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Evidence-Based Integrative Medicine

Chinese Herbal Medicine for Osteosarcoma in the Mouse: A Systematic Review and Meta-Analysis*

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ABSTRACT **Objective:** To summarize and critically assess the inhibitory effects of Chinese herbal medicine (CHM) on tumor volume and tumor weight for the treatment of osteosarcoma (OS) in mouse models. **Methods:** PubMed, Embase, Web of Science, China Knowledge Resource Integrated Database (CNKI), Wanfang Database, VIP Database, and Chinese BioMedical (CBM) were searched since their inception dates to March 10, 2016. Two reviewers independently selected the controlled studies estimating effects of CHM on mouse OS by administration *in vivo*. A pair-wise meta-analysis was performed. Twenty-five studies with adequate randomization were included in the systematic review. **Results:** CHM may significantly inhibit OS growth in mice, as assessed using the tumor weight [20 studies, n=443; 290 for CHM and 153 for the control; pooled mean difference (MD)=–2.90; 95% confidence interval (CI): –3.50 to –2.31; P<0.01], tumor volume (16 studies, n=382; 257 for CHM and 125 for the control; pooled MD =–2.57; 95% CI: –3.33 to –1.80; P<0.01) and tumor growth inhibition rate. **Conclusion:** CHM could significantly inhibit the growth of OS in mouse models, which might be supportive for the design of preclinical and clinical trials in future.

KEYWORDS Chinese herbal medicine, osteosarcoma, systematic review, meta-analysis, mouse, xenograft model

Osteosarcoma (OS) is the most common primary cancer arising from bone and the fifth most prevalent carcinoma in children and young adults. The standard treatment of OS is the surgical resection with adjuvant chemotherapy including 4 agents: methotrexate, doxorubicin, cisplatin or ifosfamide.[1] The combined treatment has significantly improved survival of nonmetastatic patients over the past several decades,[2] patients with localized disease have 5-year survival rates of about 70%. However, the 5-year survival for patients with metastatic disease is 20%, currently about 20% of patients exhibit metastases and almost all patients with recurrent OS present metastatic disease.[3,4]

Meanwhile, a considerable number of patients are either not sensitive to chemotherapy or develop drug-resistant.[5] Although targeted therapies and substantial advances in understanding of OS genetics and biology have revolutionized the therapeutic approach to malignancies, translation of selective inhibitors has been slow or poorly effective in the treatment of OS,[6] and there have been no significant improvements in therapy in the past 30 years.[7,8] This disappointing outcome strongly suggests that the evaluation of alternative anti-cancer agents with increased drug-response rates, avoided chemo-resistance, reduced toxicity is urgently needed to prevent OS progression and improve clinical outcomes.[9-11]

Chinese herbal medicines (CHM), with multi-biologically active natural components and therapeutic efficacy, has minimal side effects and provides unique sources for development of front-line anticancer drugs.[12,13]

Given lots of CHM that are reportedly found to be beneficial in animal models of OS, therefore, the aim of this study is to carry out a comprehensive systematic review and meta-analysis about the efficacy of CHM for

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OS in mouse models, which may be helpful in future for the design of preclinical and clinical trials.

METHODS

Study Selection

A systematic literature was retrieved from 7 databases including PubMed, Embase, Web of Science, China Knowledge Resource Integrated Database (CNKI), Wanfang Database, VIP Database, and Chinese BioMedical (CBM) since their inception dates to March 10, 2016. Key words used for search were as the following: Chinese medicine OR herb OR herbal medicine OR herbs OR Chinese herbal medicine OR Chinese herbal drug OR Chinese herbal drugs OR herbal drug OR herbal drugs OR botanical OR medicinal herbs OR medicinal plants OR medicinal plant OR herbal preparations OR herbal preparation OR plants extract OR traditional medicine OR phytomedicine AND osteosarcoma AND mice OR mouse, regardless of publication status and the language.

The studies were selected independently by two reviewers (Chang JL and Li YM) by screening the abstracts and full texts according to the eligibility criteria. Disagreements were resolved by consensus with a third author (Yang YP). Only studies satisfied criteria were included in the meta-analysis.

Eligibility Criteria

Types of Studies

Controlled studies estimating effects of CHM on mice with OS by administration *in vivo* were searched. All clinical case reports and studies only having *in vitro* experiments were excluded. There were no language, publication status or publication date restrictions.

