecule of vascular endothelial cells. In addition to promoting tumor cell adhesion, VE-cadherin transports intracellular signaling molecules to help maintain endothelial integrity and vascular stability, which is critical for the migration and colonization of tumor cells. Osteosarcoma cells have more potential to transdifferentiate and form blood vessels compared with endothelial cells, in which VE-cadherin may be involved in transdifferentiation and mimicking the endothelial function of osteosarcoma.[71] The siRNA-mediated knockdown of VE-cadherin can block the migration of osteosarcoma cells.[72]

Yu, et al[29] showed that berberine decreased the expression of VE-cadherin and integrin β 3 *in vitro*, preventing the migration of MG63 cells and formation of vascular networks.[29] It suggested that berberine could inhibit the transdifferentiation of osteosarcoma cells into endothelial cells and adhesion after transdifferentiation in early angiogenesis.

Inhibition of Angiogenesis

The growth and metastasis of sarcoma depend on angiogenesis.[73] Due to the heterogeneity of tumor mesenchymal origin, osteosarcoma has a characteristic of blood-derived spread.[23] Abnormal vasculatures are an important source of tumor metastatic spread and also constitute a huge obstacle to the delivery of therapeutic drugs.[74] VEGF is a key regulator of angiogenesis and can induce the formation of different types of tumor blood vessels.[75] The level of VEGF expression is significantly related to the overall survival rate of patients with osteosarcoma.[76] Further studies highlighted that the overexpression of VEGF was associated with the high grade and metastasis of osteosarcoma.[77] The overexpression of VEGF and MMP-inducible factor was detected in tumor tissues from patients with osteosarcoma. Overall survival and disease-free survival were significantly reduced in these patients.[78] An *in vitro* study confirmed that VEGF could interact with metalloproteinases to form a tumor matrix that promoted tumor growth and metastasis.[79]

Sinomenine is an effective anti-inflammatory analgesic ingredient derived from the Chinese medicinal plant *Sinomenium acutum* Rehd. et Wils (Figure 2J). The inhibitory effect of sinomenine on tumor invasion and migration has been demonstrated in osteosarcoma cell lines (HOS and U2OS). It induced a reduced migration and invasion while inhibiting tumor tubular formation in U2OS cells.[37] The reduction of VEGF expression through CXCR4-STAT3 signaling pathways may contribute to the hindering of VEGF-related neovascularization and bone destruction.[37]

Prospects

The difficulty in treating osteosarcoma metastasis is largely due to the lack of specific targeting agents. Therefore, understanding the molecular biology of osteosarcoma metastasis is critical for establishing a new treatment. From the 1940s to the end of 2014, 175 small-molecule antineoplastic drugs were approved by the Food and Drug Administration and similar organizations, of which 49% were natural products or derived directly from natural products.[80] Natural products have been vital in discovering the potential of drugs for treating human diseases.[81] The constituents of herbs exhibit promising anticancer effects in osteosarcoma

cells.[82] However, the studies on the application of these herbs are still limited by the heterogeneity of this disease and the complexity of the natural plant extracts.

Future studies need to quickly and comprehensively clarify the safety, efficacy, and stability of the constituents of herbs and understand their anticancer effects on the pathogenesis of osteosarcoma. Complex signaling is one of the characteristics of recurrent osteosarcoma.[81] Hence, the tumorigenesis of osteosarcoma does not follow a simple pattern.[83] Phenotypic screening in natural product research may be more favorable.[84] In addition, the development of reliable animal models is critical to evaluating the drug activity and studying tumor biology.[85] Transplanted and genetically engineered mice are commonly used in osteosarcoma studies for cancer drug discovery.

In conclusion, the constituents of herbs provide a new approach to reduce the incidence and mortality of osteosarcoma. However, it is still a great challenge to clarify the anti-osteosarcoma mechanism of each constituent and evaluate the safety of their application in the clinic.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Su QH and Bian SS conceived and participated in manuscriptwriting. Xu XQ and Luan JW also contributed to manuscript writing. Luan JW, Huang HY and Ren X were engaged in correcting the text.

