MicroRNAs for osteosarcoma in the mouse: a meta-analysis

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ABSTRACT

Osteosarcoma (OS) is the most common primary malignant bone carcinoma with high morbidity that happens mainly in children and young adults. As the key components of gene-regulatory networks, microRNAs (miRNAs) control many critical pathophysiological processes, including initiation and progression of cancers. The objective of this study is to summarize and evaluate the potential of miRNAs as targets for prevention and treatment of OS in mouse models, and to explore the methodological quality of current studies. We searched PubMed, Web of Science, Embase, Wan Fang Database, VIP Database, China Knowledge Resource Integrated Database, and Chinese BioMedical since their beginning date to 10 May 2016. Two reviewers separately screened the controlled studies, which estimate the effects of miRNAs on osteosarcoma in mice. A pair-wise analysis was performed. Thirty six studies with enough randomization were selected and included in the meta-analysis. We found that blocking oncogenic or restoring decreased miRNAs in cancer cells could significantly suppress the progression of OS in vivo, as assessed by tumor volume and tumor weight. This meta-analysis suggests that miRNAs are potential therapeutic targets for OS and correction of the altered expression of miRNAs significantly suppresses the progression of OS in mouse models, however, the overall methodological quality of studies included here was low, and more animal studies with the rigourous design must be carried out before a miRNA-based treatment could be translated from animal studies to clinical trials.

INTRODUCTION

Osteosarcoma (OS), is a most frequent primary malignant bone tumor, accounts for 60% of all malignant childhood bone tumors, and is the second highest reason of cancer-associated death in adolescents [1, 2]. Although OS can happen in any bone, the most common sites of primary bone malignancies are the proximal tibia, proximal humerus and distal femur [1]. Typical symptoms and signs include pain history, localized swelling, joint movement limitations and typical findings of normal trabecular bone destruction on X-rays [3, 4].

Despite the neoadjuvant therapeutic strategies combined with aggressive tumor resection, the prognosis for OS patients still remains poor due to the risk of local relapse and development of pulmonary metastasis [5, 6]. For all the children diagnosed with OS, only 70% of them will survive beyond 5 years; less than 50% of them will live for more than 10 years [7-9]. Therefore, the clinical need for developing the new therapeutic approaches targeting the treatment of OS remains urgent but unmet.

MiRNAs are a class of non-coding RNAs containing about 22 nucleotides and can regulate the expression of more than 30% of all genes by imperfect base pairing with 3'-untranslated region (3'-UTR) of the target mRNAs at post-transcriptional level [10, 11]. Growing evidences show that abnormal miRNA expression has been detected in almost all human cancers [12, 13] and contributes to tumor initiation, cancer progression and clinical outcome of cancer patients [13, 14], which suggests that miRNAs could be potential targets for cancer therapy, and studies on miRNAs have provided a new possibility for the treatment of cancer.

MiRNAs can either function as oncogenes or tumor suppressors, in accordance with their expression in malignancies and the role in cellular transformation.

Overexpression of oncogenic miRNAs is to be related with transformation, metastasis, increased cell viability and proliferation in many solid malignancies. Some miRNAs have been shown to possess tumor suppressor character, as loss of function of them promotes tumourigenesis [15, 16]. In this regard, therapeutic potentials of RNA oligonucleotides have been proposed as the most direct way for molecules to correct the abnormally expressed miRNAs, including two possible approaches of blocking oncogenic miRNAs using antimiRNA oligonucleotides or replacing tumor suppressor miRNAs using miRNA mimetics [17].

However, in contrast to some other types of cancer, such as breast cancer and colorectal cancer, little is identified about the function of miRNAs in the pathogenesis of OS. It was found that OS cell lines in general are extremely tumorigenic by evaluating the *in vivo* tumorigenicity, *in vitro* colony-forming potential, invasive/migratory capacity and proliferation ability of 22 OS cell lines. There was a strong association among motility, invasion and colony formation, especially for the exceedingly aggressive OS cell lines, such as HOS-143B. Comparing the miRNA expression profiles of high (such as MG-63, HOS and OSA) and low (such

as HAL, IOR/MOS, IOR/OS9, IOR/OS14 and ZK-58) clonogenic OS cell lines discovered that miRNAs were differentially expressed between the two groups. One of them was miR-155-5p, which was highly expressed in all OS cell lines that formed a high number of colonies, and less expressed or absent in OS cell lines having a low clonogenic capability [18]. Tumorigenic and non-tumorigenic OS cell subpopulations also exhibit distinct miRNA expression profiles. A total of 268 miRNAs were identified significantly dysregulated in OS cell line MG-63 compared with the osteoblast cell line HOB [19].