Types of Participants

Laboratory mice of any age, gender, or strain exposed to OS induced by injection of OS cells (subcutaneous or intratibial) were included.

Types of Intervention

Any type of CHM intervention compared to placebo control was included. Dosage, formulation, route of administration, and CHM administration time were not limited. Placebo control included dimethyl sulfoxide (DMSO), physiological saline, or no treatment.

Type of Outcome Measure

Human cancer xenograft models, which are derived from human tumor cell lines, are classified according to the transplant site, such as ectopic xenograft and orthotopic xenograft, play key roles in evaluation and screening of agents for new anticancer candidates. These models are also utilized to evaluate the toxicity and therapeutic efficacy. Standard animal models save money and time, and provide evidence to support clinical trials during the discovery of anticancer drugs.[11] Therefore, tumor weight and tumor volume commonly are used to evaluate the anticancer efficacy in xenograft models of cancers. Xenograft models included in this meta-analysis were established via subcutaneous or intratibial injection of OS cells.

Tumor Volume

Tumor volume was calculated from the digital vernier caliper measurements by the following formula: $0.5 \times a \times b^2$, where a is the largest dimension and b is the perpendicular diameter.[5]

Tumor Weight

Tumors were removed and weighed at the end of experiments when mice were sacrificed.

Tumor Growth Inhibition Ratio

The tumor growth inhibition ratio was expressed according to the following formula: (mean tumor weight of control group–mean tumor weight of treated group)/ the mean tumor weight of control group $\times$ 100 %.

Data Extraction

The detail information was extracted independently by 2 authors (Chang JL and Li YM) from included studies in this meta-analysis using a predefined form. Details included first author's name, publication year, animal strain, animal age (as reported in the study), animal gender, number of mice in each group, method used to induce OS, CHM usage (dosage, administration method, and time point of initiation administration time) and measured outcomes. Data were collected by mean outcome and standard deviation (SD) for each comparison. Any data were shown by graphs instead of text were estimated using GetData Graph Digitizer 2.24. A third reviewer (Yang YP) resolved any disagreements between the two reviewers.

Assessment of the Risk of Bias in Individual Studies

Since there is no established reliable and valid

tools to assess bias or methodological quality in animal studies, the initial Stroke Therapy Academic Industry Roundtable (STAIR) was used, which was updated by the STAIR group to assess the design and reporting quality of included studies. The list of STAIR includes: (1) sample-size calculation; (2) inclusion and exclusion criteria; (3) randomization; (4) allocation concealment; (5) reporting of animals excluded from analysis; (6) blinded assessment of outcome; and (7) reporting potential conflicts of interest and study funding.[14] The risk of bias in each study was assessed by two authors (Chang JL and Li YM) and presented as a low or high risk. The "unclear" indicated that the risk of bias was not clear.

Statistical Analysis

Data were pooled if outcomes were reported by more than 2 studies. Two treatment outcomes (tumor weight and tumor volume) were analyzed separately. Pair-wise meta-analysis for studies that directly compared a CHM group and a placebo control group was performed. For each measurement outcome of interest, pair-wise meta-analysis was performed to determine the pooled relative effect of each intervention relative to the other effects, and the mean differences (MDs) of the post-intervention values from the different interventions were determined. The post-intervention values were adopted in meta-analysis based on the baseline values being comparable between CHM group and placebo control group, as indicated by a Cochrane review.[15]

Final results from the studies to assess differences between the intervention and control group were analyzed using the REVIEW MANAGER 5.1.2 software provided by the Cochrane Collaboration), and Cochrane's I^2 was used to evaluate the heterogeneity. Heterogeneity was assumed if the *P* value was less than 0.10 using the Chi-square (χ^2) test. If the I^2 value was above 50%, the result was considered to have a high level of heterogeneity.[15] Clinically and statistically homogeneous studies were pooled using the fixed-effects model.[15] Clinically homogeneous and statistically heterogeneous studies were pooled using the random-effects model.[15] When studies used different instruments to measure the same thing, a standardized mean difference (SMD) was used in the meta-analysis to combine continuous data. Funnel plots were made to evaluate the publication bias, when at least 10 studies were included in this meta-analysis.