REFERENCES

1. Vander Griend RA. Osteosarcoma and its variants. Orthop Clin North Am 1996;27:575-581.
2. Krishnan K, Khanna C, Helman LJ. The biology of metastases in pediatric sarcomas. Cancer J 2005;11:306-313.
3. Yang Z, Li X, Yang Y, He Z, Qu X, Zhang Y. Long noncoding RNAs in the progression, metastasis, and prognosis of osteosarcoma. Cell Death Dis 2016;7:e2389.
4. Isakoff MS, Bielack SS, Meltzer P, Gorlick R. Osteosarcoma: current treatment and a collaborative pathway to success. J Clin Oncol 2015;33:3029-3035.
5. Gorlick R, Anderson P, Andrulis I, Arndt C, Beardsley GP, Bernstein M, et al. Biology of childhood osteogenic sarcoma and potential targets for therapeutic development: meeting summary. Clin Cancer Res 2003;9:5442-5453.
6. Yarla NS, Bishayee A, Sethi G, Reddanna P, Kalle AM, Dhananjaya BL, et al. Targeting arachidonic acid pathway by natural products for cancer prevention and therapy. Semin Cancer Biol 2016;40-41:48-81.
7. Tundis R, Xiao J, Loizzo MR. Annona species (Annonaceae): a rich source of potential antitumor agents? Ann N Y Acad Sci 2017;1398:30-36.
8. Hua F, Shang S, Hu ZW. Seeking new anti-cancer agents from autophagy-regulating natural products. J Asian Nat Prod Res 2017;19:305-313.
9. Mishra BB, Tiwari VK. Natural products: an evolving role in future drug discovery. Eur J Med Chem 2011;46:4769-4807.
10. Taylor WF, Jabbarzadeh E. The use of natural products to target cancer stem cells. Am J Cancer Res 2017;7:1588-1605.
11. Zhang Q, Feng Y, Kennedy D. Multidrug-resistant cancer cells and cancer stem cells hijack cellular systems to circumvent systemic therapies, can natural products reverse this? Cell Mol Life Sci 2017;74:777-801.
12. Suh N, Luyengi L, Fong HH, Kinghorn AD, Pezzuto JM. Discovery of natural product chemopreventive agents utilizing HL-60 cell differentiation as a model. Anticancer Res 1995;15:233-239.
13. Mutsaers AJ, Walkley CR. Cells of origin in osteosarcoma: mesenchymal stem cells or osteoblast committed cells? Bone 2014;62:56-63.
14. Bacci G, Bertoni F, Longhi A, Ferrari S, Forni C, Biagini R, et al. Neoadjuvant chemotherapy for high-grade central osteosarcoma of the extremity. Histologic response to preoperative chemotherapy correlates with histologic subtype of the tumor. Cancer 2003;97:3068-3075.
15. Kuijjer ML, Hogendoorn PC, Cleton-Jansen AM. Genome-wide analyses on high-grade osteosarcoma: making sense of a genomically most unstable tumor. Int J Cancer 2013;133:2512-2521.
16. Ren L, Mendoza A, Zhu J, Briggs JW, Halsey C, Hong ES, et al. Characterization of the metastatic phenotype of a panel of established osteosarcoma cells. Oncotarget 2015;6:29469-29481.
17. Jones MJ, Jallepalli PV. Chromothripsis: chromosomes in crisis. Dev Cell 2012;23:908-917.
18. Stephens PJ, Greenman CD, Fu B, Yang F, Bignell GR, Mudie LJ, et al. Massive genomic rearrangement acquired in a single catastrophic event during cancer development. Cell 2011;144:27-40.
19. Atiye J, Wolf M, Kaur S, Monni O, Bohling T, Kivioja A, et al. Gene amplifications in osteosarcoma-CGH microarray analysis. Genes Chromosomes Cancer 2005;42:158-163.
20. Kansara M, Teng MW, Smyth MJ, Thomas DM. Translational biology of osteosarcoma. Nat Rev Cancer 2014;14:722-735.
21. Khan J, Wei JS, Ringner M, Saal LH, Ladanyi M, Westermann F, et al. Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. Nat Med 2001;7:673-679.