The aim of this meta-analysis is to evaluate the potential value of miRNAs as therapeutic targets for OS based on the published literatures, and to explore the methodological quality of current studies, with the intention to guide the rigour of preclinical experimental design and the future clinical trials.

RESULTS

Literature selection

The outline of literature selection process is shown in Figure 1. Our database search retrieved 1171 publications following the search strategy described in the section of methods and 20 of the duplicated ones





Study	Animals	Number of animals	Osteosarcoma xerograph method	miRNA	Experimental groups	Control group	Outcome
Lei Fan 2013[65]	16 female or male BALB/c nude mice	8/8	Subcutaneous	miR-145	MG-63+miR-145	MG-63	Tumor volume Tumor weight
Jie Gao 2012[64]	10 female BALB/c nude mice (4 weeks)	5/5	Intratibial	miR-195	F5M2+pSilencer 4.1-CMV-miR-195	F5M2+pSilencer 4.1-CMV-NC	Tumor volume Tumor weight
Jie Jin 2013[63]	15 SCID nude mice	5/5/5	Subcutaneous	miR-218	Saos-2+pcDNA3.1- miR-218	A:Saos-2 B:Saos- 2+pcDNA3.1-NC	Tumor volume
Fang Ji 2013(a)[40]	8 BALB/c nude mice (4 weeks)	4/4	Subcutaneous	miR-133a	MG-63+miR-133a	MG-63+NC	Tumor volume
Fang Ji 2013(b)[40]	8 BALB/c nude mice (4 weeks)	4/4	Subcutaneous	miR-133a	U2 OS+miR-133a	U2 OS+NC	Tumor volume
Chi Cheng 2014 [44]	12 BALB/c nude mice (4 weeks)	6/6	Subcutaneous	miR-320	U2 OS+miR-320	U2 OS+NC	Tumor volume Tumor weight
Guoxing Xu 2014 [35]	10	5/5	Subcutaneous	miR-142- 3p	HOS+pcDNA3.1- miR-142-3p	HOS+pcDNA3.1	Tumor volume Tumor weight
Hao Zhang 2010(a) [41]	12 female BALB/c nude mice (4 weeks)	6/6	Subcutaneous	miR-143	MG-63+miR-143	MG-63+NC	Tumor volume
Hao Zhang 2010(b) [41]	12 female BALB/c nude mice (4 weeks)	6/6	Subcutaneous	miR-143	U2 OS+miR-143	U2 OS+NC	Tumor volume
Tomohiro Fujiwara 2014 [43]	25 athymic nude mice (5weeks)	5/5/5/5/5	Intratibial	miR-133a	A:143B, LNA-miR- 133a/Saline B:143B, LNA-NC/CDDP C:143B, LNA-miR- 133a/CDDP Injected via the tail vain	A: 143B, Saline/ Saline B:143B, LNA-NC/Saline Injected via the tail vain	Tumor weight
Lei Song 2013[38]	8 female BALB/c nude mice (5-6weeks)	4/4	Subcutaneous	miR-24	MG-63 +lentiviruse- miR-24	MG-63+lentiviruse- NC	Tumor volume
Xinyu Wu 2013(a) [36]	18 female BALB/c nude mice (4-6weeks)	6/6/6	Subcutaneous	miR-34a	MG-63+pcDNA3.1 -miR-34a	A:MG-63 B:MG- 63+pcDNA3.1	Tumor volume
Jin Wang 2014 [37]	10 BALB/c nude mice	5/5	Subcutaneous	miR-132	143B+lentiviruse- miR-132	143B+lentiviruse- NC	Tumor volume Tumor weight
Guodong LI 2012 [39]	18 nude mice (4-6weeks)	6/6/6	Subcutaneous	miR-223	MG-63, pcDNA- miR-223 Intratumor injection	A:MG-63,PBS B:MG-63,pcDNA3.1 Intratumor injection	Tumor volume
Lei Chen 2013 [45]	10 male BALB/c nude mice (5weeks)	5/5	Subcutaneous	miR-16	U2 OS +lentiviruse- miR-16	U2 OS+lentiviruse- NC	Tumor volume Tumor weight
Zhengyu Xu 2014[34]	12 BALB/c nude mice	6/6	Subcutaneous	miR-214	Saos-2 +lentiviruse- miR-214	Saos-2+lentiviruse- NC	Tumor volume Tumor weight
Xinyu Wu 2013(b) [36]	18 female BALB/c nude mice (4-6weeks)	6/6/6	Subcutaneous	miR-34a	Saos-2+pcDNA3.1 -miR-34a	A:Saos-2 B:Saos- 2+pcDNA3.1	Tumor volume
Kang Yan 2012 [33]	12 female BALB/c nude mice (4weeks)	6/6	Intratibial	miR-34a	SOSP-9607+pcDNA- miR-34a	SOSP- 9607+pcDNA3.1	Tumor volume Tumor weight
Mitsuhiko Osaki 2011 [17]	20 male nude mice (5-6weeks)	10/10	Intratibial	miR-143	143B+Luc, miR-143 Injected via the tail vain	143B+Luc, NC Injected via the tail vain	Tumor weight
Kang Han 2014 [42]	30 female BALB/c nude mice (4weeks)	10/10/10	Intratibial	miR-194	SOSP- 9607+lentiviruse- miR-194	A:SOSP-9607 B:SOSP- 9607+lentiviruse- NC	Tumor volume Tumor weight
Xin Zhou 2013 [32]	12 BALB/c nude mice (5weeks)	6/6	Subcutaneous	miR-340	Saos-2+lentiviruse- miR-340	Saos-2+lentiviruse- NC	Tumor weight
Masanori Kawano 2015[56]	21 BALB/c nude mice (6 weeks)	7/7/7	Subcutaneous	miR-93	Saos-2+miR-93	A:Saos-2+NC B:Untreated	Tumor volume
Yong Zhao 2015 [47]	12 male athymic nude mice (4–6-weeks)	6/6	Subcutaneous	miR-34a	143B, miR-34a Injected via tail vein	Vehicle Injected via tail vein	Tumor volume Tumor weight
K Tian 2015 [51]	30 C57BL/6 mice (8 weeks)	10/10/10	Subcutaneous	miR-23a	HOS58+ pGL3- miR23a-EGFP	A:HOS58 B: HOS58+ pGL3- Ctrl-EGFP	Tumor volume Tumor weight