RESULTS

Selection of Studies

The flow of literature identification and selection process is shown in Figure 1.

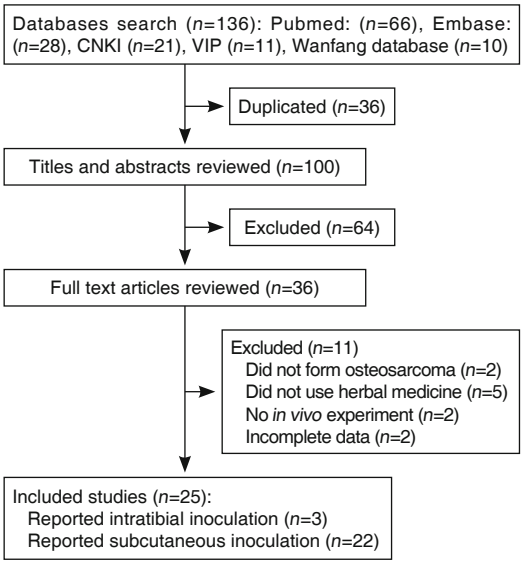

Figure 1. Flow of Literature Identification and Selection Process

Characteristics of the Included Studies

Among all the 25 included studies,[16-40] most of the studies used nude mice (17[16,18-21,24-28,30-32,34,38-40] of them used BALB/c mice, 1[17] of them used C3H/He mice, and the strain of mice used in other 4 studies[22,23,29,35] was not clear), while Kunming mice were used in 3[33,36,37] of the remaining included studies; 11 studies[16,18,19,27,29-31,35,37,39,40] used female mice, 7 studies[17,20,22,23,25,32,34] used males, 5 studies[21,24,33,36,38] used both female and male mice, and the gender of mice in 2 studies[26,28] was not presented.

Median sample size for the 25 studies[16-40] was 21 mice (range from 10 to 50). The main composition of background diet in the included studies was not reported. OS xenograft models of the mice used in 20 studies[16-21,25,26,28-34,36-40] were induced by subcutaneous injection, in 3 studies[24,27,35] were induced by intratibial injection, and the OS inoculation methods in 2 studies[22,23] were not reported.

CHM was administrated intraperitoneally (14 of 25 included studies),[16,18,19,21,25-28,31,32,34,36,39,40] intragastrically (8 of 25 included studies),[17,20,24,29,33,35,37,38] or intravenously,[30] except in 2 studies[22,23] where CHM was supplemented in diet. Ten studies[16,17,22-24,36-40] administered CHM shortly after inoculation of the

OS cells (range from 12 to 24 h), 5 studies[18,27,32,33,35] started to treat OS several days after inoculation of the OS cells (range from 6 to 14 days), and 10 studies[19-21,25,26,28-31,34] started to treat OS when the tumor size/volume reached certain values. The duration of CHM administration ranged from 10 to 42 days.

Most of the studies reported the outcomes of tumor weight and tumor volume, and some of the studies also reported tumor inhibition ratio (Appendix 1).

Risk of Bias within Studies

The risk of bias that reported for each publication included in this meta-analysis were described (Appendix 2). Overall, the methodological quality of the studies was not high. None of the studies utilized here described a sample-size calculation, allocation, concealment, reporting of animals excluded from analysis or blinded assessment of outcome. Nine studies[19-21,25,26,28,33-35] reported inclusion and exclusion criteria. Sixteen studies[16,18,20-23,25,26,28,29,31,33,35,38-40] reported randomization, whereas 14 studies[16,17,21,25-33,39,40] reported potential conflicts of interest and study funding.

Effects of the Interventions

Inhibitory Effects of CHM on Tumor Weight of OS Xenografts

Subgrouped by Injection Sites of OS cells

Nineteen[16-20,22,23,25,26,28-30,32,33,36-40] of the 20 studies, that included measurements of the tumor weight, used OS xenograft models induced by subcutaneous injection. These 19 studies were combined for a meta-analysis, and a total of 280 mice in the intervention arm and 143 in the control arm were included. Since there were high heterogeneity among the studies, a random-effects model was used, and the tumor weight significantly decreased with the treatment of CHM [pooled MD=–2.99; 95% confidence interval (CI): –3.62 to –2.36; *P*<0.01; Appendix 3].