22. Angulo P, Kaushik G, Subramaniam D, Dandawate P, Neville K, Chastain K, et al. Natural compounds targeting major cell signaling pathways: a novel paradigm for osteosarcoma therapy. J Hematol Oncol 2017;10:10.
23. Steeg PS. Targeting metastasis. Nat Rev Cancer 2016;16:201-218.
24. Reed DE, Shokat KM. Targeting osteosarcoma. Proc Natl Acad Sci U S A 2014;111:18100-18101.
25. Yang D, Wang P, Ren X. Apoptosis induced by chamaejasmine in human osteosarcoma cells through p53 pathway. Tumour Biol 2015;36:5433-5439.
26. Zhu Y, Ma N, Li HX, Tian L, Ba YF, Hao B. Berberine induces apoptosis and DNA damage in MG63 human osteosarcoma cells. Mol Med Rep 2014;10:1734-1738.
27. Chen ZZ. Berberine induced apoptosis of human osteosarcoma cells by inhibiting phosphoinositide 3 kinase/protein kinase B (PI3K/Akt) signal pathway activation. Iran J Public Health 2016;45:578-585.
28. Liu Z, Liu Q, Xu B, Wu J, Guo C, Zhu F, et al. Berberine induces p53-dependent cell cycle arrest and apoptosis of human osteosarcoma cells by inflicting DNA damage. Mutat Res 2009;662:75-83.
29. Yu D, Fu S, Cao Z, Bao M, Zhang G, Pan Y, et al. Unraveling the novel anti-osteosarcoma function of coptisine and its mechanisms. Toxicol Lett 2014;226:328-336.
30. Ji Y, Zhang YC, Pei LB, Shi LL, Yan JL, Ma XH. Anti-tumor effects of dihydroartemisinin on human osteosarcoma. Mol Cell Biochem 2011;351:99-108.
31. Niu F, Zhao S, Xu CY, Sha H, Bi GB, Chen L, et al. Potentiation of the antitumor activity of adriamycin against osteosarcoma by cannabinoid WIN-55,212-2. Oncol Lett 2015;10:2415-2421.
32. Deng B, Qiu B. Shikonin inhibits invasiveness of osteosarcoma through MMP13 suppression. Tumour Biol 2015;36:9311-9317.
33. Shang HS, Chang JB, Lin JH, Lin JP, Hsu SC, Liu CM, et al. Deguelin inhibits the migration and invasion of U-2 OS human osteosarcoma cells via the inhibition of matrix metalloproteinase-2/-9 *in vitro*. Molecules 2014;19:16588-16608.
34. Zhang J, Zhu X, Li H, Li B, Sun L, Xie T, et al. Piperine inhibits proliferation of human osteosarcoma cells via G_2/M phase arrest and metastasis by suppressing MMP-2/-9 expression. Int Immunopharmacol 2015;24:50-58.
35. Cheng G, Gao F, Sun X, Bi H, Zhu Y. Paris saponin Ⅶ suppresses osteosarcoma cell migration and invasion by inhibiting MMP2/9 production via the p38 MAPK signaling pathway. Mol Med Rep 2016;14:3199-3205.
36. Liu JF, Hou CH, Lin FL, Tsao YT, Hou SM. Nimbolide induces ROS-regulated apoptosis and inhibits cell migration in osteosarcoma. Int J Mol Sci 2015;16:23405-23424.
37. Xie T, Ren HY, Lin HQ, Mao JP, Zhu T, Wang SD, et al. Sinomenine prevents metastasis of human osteosarcoma cells via S phase arrest and suppression of tumor-related neovascularization and osteolysis through the CXCR4-STAT3 pathway. Int J Oncol 2016;48:2098-2112.