Table 1: Description	of the characteristics of st	udies included in the	e meta-analysis. (NC	= negative control)
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Guoqing Duan 2015 [60]	14 female BALB/c nude mice (6 weeks)	7/7	Subcutaneous	miR-26b	U2OS+ pcDNA3.1-miR-26b	U2OS+pcDNA3.1- anti-miR-26b	Tumor volume
Jiahui Zhou (a)2015 [46]	18 BALB/c nude mice(20g)	9/9	Subcutaneous	miR-143	Saos-2, AdmiR-143 intratumorally	Saos-2, ADNC intratumorally	Tumor weight
Jiahui Zhou (b) 2015 [46]	18 BALB/c nude mice(20g)	9/9	Subcutaneous	miR-143	U2OS, AdmiR-143 intratumorally	U2OS, ADNC intratumorally	Tumor weight
Wei Wang 2015 [61]	12 BALB/c nude mice (4 weeks)	6/6	Subcutaneous	miR-144	143B+ lentiviruse-miR-144	143B+ lentiviruse- NC	Tumor volume Tumor weight
Xiaoji Luo 2014 [54]	10 male BALB/c nude mice (4 weeks)	5/5	Subcutaneous	miR-212	MG-63+ miR-212	MG-63+NC	Tumor volume Tumor weight
Xuming Wang 2014 [50]	8 BALB/c nude mice (4-6 weeks)	4/4	Subcutaneous	miR-214	Saos-2+pcDNA3.1-miR-214	Saos-2+pcDNA3.1	Tumor volume Tumor weight
Wei Liu 2015 [55]	10 BALB/c nude mice(6 weeks)	5/5	Subcutaneous	miR-49 0-3p	Saos-2+ miR-49 0-3p	Saos-2+NC	Tumor volume Tumor weight
Liang Ge 2016 [59]	20 male BALB/c mice(5-6 weeks)	10/10	Subcutaneous	miR-497	MG-63+ miR-497	MG-63+NC	Tumor volume Tumor weight
Xiuhui Wang 2014[49]	12 male BALB/c nude mice (4 weeks)	6/6	Subcutaneous	miR-25	Saos-2+ miR-25	Saos-2+NC	Tumor weight
Yu He 2014[58]	16 male BALB/c nude mice (5 weeks)	8/8	Subcutaneous	miR-23a	MG-63, miR-23a intratumorally	MG-63, NC	Tumor volume Tumor weight
Xiaohui Sun 2015[52]	12 male BALB/c nude mice	6/6	Subcutaneous	miR-155	U2OS, anti-miR-155 intratumorally	U2OS, anti-NC intratumorally	Tumor volume Tumor weight
Zhengwen Sun	10 male BALB/c nude	5/5				UOS lantivimusa	- ·
2014[25]	mice (4 weeks)	5/5	Subcutaneous	miR-202	HOS+ lentiviruse-miR-202	NC	Tumor volume Tumor weight
Meng Xu 2014[48]	mice (4 weeks) 16 female athymic nude mice(6 weeks)	8/8	Subcutaneous	miR-202 miR-382	HOS+ lentiviruse-miR-202 CD133high OS primary tumor cell+miR-382	CD133high OS primary tumor cell+NC	Tumor volume Tumor weight
2014[25] Meng Xu 2014[48] Baoyong Sun 2015[53]	mice (4 weeks) 16 female athymic nude mice(6 weeks) 16 female BALB/c athymic nude mice (3–4 weeks)	8/8 8/8	Subcutaneous Subcutaneous Subcutaneous	miR-202 miR-382 miR-217	HOS+ lentiviruse-miR-202 CD133high OS primary tumor cell+miR-382 MG-63+ lentiviruse -miR- 217	NC CD133high OS primary tumor cell+NC MG-63+ lentiviruse- NC	Tumor volume Tumor volume Tumor volume Tumor volume
2014[25]Meng 2014[48]XuBaoyong 2015[53]SunTatsuya Iwasaki 2015[57]	mice (4 weeks) 16 female athymic nude mice(6 weeks) 16 female BALB/c athymic nude mice (3–4 weeks) 21 nude mice	8/8 8/8 7/7/7	Subcutaneous Subcutaneous Subcutaneous Subcutaneous	miR-202 miR-382 miR-217 miR-let-7a	HOS+ lentiviruse-miR-202 CD133high OS primary tumor cell+miR-382 MG-63+ lentiviruse -miR- 217 MG-63+ miR-let-7a	CD133high OS primary tumor cell+NC MG-63+ lentiviruse-NC A: MG-63 B: MG-63+NC	Tumor volume Tumor volume Tumor volume Tumor volume Tumor volume