Only 1 study[30] reported that an OS xenograft model induced by intratibial injection of OS cells was used, so the results could not be pooled. The study reported that the posterior limbs with primary tumor in shikonin group were lighter than that in control group (*P*<0.01), shikonin had prompt but profound anti-tumor effect.

Subgrouped by the Ingredients of CHM

Since the CHM includes a diverse set of therapies, the inhibitory effects after subgrouped by its ingredients were further analyzed. Fourteen studies[16-20,25-30,32,39,40] used isolated products and thus were combined for a meta-analysis. A total of 190 mice in the inter formulas of CHM intervention arm and 103 in the control arm were included. A random-effects model was used due to high heterogeneity among the studies, and the tumor weight significantly decreased with the treatment of CHM (pooled MD= –3.10; 95% CI: –3.76 to –2.44; *P*<0.01; Appendix 4, upper part).

The remaining 6 studies[22,23,33,36-38] used formulas of CHM and were combined together for a meta-analysis. A total of 100 mice in the intervention arm and 50 in the control arm were included. A random-effects model was used because of the high heterogeneity among studies, and the tumor weight was considerably suppressed by formulas of CHM (pooled MD=–2.43; 95% CI: –3.57 to –1.28; *P*<0.01; Appendix 4, lower part).

Subgrouped by the Initiation Intervention Time Point

Seven studies[19,20,25,26,28-30] initiated the intervention with CHM when tumor volume reached a certain size and were combined for a meta-analysis. A total of 99 mice in the intervention arm and 55 in the control arm were included. A random-effects model was used due to high heterogeneity among the studies, and the MD=–2.84; 95% CI: –3.58 to –2.11; *P*<0.01; Appendix 5, upper part).

Four studies[18,27,32,33] performed the initiating treatment several days after inoculation of the OS cells (range from 6 to 14 days) and thus were combined together for the meta-analysis. A total of 67 mice in the intervention arm and 31 in the control arm were included. A random-effects model was used and the tumor weight also significantly decreased with the treatment of CHM (pooled MD=–2.92; 95% CI: –4.36 to –1.47; *P*<0.01; Appendix 5, middle part);

In other 9 studies,[16,17,22,23,36-40] mice were treated with CHM within 24 h of OS inoculation and were combined for a meta-analysis. A total of 124 mice in the intervention arm and 67 in the control arm were included. A random-effects model was used due to high heterogeneity among the studies, and the tumor weight significantly decreased with the treatment of CHM (pooled MD=–3.04; 95% CI: –4.15 to –1.92; *P*<0.01; Appendix 5, lower part).

The overall effect on the inhibition of tumor weight also showed a significant trend after pooling together all the 20 studies[16-20,22,23,25-30,32,33,36-40] for analysis, which continuously support the anti-tumor effect of CHM at (pool MD=–2.90, 95% CI: –3.50 to –2.31; Appendix 6).

Inhibitory Effects of CHM on Tumor Volume of OS Xenografts

When Subgrouped by Injection Sites of OS Cells

Sixteen studies[17-21,24-26,28,30,31,34-37,40] that included measurements of tumor volume were divided into two subgroups according to the OS inoculation methods.

Fourteen studies[17-21,25,26,28,30,31,34,36,37,40] that compared the effects of CHM versus the control in OS xenograft models induced by subcutaneous injection, including a total of 218 mice in the intervention arm and 112 in the control arm. The tumor volume significantly decreased after treatment with CHM (pooled MD=–2.80; 95% CI: –3.70 to –1.89; *P*<0.01; Appendix 7, upper part) in a random-effects model because of the high heterogeneity.

Another subgroup consisted of 2 studies[24,35] that compared the effects of CHM versus the control in OS xenograft models induced by intratibial injection, including a total of 39 mice in the intervention arm and 13 in the control arm. The tumor volume significantly decreased after treatment with CHM (pooled MD=–1.38; 95% CI: –2.07 to –0.69; *P*<0.01; Appendix 7, lower part) in a random-effects model because of the high heterogeneity.

Subgrouped by the Ingredients of CHM

Sixteen studies[17-21,24-26,28,30,31,34-37,40] that included measurements of tumor volume were divided into two subgroups according to the ingredients of CHM.