38. Yan X, Yan Z, Liu W, Ding H, Qiao S, Chen G, et al. External application of traditional Chinese medicine in the treatment of bone cancer pain: a meta-analysis. Support Care Cancer 2016;24:11-17.
39. Roger L, Gadea G, Roux P. Control of cell migration: a tumour suppressor function for p53? Biol Cell 2006;98:141-152.
40. Wolf NK, Largaespada DA, Moriarity BS. Coping with cancer genes altered by copy number. Oncotarget 2015;6:35155-35156.
41. Susanne L, Tale B, Sun J, Nome T, Vodák D, Bryne JC, et al. Unscrambling the genomic chaos of osteosarcoma reveals extensive transcript fusion, recurrent rearrangements and frequent novel TP53 aberrations. Oncotarget 2016;5:5273-5288.
42. Varki A, Varki NM, Borsig L. Molecular basis of metastasis. N Engl J Med 2009;360:1678-1679.
43. Chen X, Bahrami A, Pappo A, Easton J, Dalton J, Hedlund E, et al. Recurrent somatic structural variations contribute to tumorigenesis in pediatric osteosarcoma. Cell Rep 2014;7:104-112.
44. Kansara M, Teng MW, Smyth MJ, Thomas DM. Translational biology of osteosarcoma. Nat Rev Cancer 2014;14:722-735.
45. Kojima K, Ishizawa J, Andreeff M. Pharmacological activation of wild-type p53 in the therapy of leukemia. Exp Hematol 2016;9:791-798.
46. Khoury K, Dömling A. P53 Mdm2 inhibitors. Curr Pharm Des 2012;30:4668.
47. Fu HL, Shao L, Wang Q, Jia T, Li M, Yang DP. A systematic review of p53 as a biomarker of survival in patients with osteosarcoma. Tumour Biol 2013;34:3817-3821.
48. Song R, Tian K, Wang W, Wang L. P53 suppresses cell proliferation, metastasis, and angiogenesis of osteosarcoma through inhibition of the PI3K/AKT/mTOR pathway. Int J Surg 2015;20:80-87.
49. Muller PA, Vousden KH. p53 mutations in cancer. Nat Cell Biol 2013;1:2-8.
50. Jin H, Jin X, Cao B, Wang W. Berberine affects osteosarcoma via downregulating the caspase-1/IL-1beta signaling axis. Oncol Rep 2017;37:729-736.
51. Cabello CM, Lamore SD, Bair WB, 3rd, Qiao S, Azimian S, Lesson JL, et al. The redox antimalarial dihydroartemisinin targets human metastatic melanoma cells but not primary melanocytes with induction of NOXA-dependent apoptosis. Invest New Drugs 2012;30:1289-1301.
52. Tong Y, Liu Y, Zheng H, Zheng L, Liu W, Wu J, et al. Artemisinin and its derivatives can significantly inhibit lung tumorigenesis and tumor metastasis through Wnt/beta-catenin signaling. Oncotarget 2016;7:31413-31428.
53. Gao X, Luo Z, Xiang T, Wang K, Li J, Wang P. Dihydroartemisinin induces endoplasmic reticulum stress-mediated apoptosis in HepG2 human hepatoma cells. Tumori 2011;97:771-780.