were excluded. After reading the titles and abstracts, 1103 publications were excluded. By a full text review of the 48 publications, 12 studies were further excluded due to they had no *in vivo* experiments [20-22](n = 3) or exhibited incomplete data [23-31](n = 9). Thirty six of the publications met the criteria were included in the final meta-analysis. Thirty three of the publications were reported in English [17, 25, 32-62], and 3 of them were reported in Chinese [63-65].

Study characteristics

Among all the 36 included studies, 34 of them used nude mice, while the strain of mice used in 2 studies was not clear. Ten studies used female mice, 9 studies used males, 1 study used female or male mice, and the gender of mice in 16 studies was not presented in the literatures.

Median sample size of mice for the 36 included

studies was 16 (range from 8 to 30). The main composition of background diet used in the included studies was not reported. OS xenograft models of the mice used in 30 studies were established by subcutaneous injection, and in 6 studies were established by intratibial injection.

MiRNAs were transfected into human OS cells before inoculating mice (29 of 36 included studies) [25, 32-38, 40-42, 44, 45, 48-50, 53-65], injected into the tumor (4 of 36 included studies) [39, 46, 52, 58], systemic administrated by tail vain injection(3 of 36 included studies) [17, 43, 47]. The included studies reported the outcomes of tumor weight, tumor volume, or both of them (Table 1).

Quality assessments of the included experiments

The quality assessment of each included publication in this meta-analysis is shown in Table 2. No studies

Table 2: Quality	assessment of	the included	experiments
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Study	Sample- size calculation	Inclusion and exclusion criteria	Randomization	Allocation concealment	Reporting of animals excluded from analysis	Blinded assessment of outcome	Reporting potential conflicts of interest and study funding
Lei Fan 2013 [65]	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Unclear
Jie Gao 2012 [64]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Jie Jin 2013 [63]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Fang Ji 2013[40]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Chi Cheng 2014 [44]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Tomohiro Fujiwara 2014 [43]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Hao Zhang 2010 [41]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Lei Song 2013 [38]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Xinyu Wu 2013 [36]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Kang Yan 2012[33]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Mitsuhiko Osaki 2011[17]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Kang Han 2014 [42]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Xin Zhou 2013 [32]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Zhengyu Xu 2014[34]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Lei Chen 2013 [45]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Jin Wang 2014 [37]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Guoxing Xu 2014 [35]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Guodong LI 2013 [39]	Unclear	Yes	Yes	Unclear	Unclear	Unclear	Yes
Masanori Kawano 2015[56]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Yong Zhao 2015[47]	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes
K Tian 2015[51]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Guoqing Duan 2015[60]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Jiahui Zhou 2015[46]	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes
Wei Wang 2015[61]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Xiaoji Luo 2014[54]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Xuming Wang 2014[50]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Wei Liu 2015[55]	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes
Liang Ge 2016[59]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Xiuhui Wang 2014[49]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Yu He 2014[58]	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes
Xiaohui Sun 2015[52]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Zhengwen Sun 2014[25]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Meng Xu 2014[48]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Baoyong Sun 2015[53]	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes
Tatsuya Iwasaki 2015[57]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Kang Han 2015[62]	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Yes