One subgroup included 13 studies[17-20,24-26,28,30,31,34,35,40] that used isolated products, including a total of 201 mice in the intervention arm and 95 in the control arm. The tumor volume significantly decreased after treatment with the isolated products of CHM (pooled MD=–2.82; 95% CI: –3.74 to –1.90; *P*<0.01; Appendix 8, upper part) in a random-effects model because of the high heterogeneity.

Another subgroup included 3 studies[21,36,37] that used the formulas of CHM, including a total of 56 mice in the intervention arm and 30 in the control arm. The tumor volume significantly decreased after treatment with the formulas of CHM (pooled MD=–1.69; 95% CI: –3.24 to –0.14; *P*<0.01; Appendix 8, lower part) in a random-effects model because of the high heterogeneity.

Subgrouped by the Initiation Intervention Time Point

Sixteen studies[17-21,24-26,28,30,31,34-37,40] that included measurements of tumor volume were divided into 3 subgroups according to the initiation time point of CHM administration.

Nine studies[19-21,25,26,28,30,31,34] that compared the effects of CHM started administration when tumor volume reached certain size versus the control in OS xenograft models, including a total of 124 mice in the intervention arm and 71 in the control arm. The tumor volume significantly decreased after treatment with CHM (pooled MD=–3.34; 95% CI:–4.74 to –1.93; *P*<0.01; Appendix 9, upper part) in a random-effects model because of the high heterogeneity.

The second subgroup included 2 studies[18,35] that compared the effects of CHM started administration several days after inoculation of the OS cells (range from 7 to 10 days), including a total of 36 mice in the intervention arm and 14 in the control arm. The tumor volume significantly decreased after treatment with CHM (pooled MD=–2.31; 95% confidence interval CI: –4.18 to –0.43; *P*=0.020; Appendix 9, middle part) in a random-effects model because of the high heterogeneity.

The third subgroup consisted of 5 studies[17,24,36,37,40] that compared the effects of CHM started administration within 24 h of OS inoculation versus the control in OS xenograft models, including a total of 97 mice in the intervention arm and 40 in the control arm. The tumor volume significantly decreased after treatment with CHM (pooled MD=–1.54; 95% CI: –2.37 to –0.71; *P*<0.01; Appendix 9, lower part) in a random-effects model because of the high heterogeneity.

When all the 16 studies[17-21,24-26,28,30,31,34-37,40] were pooled together for the analysis, the overall effect estimate continuously showed a significant trend, supporting the treatment of CHM at (pooled MD=–2.57, 95% CI: –3.33 to –1.80; Appendix 10).

Tumor Growth Inhibition Rate of CHM in OS Xenografts

Only 6 studies[21,29,33,36,38,40] reported tumor

growth inhibition rate of CHM in OS xenografts, and these data can not be pooled. First study reported that compared with the control group, treatment with 5, 10 or 20 mg/kg oxymatrine significantly inhibited tumor growth at a rate of 34.48%, 45.69% or 56.03%, respectively.[40] Second study reported that compared with the control group, treatment with Pientzehuang (片仔癀) significantly inhibited tumor growth at a rate of 34.1%.[21] The third study reported that compared with the control group, treatment with Duhuo Jisheng Decoction (独活寄生汤, DJD, low), DJD (medium) or DJD (high) significantly inhibited tumor growth at a rate of 32.45%, 43.75% or 40.26%, respectively.[33] The fourth study reported that compared with the control group, treatment with Ping-Tiao-Yin (平调饮, low) or Ping-Tiao-Yin (high) significantly inhibited tumor growth at a rate of 32.1% or 43.97%, respectively.[38] The fifth study reported that compared with the control group, treatment with Huangqi Injection (0.2 mL/d) significantly inhibited tumor growth at a rate of 20.52%.[36] The sixth study reported that compared with the control group, treatment with aspidin PB (15 and 30 mg/kg) significantly inhibited tumor growth at a rate of 74.01% or 51.43%.[29]

Publication Bias Assessment

Funnel plots were made to assess the publication bias. The standard error was plotted against the tumor weight (20 publications evaluating the inhibitory effects of CHM on tumor weight, Appendix 11A), and tumor volume (16 publications evaluating the inhibitory effects of CHM on tumor volume, Appendix 11B), No skewed distribution was observed, suggesting no serious publication bias, and more samples are necessary for further conclusions.