54. Roy R, Yang J, Moses MA. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J Clin Oncol 2009;27:5287-5297.
55. Bjornland K, Flatmark K, Pettersen S, Aaasen AO, Fodstad O, Maelandsmo GM. Matrix metalloproteinases participate in osteosarcoma invasion. J Surg Res 2005;127:151-156.
56. Liu Y, Wang Y, Teng Z, Chen J, Li Y, Chen Z, et al. Matrix metalloproteinase 9 expression and survival of patients with osteosarcoma: a meta-analysis. Eur J Cancer Care 2017;26.
57. Sun W, Ma X, Shen J, Yin F, Wang C, Cai Z. Bioinformatics analysis of differentially expressed pathways related to the metastatic characteristics of osteosarcoma. Int J Mol Med 2016;38:466-474.
58. Zhang M, Zhang X. Association of MMP-2 expression and prognosis in osteosarcoma patients. Int J Clin Exp Pathol 2015;8:14965-14970.
59. Bjornland K, Flatmark K, Pettersen S, Aaasen AO, Fodstad O, Maelandsmo GM. Matrix metalloproteinases participate in osteosarcoma invasion. J Surg Res 2005;127:151-156.
60. Hirahata M, Osaki M, Kanda Y, Sugimoto Y, Yoshioka Y, Kosaka N, et al. PAI-1, a target gene of miR-143, regulates invasion and metastasis by upregulating MMP-13 expression of human osteosarcoma. Cancer Med 2016;5:892-902.
61. Andujar I, Rios JL, Giner RM, Recio MC. Pharmacological properties of shikonin—a review of literature since 2002. Planta Med 2013;79:1685-1697.
62. Fu Z, Deng B, Liao Y, Shan L, Yin F, Wang Z, et al. The anti-tumor effect of shikonin on osteosarcoma by inducing RIP1 and RIP3 dependent necroptosis. BMC Cancer 2013;13:580.
63. Zheng W, Lu S, Cai H, Kang M, Qin W, Li C, et al. Deguelin inhibits proliferation and migration of human pancreatic cancer cells *in vitro* targeting hedgehog pathway. Oncol Lett 2016;12:2761-2765.
64. Yan B, Zhao D, Yao Y, Bao Z, Lu G, Zhou J. Deguelin induces the apoptosis of lung squamous cell carcinoma cells through regulating the expression of galectin-1. Int J Biol Sci 2016;12:850-860.
65. Robles AJ, Cai S, Cichewicz RH, Mooberry SL. Selective activity of deguelin identifies therapeutic targets for androgen receptor-positive breast cancer. Breast Cancer Res Treat 2016;157:475-488.
66. Ganguly KK, Pal S, Moulik S, Chatterjee A. Integrins and metastasis. Cell Adh Migr 2013;7:251-261.
67. Huang CY, Lee CY, Chen MY, Tsai HC, Hsu HC, Tang CH. Nephroblastoma overexpressed gene (NOV) enhances cell motility and COX-2 upregulation of human osteosarcoma involves alphavbeta5 integrin, ILK and AP-1-dependent pathways. Biochem Pharmacol 2011;81:577-585.
68. Witlox AM, van Beusechem VW, Molenaar B, Bras H, Schaap GR, Alemany R, et al. Conditionally replicative adenovirus with tropism expanded towards integrins inhibits osteosarcoma tumor growth *in vitro* and *in vivo*. Clin Cancer Res 2004;10:61-67.
69. Bodduluru LN, Kasala ER, Thota N, Barua CC, Sistla R. Chemopreventive and therapeutic effects of nimbolide in cancer: the underlying mechanisms. Toxicol *In Vitro* 2014;28:1026-1035.
70. Yang J, Wang N. Analysis of the molecular mechanism of osteosarcoma using a bioinformatics approach. Oncol Lett 2016;12:3075-3080.
71. Zhang LZ, Mei J, Qian ZK, Cai XS, Jiang Y, Huang WD. The role of VE-cadherin in osteosarcoma cells. Pathol Oncol Res 2010;16:111-117.
72. Gavard J. Breaking the VE-cadherin bonds. FEBS Lett 2009;583:1-6.
73. DuBois S, Demetri G. Markers of angiogenesis and clinical features in patients with sarcoma. Cancer 2007;109:813-819.
74. Viallard C, Larrivée B. Tumor angiogenesis and vascular normalization: alternative therapeutic targets. Angiogenesis 2017;4:1-18.
75. Dvorak HF. Tumor stroma, tumor blood vessels, and antiangiogenesis therapy. Cancer J 2015;21:237-243.
76. Yu XW, Wu TY, Yi X, Ren WP, Zhou ZB, Sun YQ, et al. Prognostic significance of VEGF expression in osteosarcoma: a meta-analysis. Tumour Biol 2014;35:155-160.
77. Han G, Wang Y, Bi W, Jia J, Wang W, Xu M. Effects of vascular endothelial growth factor expression on pathological characteristics and prognosis of osteosarcoma. Clin Exp Med 2016;16:577-584.
78. Zhou Q, Zhu Y, Deng Z, Long H, Zhang S, Chen X. VEGF and EMMPRIN expression correlates with survival of patients with osteosarcoma. Surg Oncol 2011;20:13-19.
79. Stetler-Stevenson WG. The tumor microenvironment: regulation by MMP-independent effects of tissue inhibitor of metalloproteinases-2. Cancer Metastasis Rev 2008;27:57-66.
80. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 2016;79:629-661.
81. Subbiah V, Wagner MJ, McGuire MF, Sarwari NM, Devarajan E, Lewis VO, et al. Personalized comprehensive molecular profiling of high risk osteosarcoma: implications and limitations for precision medicine. Oncotarget 2015;6:40642-40654.
82. Vina D, Serra S, Lamela M, Delogu G. Herbal natural products as a source of monoamine oxidase inhibitors: a review. Curr Top Med Chem 2012;12:2131-2144.
83. Moriarity BS, Otto GM, Rahrmann EP, Rathe SK, Wolf NK, Weg MT, et al. A sleeping beauty forward genetic screen identifies new genes and pathways driving osteosarcoma development and metastasis. Nat Genet 2015;47:615-624.
84. Moffat JG, Rudolph J, Bailey D. Phenotypic screening in cancer drug discovery—past, present and future. Nat Rev Drug Discov 2014;13:588-602.
85. Talmadge JE, Singh RK, Fidler IJ, Raz A. Murine models to evaluate novel and conventional therapeutic strategies for cancer. Am J Pathol 2007;170:793-804.

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