included here described allocation concealment and sample-size calculation, reported animals excluded from analysis or blinded assessment of outcome. One study reported inclusion and exclusion criteria [39]. Six studies reported randomization [39, 46, 47, 55, 58, 65], whereas 23 studies reported potential conflicts of interest and study funding [25, 33, 36, 37, 39, 40, 43, 44, 46-48, 50, 52-58, 60-62, 64]. Only one study reported blinded assessment of outcome [53]. Therefore, the methodological quality of studies included here was not satisfied.

Inhibitory effects on the tumor growth (tumor weight/ tumor volume) of osteosarcoma xenograft models by correction of the abnormally expressed miRNAs

Due to the data used for this systematic review and meta-analysis were experiment-levels, different major outcome measures (tumor weight or tumor volume); different types(miR-195, miR-143, miR-34a, miR-214, miR-23a, miR-133a, and so on) or functions(oncogenes or tumor suppressors) of miRNAs; different miRNA intervention methods(directly transfected into OS cells, transfected into OS cells with plasmid vectors, infected into OS cells by lentivirus vectors, systematic administration or injected into tumor directly); different OS cells(MG-63, U2 OS, Saos-2,143B or SOSP-9607) and inoculation sites for producing xenograft models (intratibial inoculation or subcutaneous inoculation) were used in the included studies, these factors all could potentially produce a high heterogeneity. However, if all the baseline characteristics among groups were balanced, the data could not be evaluated. Therefore, stratifications were performed based on these factors to minimize the heterogeneity. Meanwhile, random-effects models were used for the analyses.

	n	irna		0	Control		:	Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% Cl
1.1.1 Tumor suppressor									
Baoyong Sun 2015	0.328	0.221	8	1.459	0.303	8	5.2%	-4.03 [-5.92, -2.15]	•
Chi Cheng 2014	0.2737	0.0253	6	0.5516	0.0295	6	2.5%	-9.33 [-14.03, -4.64]	
Guoxing Xu 2014	0.3123	0.0571	5	0.6884	0.0907	5	4.1%	-4.48 [-7.29, -1.67]	
Jiahui Zhou 2015 (a)	0.467	0.101	9	1.457	0.209	9	4.7%	-5.74 [-8.06, -3.43]	-
Jiahui Zhou 2015 (b)	0.41	0.162	9	1.366	0.202	9	5.0%	-4.97 [-7.03, -2.92]	-
Jie Gao 2012	1.2457	0.2465	5	1.7431	0.2984	5	5.6%	-1.64 [-3.19, -0.10]	-
Jin Wang 2014	0.5112	0.0599	5	1.1882	0.0921	5	2.5%	-7.87 [-12.47, -3.27]	
Kang Han 2014	1.0437	0.085	10	1.6019	0.1131	20	5.6%	-5.18 [-6.78, -3.58]	-
Kang Han 2015	1.006	0.229	10	1.585	0.198	10	6.0%	-2.59 [-3.84, -1.34]	-
Kang Yan 2012	1.1373	0.1742	6	1.7725	0.3381	6	5.6%	-2.18 [-3.73, -0.63]	-
Lei Chen 2013	0.2095	0.0254	5	0.4698	0.0302	5	2.3%	-8.43 [-13.33, -3.52]	
Lei Fan 2013	1	0.2	3	1.8	0.4	7	5.4%	-2.00 [-3.77, -0.24]	-
Liang Ge 2016	0.36	0.083	10	0.911	0.122	10	5.2%	-5.06 [-7.01, -3.10]	-
Mitsuhiko Osaki 2011	3.32	0.65	10	3.67	0.59	10	6.3%	-0.54 [-1.44, 0.36]	1
Tomohiro Fujiwara 2014	2.155	0.6465	15	3.09	0.6346	10	6.3%	-1.41 [-2.31, -0.50]	-
Wei Liu 2015	0.279	0.051	5	0.641	0.079	5	3.9%	-4.92 [-7.95, -1.88]	-
Wei Wang 2015	0.429	0.072	6	0.97	0.099	6	3.9%	-5.77 [-8.80, -2.73]	+
Xiaoji Luo 2014	0.369	0.021	5	0.674	0.042	5	2.4%	-8.30 [-13.13, -3.46]	
Xin Zhou 2013	0.3595	0.0607	6	0.9105	0.1027	6	3.8%	-6.03 [-9.18, -2.88]	-
Yong Zhao 2015	1.738	0.398	6	2.827	0.482	6	5.6%	-2.27 [-3.86, -0.69]	•
Yu He 2014	0.375	0.075	8	0.998	0.219	8	5.4%	-3.60 [-5.34, -1.86]	-
Zhengwen Sun 2014	0.144	0.011	5	0.326	0.029	5	2.7%	-7.50 [-11.89, -3.10]	
Subtotal (95% CI)			157			166	100.0%	-4.05 [-4.97, -3.13]	•
Heterogeneity: Tau ² = 3.30	; Chi ² = 101	1.55, df =	21 (P <	0.00001); I ² = 79	%			
Test for overall effect: Z = 8	3.61 (P < 0.0	00001)							
1.1.2 Oncogenesis									
K Tian 2015	1.497	0.103	10	1.1885	0.1999	20	31.9%	1.72 [0.83, 2.61]	•
Xiuhui Wang 2014	0.753	0.064	6	0.349	0.03	6	20.7%	7.46 [3.65, 11.28]	+
Xuming Wang 2014	154.878	15.244	4	100	15.244	4	26.0%	3.13 [0.57, 5.69]	-
Zhengyu Xu 2014	1.121	0.1203	6	0.4522	0.0289	6	21.4%	7.06 [3.43, 10.68]	
Subtotal (95% CI)			26			36	100.0%	4.42 [1.57, 7.26]	◆
Heterogeneity: Tau ² = 6.38	; Chi ² = 15.	66, df = 3	(P = 0	.001); I ² =	81%				
Test for overall effect: Z = 3	3.05 (P = 0.0	002)							
									-50 -25 U 25 50
Test for subaroup differen	ces: Chi² =	30.85. df	= 1 (P	< 0.0000	1). I² = 98	ò.8%			Favours milking Favours control