DISCUSSION

This review extracted the data from 25 studies[16-40] that compared CHM to placebo control. The overall methodological quality of all the selected studies was low. Treatment with CHM led to a significant inhibition of OS growth using the tumor weight, tumor volume compared to placebo control. Only 6 studies[21,29,33,36,38,40] reported tumor growth inhibition rate, which was from 20.52% to 74.01%.

The limitations of the original studies were also shared in our study as any other meta-analysis. Although we searched both English and Chinese databases, we are still not sure that all related studies were found. Furthermore, other major reasons of bias that must be considered are selective reporting and publishing,[41] since positive results are more likely to be published, these estimations may be overstated given the evidence for publication bias. Further, some journals have restriction of spaces, so some key details and important information may have been omitted by the authors or been deleted during publication process.

Heterogeneity is acceptable in meta-analysis, it would be surprising if many studies completed by different labs in different places with different methods, all ended up by evaluating the same fundamental parameter. In addition, animal studies are generally small (with a sample size of about 10 each group). Then the challenge is to decide on the most suitable approach to analyze heterogeneous studies. When heterogeneity cannot be ignored, one analytical method is to integrate it into a random-effects model, which involves a postulation that the effects being evaluated in different studies are not identical, however follow some distribution.[42,43]

In this systematic review and meta-analysis, different animal species (Kunming mice or BALB/c nude mice), genders (male, female or both) and ages (3 to 6 weeks); different osteosarcoma cell injection sites for producing xenograft models (intratibial inoculation or subcutaneous inoculation); the wide diversity of CHM components (isolated product and formula), doses and sources; the varied administration routes (intragastric administration, intraperitoneal or intravenously injection); different initiation times for treatment (when tumor size/volume reached certain values, within 24 h or several days after osteosarcoma cell inoculation), and different measurement standards and calculation methods for the tumor volume were used in the included studies, these factors all could cause a high heterogeneity, therefore, random-effects models were used for the analyses. Due to these data used for analysis were experiment-level, and thus if all baseline characteristics among groups were balanced could not be evaluated. And more studies on a single CHM intervention for OS are necessary in the future.

With the urgent to found new therapeutics for OS, it is recognized that the process of bringing forth a new human therapy takes long time. Novel therapeutics requires substantive safety evaluations, which add expense and time to the translation of

new treatments. Based on this, the idea of exploring CHM for the treatment of OS that is already in human clinical application remains an attractive strategy.

When reading the large amount of pre-clinical data, systematic review and meta-analysis have been shown to be very important to answer "which is the most hopeful one that could be translated from animal studies to human trials", the noticeable issue was faced.

In clinical trials, meta-analysis and systematic reviews have made critical contributions for us to understand the sources of bias, and led to the improved quality of clinical trials. We believe that the same protocol can be used to improve study quality of animal experiments by increasing our understanding of the sources of bias. Whatever the merits of animal experiments, for many diseases, the benefits observed in animal models have been lost during translation. All available tools including systematic reviews should be used to determine the reasons for this discrepancy.

This review summarizes the available preclinical literature on the use of CHM in *in-vivo* models of mice. It is not a comprehensive list of every therapy that has ever been tried in pre-clinical models of OS, but rather, a systematic review of specific therapies that are currently being considered for human translation.

In spite of poor methodological quality of the studies, CHM was shown to significantly inhibit the growth of OS. Nevertheless, a public methodological quality evaluation checklist for the animal studies is required and the STAIR list may be an candidate for this function. Evidence that suggested the existence of publication bias has also been found, although we still cannot count its impact. Systematic review and meta-analysis presented here supply a framework for the design of animal studies and clinical trials, and for an evidence-based way to the development of new therapeutics for OS in the future.

Conflict of Interest

No conflict of interest was declared.

Author Contributions

Yang YP and Wang YJ conceived the study. Chang JL and HU SP drafted the manuscript. Wang WY, Li YM and Zhi WL searched and analyzed the literature. Yao M, Cui XJ and Shi Q provided the technical support for the study. Under the guidance of Yang YP, all authors participated in the study and approved the final version.