Figure 2: Meta-analysis of studies evaluating the inhibitory effects on tumor weight after the aberrantly expressed miRNAs were corrected, when all included studies used tumor weight as the major outcome measure were stratified by the function of miRNAs in the pathogenesis of osteosarcoma. SD, standard deviation; CI, confidence interval.

When all the included studies used tumor weight as the major outcome measure were stratified by whether the aberrantly expressed miRNAs function as oncogenes or tumor suppressors in the pathogenesis of osteosarcoma

Since the mechanisms of oncogenes or tumor suppressors in the pathogenesis of OS are different, we conducted the delaminating analysis.

Twenty-one of the 25 studies reported that miRNAs function as the tumor suppressors and thus the data were combined for a meta-analysis [17, 25, 32, 33, 35, 37, 42-47, 53-55, 58, 59, 61, 62, 64, 65]. A total of 157 mice in the intervention arm and 166 in the control arm were included. The results suggested that restoring the decreased tumor suppressor miRNAs was able to restrain the progression of OS *in vivo* when a random-effects model was used. And the pooled MD = [-4.05]; 95% confidence interval [CI]: [-4.97]- [-3.13]; p < 0.00001(Figure 2 upper part).

Four of the 25 studies reported that miRNAs functions as onco-miRNAs in OS [34, 49-51]. A total of 26 mice in the intervention arm and 36 in the control arm were included. The results suggested that decreasing the tumor onco-miRNAs was also able to restrain the OS progression *in vivo* when a random-effects model was used. And the pooled MD = [4.42]; 95% confidence interval [CI]: [1.57]- [7.26]; p = 0.001; Figure 2, lower part.

When above included studies that reported miRNAs as tumor suppressors or oncogenes were further stratified respectively by the following factors

The miRNA delivery method

There were 21 studies which reported that tumor weight as the major outcome measure and miRNAs as tumor suppressors. MiRNAs were directly transfected into the OS cells in 5 studies, and thus the data were combined for a meta-analysis [44, 54, 55, 59, 65]. There were 29 mice in the intervention arm and 33 mice in the control arm. The tumor weight showed a significant statistical difference when the decreased tumor suppressor miRNAs were corrected (pooled MD = [-5.28]; 95% confidence interval [CI]: [-7.69]- [-2.87]; p = 0.006; Figure 3A, part 1) in a random-effects model. MiRNAs with plasmid vectors were transfected into the OS cells in 3 studies, and were combined for a meta-analysis. There were 16 mice in both the intervention and control arms. Tumor weight was also significantly inhibited after the correction of the decreased tumor suppressor miRNAs (pooled MD = [-2.37]; 95% confidence interval [CI]: [-3.68]- [1.06]; p = 0.22; Figure 3A, part 2) in a random-effects model [33, 35, 64]. MiRNAs were infected into the OS cells by lentivirus vectors in 8 studies, and were combined for a meta-analysis. There were 55 mice in the intervention arm and 65 in the control arm. The tumor weight significantly decreased when the decreased tumor suppressor miRNAs were corrected (pooled MD = [-5.17]; 95% confidence interval [CI]: [-6.60]- [-3.74]; p = 0.01; Figure 3A, part 3)in a random-effects model [25, 32, 37, 42, 45, 53, 61, 62]. MiRNAs were delivered by systematic administration after inoculation with OS cells in other 3 studies [17, 43, 47]. There were 31 mice in the intervention arm and 26 mice in the control arm. Tumor weight also showed a statistically significant decrease after the aberrantly expressed miRNAs were corrected (pooled MD = [-1.25]; 95% confidence interval [CI]: [-2.13]-[-0.37]; p = 0.13; Figure 3A, part 4) in a random-effects model. MiRNAs were injected into tumor directly in 2 studies, and were combined for a meta-analysis. There were 26 mice in both of the intervention and control arms. The tumor weight significantly decreased when the aberrantly expressed miRNAs were corrected (pooled MD = [-4.59]; 95% confidence interval [CI]: [-5.84]- [-3.34]; *p* = 0.31; Figure 3A, part 5)in a random-effects model [46, 58].

There were 4 studies which reported that tumor weight as the major outcome measure and miRNAs as oncogenes. MiRNAs were transfected into OS cells with plasmid vectors in 2 studies, and the data were combined for a meta-analysis. There were 14 mice in the intervention arm and 24 in the control arm. Tumor weight was also significantly inhibited after correction of the up-regulated oncogene miRNAs (pooled MD = [1.89]; 95% confidence interval [CI]: [0.98]- [2.81]; p = 0.31; Figure 3B) in a random-effects model [50, 51].

Only 1 study respectively reported that miRNA was either directly transfected into the OS cells or infected into the OS cells by lentivirus vectors, therefore the data could not be pooled. One of the 2 studies reported that miR-25 was transfected into OS cells directly [49]. This study confirmed that miR-25 was frequently over-expressed in OS, and up-regulation of miR-25 promoted cell proliferation in vitro and tumor growth in a xenograftmouse model. The other study reported that miR-214 was infected into OS cells by lentivirus vectors [34]. This study showed that miR-214 was frequently up-regulated in OS specimens than noncancerous, and over-expression of miR-214 could promote OS cell proliferation, invasion and tumor growth in nude mice. The weight of miR-214-overexpressing tumor was > 2-fold higher than that of the controls.

As we could see in Figure 3A, the overall effect on reducing the tumor weight *via* miRNAs being infected into OS cells with lentivirus vectors or transfected into OS cells directly were the best and comparable, then direct injecting miRNAs into tumors, and followed by being transfected into OS cells with plasmid vectors or systematic administration of miRNAs.

The names of miRNAs

In order to find out if different miRNA has different influence on OS growth, data of same miRNA from more than 2 studies (if have), which reported that tumor weight was the major outcome measure, were combined