Electronic Supplementary Material: Supplementary materials (Appendixes) are available in the online version of this article at https://doi.org/10.1007/s11655-018-2565-6

REFERENCES

1. Marano A, Priora F, Lenti LM, et al. Spinoglio G. Application of fluorescence in robotic general surgery: review of the literature and state of the art. World J Sur 2013;37:2800-2811.
2. Touati F, Tabish R. u-Healthcare system: state-of-the-art review and challenges. J Med Syst 2013;37:9949.
3. Felix R, Lackner K, Thurn P. CT and the heart: present state of the art and its future. A review. Der Radiologe 1980;20:50-55.
4. Gregory JM, Knapp DE. State-of-the-art of drug usage review. Am J Hosp Pharm 1976;33:925-928.
5. Murray JF. Review of the state of the art in intermittent positive pressure breathing therapy. Am Rev Respir Dis 1974;110:193-1999.
6. Alford CA Jr, Whitley RJ. Treatment of infections due to herpesvirus in humans: a critical review of the state of the art. J Infect Dis 1976;133 Suppl: A101-108.
7. Brodman E, Johnson MF Jr. Medical serials control systems by computer–a state of the art review. Bull Med Libr Assoc 1976;64:12-19.
8. Bowman MC. Editorial: Analysis of pesticides A state-of-the-art review. Part Ⅱ. J Chromatogr Sci 1975;13:255.
9. Staub NC. "State of the art" review. Pathogenesis of pulmonary edema. Am Rev Respir Dis 1974;109:358-372.
10. Yakimenko SA, Logai IM. State of art of the open keratoprosthesis (literature review). Oftalmologicheskii Zhurnal 1974;29:409-416.
11. Low SA, Yang J, Kopecek J. Bone-targeted acid-sensitive doxorubicin conjugate micelles as potential osteosarcoma therapeutics. Bioconjugate Chem 2014;25:2012-2020.
12. Mishra BB, Tiwari VK. Natural products: an evolving role in future drug discovery. Eur J Med Chem 2011;46:4769-4807.
13. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nature Rev Drug Discovery 2005;4:206-220.
14. Hughes DP. Strategies for the targeted delivery of therapeutics for osteosarcoma. Expert Opinion Drug Deliv 2009;6:1311-21.
15. Anninga JK, Gelderblom H, Fiocco M, et al. Chemotherapeutic adjuvant treatment for osteosarcoma: where do we stand? Eur J Cancer 2011;47:2431-2445.
16. Yu D, Fu S, Cao Z, et al. Unraveling the novel anti-osteosarcoma function of coptisine and its mechanisms. Toxicol Letters 2014;226:328-336.
17. Yoshiyuki KMS. Anti-tumor and anti-metastatic actions of

wogonin isolated from *Scutellaria baicalensis* roots through anti-lymphangiogenesis. Phytomedicine 2013;20:328-336.
18. Xie XB, Yin JQ, Wen LL, et al. Critical role of heat shock protein 27 in bufalin-induced apoptosis in human osteosarcomas: a proteomic-based research. PloS One 2012;7:e47375.
19. Wang Y, Fu Q, Zhao W. Tetramethylpyrazine inhibits osteosarcoma cell proliferation via downregulation of NF-kappa B *in vitro* and *in vivo*. Mol Med Rep 2013;8:984-988.
20. Wang Y, Chen J, Zhang D, et al. Tumoricidal effects of a selenium (Se)-polysaccharide from Ziyang green tea on human osteosarcoma U-2 OS cells. Carbohydrate Polymers 2013;98:1186-1190.
21. Ren SS, Yuan F, Liu YH, et al. Effect of p27 gene combined with Pientzehuang (片仔癀) on tumor growth in osteosarcoma-bearing nude mice. Chin J Integr Med 2015;21:830-836.
22. Roomi MW, Ivanov V, Kalinovsky T, et al. Effect of ascorbic acid, lysine, proline, and green tea extract on human osteosarcoma cell line MNNG-HOS xenografts in nude mice evaluation of tumor growth and immunohistochemistry. Med Oncol 2006;23:411-417.
23. Roomi MW, Roomi N, Ivanov V, et al. Inhibitory effect of a mixture containing ascorbic acid, lysine,proline and green tea extract on critical parameters in angiogenesis. Oncol Rep 2005;14:807-815.
24. Liu Y, Wang W, Xu J, et al. Dihydroartemisinin inhibits tumor growth of human osteosarcoma cells by suppressing Wnt/beta-catenin signaling. Oncol Rep 2013;30:1723-1730.
25. Lin CC, Chuang YJ, Yu CC, et al. Apigenin induces apoptosis through mitochondrial dysfunction in U-2 OS human osteosarcoma cells and inhibits osteosarcoma xenograft tumor growth *in vivo*. J Agricultural Food Chem 2012;60:11395-11402.
26. Jiang W, Huang Y, Wang JP, et al. The synergistic anticancer effect of artesunate combined with allicin in osteosarcoma cell line *in vitro* and *in vivo*. Asian Pacific J Cancer Prev 2013;14:4615-4619.
27. Fu Z, Deng B, Liao Y, et al. The anti-tumor effect of shikonin on osteosarcoma by inducing RIP1 and RIP3 dependent necroptosis. BMC Cancer 2013;13:580-590.
28. Chen Z, Huang C, Yang YL, et al. Inhibition of the STAT3 signaling pathway is involved in the antitumor activity of cepharanthine in $SaOS_2$ cells. Acta Pharmacol Sin 2012;33:101-108.
29. Wan D, Jiang C, Hua X, et al. Cell cycle arrest and apoptosis induced by aspidin PB through the p53/p21 and mitochondria-dependent pathways in human osteosarcoma cells. Anti-cancer Drugs 2015;26:931-941.
30. Liu Y, Bi T, Dai W, et al. Lupeol induces apoptosis and cell cycle arrest of human osteosarcoma cells through PI3K/AKT/mTOR pathway. Technol Cancer Res Treat 2016;15(6) NP16-NP24.
31. Li HY, Zhang J, Sun LL, et al. Celastrol induces apoptosis and autophagy via the ROS/JNK signaling pathway in human osteosarcoma cells: an *in vitro* and *in vivo* study. Cell Death Dis 2015;6:e1604.
32. Park KR, Yun HM, Quang TH, et al. 4-Methoxydalbergione suppresses growth and induces apoptosis in human osteosarcoma cells *in vitro* and *in vivo* xenograft model through down-regulation of the JAK2_STAT3 pathway. Oncotarget 2016;7:6960-6971.
33. Zhang RN. An Experimental Study on the effects and mechanisms of Duhuojisheng decoction in Inhibiting Carcinoma [dissertation]. Xi'an: The Fourth Military Medical University; 2004.
34. Shi QW, Li SG, Li J, et al. Anti-tumor effects of triptolide on osteosarcoma cells *in vitro* and *in vivo*: an experimental research. Chin J Integr Tradit West Med (Chin) 2013;33:659-663.
35. Liu YL. The anti-tumor effect of dihydroarteminin on human-osteasarcoma [dissertation]. Chongging: Chongqing Medical University; 2013.
36. Li XW, Du MC. Intervention of strengthening genuine qi on growth and metastasis of osteosarcoma. Chin J Cancer Prev Treat 2008;15:1721-1723.
37. Huang YM, He RR, Liu JW, et al. Effects of modified Siwu Decoction on expressions of VEGF and its receptors in osteosarcoma-bearing mice. Chin J Tradit Med Traum Orthop (Chin) 2009;17:1-3.
38. Han F, Jiang N, Yang F. Experimental research of Pingtiaoyin on presented and expressed of PCNA, Ki-67, EGF and EGFR in S180 osteosarcoma mouse. Aerospace Med 2006;17:131-132.
39. Yu D. The selective inhibitory effects and mechanisms of coptisine on osteosarcoma cell growth and vasculogenic mimicry [dissertation]. Suzhou: Sochow University; 2014.
40. Zhang Y, Sun S, Chen J, et al. Oxymatrine induces mitochondria dependent apoptosis in human osteosarcoma MNNG/HOS cells through inhibition of PI3K/Akt pathway. Tumour Biol J Int Soc Oncodev Biol Med 2014;35:1619-1625.
41. Pittler MH, Abbot NC, Harkness EF, et al. Location bias in controlled clinical trials of complementary/alternative therapies. J Clin Epidemiol 2000;53:485-489.
42. Higgins JP. Commentary: Heterogeneity in meta-analysis should be expected and appropriately quantified. Int J Epidemiol 2008;37:1158-1160.
43. Vesterinen HM, Sena ES, Egan KJ, et al. Meta-analysis of data from animal studies: a practical guide. J Neurosci Methods 2014;221:92-102.

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