ImiRNA Control Std. Mean Difference Std. Mean Difference Std. Mean Difference 12.1 miRNAs were transfected into OS cells directly 0.253 0.0253 0.0253 0.0253 0.0251 0.0295 6 14.1% -9.33 [+14.03, -4.64] Laif go 2014 0.273 0.0253 0.0251 5 0.641 0.079 5 2.00 [+3.77, -0.24] - Laif go 2010 0.279 0.0251 5 0.641 0.079 5 2.00 [+3.77, -0.24] - Wei Lu 2015 0.279 0.0251 5 0.641 0.079 5 2.00 [+3.77, -0.24] - Hetrogeneity, Tar# - 4.96, Ch#= 14.28, df = 4 (P = 0.006); P = 72% Test for werail effect 2 = 4.29 (P < 0.0001) - - 12.2 miRNAs were transfected into OS cells with plasmid vectors - - - - Guoring Xu 2014 0.312 0.0571 1 6 16 100.0% -2.37 [-3.68, -1.06] - Heterogeneity, Tar# - 0.46, Ch# = 3.02, df = 2 (P = 0.22); P = 34% Test for werail effect Z = 3.54 (P = 0.000) - 5.17.7% -4.48 [-5.9, -2.16, 7] - 1.2.3 miRNAs were infected into OS cells by lent/wrus vectors Baoyong Sun 2015 0.228 0.221 f 6 1.64 100.0% -2.37 [-3.68, -1.06] - 1.2										
Study or subgroup Mean SD Total Mean SD Total Weight M, Random 95% CI Lifar 2013 1 0.23 0.8 0.025 6 0.516 0.0295 6 14.1% -0.33 [+4.03, -4.64] +0.217 (-0.24) +0.217 (-0.24) +0.217 (-0.24) +0.217 (-0.24) +0.218 (-0.27, -0.26) +0.218 (-0.27, -0.27) +0.218 (-0.27, -0.28) +0.218 (-0.27, -0.28) +0.218 (-0.27, -0.28) +0.218 (-0.27, -0.28) +0.218 (-0.28) +0.		r	niRNA		(Control			Std. Mean Difference	Std. Mean Difference
1.2.1 miRNAs were transfected into OS cells directly Lal Fan 2013 1 0.22 3 0.0253 6 0.295 6 14.1% -0.33 [+14.03, -4.64] Lal Fan 2013 1 0.279 0.0251 5 0.641 0.079 5 20.0% -4.92 (-7.95, -1.81) Wei Liu 2015 0.279 0.0251 5 0.641 0.079 5 20.0% -4.92 (-7.95, -1.81) Wei Liu 2015 0.279 0.0251 5 0.674 0.042 5 13.7% -8.30 (+13.3, 3.46) Large activity overall effect Z = 4.29 (P < 0.0001) P = 72% Testfor overall effect Z = 4.29 (P < 0.0001) 1.2.2 miRNAs were transfected into OS cells with plasmid vectors Guoding X2 014 0.312 0.0571 16 0.0294 5 17.7% -4.48 (-7.29, -1.67) Jie 0a 2012 1.2457 0.2465 5 1.7431 0.2984 5 41.2% -1.64 (-3.19, -0.06) 1.1.2% miRNAs were indected into OS cells with plasmid vectors Bayong Sun 2012 1.1373 0.1742 6 0.207 F = 32% Testfor overall effect Z = 3.54 (P = 0.000, H = 0.207, P = 34% Testfor overall effect Z = 3.54 (P = 0.002, H = 2.07, P = 34% Testfor overall effect Z = 3.54 (P = 0.000, H = 0.002) F = 1.8% Xang Han 2015 0.028 0.0221 6 0.499 0.003 8 17.2% -4.03 (-5.92, -2.15) Jim Wang 2014 0.0412 0.0599 5 1.1882 0.0921 5 6.9% -7.87 (+1.247, -3.27) Jim Wang 2014 0.0412 0.0599 5 1.1882 0.0921 5 6.9% -7.87 (+1.247, -3.27) Jim Wang 2014 0.0412 0.0599 5 1.1882 0.0921 5 6.9% -7.87 (+1.247, -3.27) Jim Wang 2014 0.0412 0.0599 0.0254 6 0.498 0.0032 5 6.7.% -5.77 (+8.03, -7.38) Jim Wang 2014 0.0414 0.011 5 0.326 0.022 6 7.4% -5.77 (+8.03, -7.38) Wei Wang 2013 0.398 0.0607 6 10 39.6% -0.54 (+1.44, 0.36) The terrogeneity. Tat" = 0.4, 0.011 5 0.328 0.029 5 7.4% -7.50 (+1.183, -3.16) 1.2.4 miRNA were delivered by systematic administration Mitsuliko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 (+1.44, 0.36) The terrogeneity. Tat" = 0.00, Ch7 = 0.0009 1.2.5 miRNAs were injected into turmor directly Jahu Zhou 2015 (0, 0.467 0.0101 9 1.457 0.209 9 2.5%, -5.74 (+8.06, -3.43] 4.59 (-5.84, -3.43] 5.50 (-5.53, -1.18) Favours miRNA = avo	Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% Cl
Chi Cheng 2014 0.2737 0.0253 6 0.5516 0.0295 6 14.1% -9.33 [14.03, -4.44] Liang 06 2016 0.36 0.083 10 0.911 0.122 10 25.4% -5.06 [-7.07, -0.24] Liang 06 2016 0.36 0.083 10 0.911 0.122 10 25.4% -5.06 [-7.07, -0.24] Wel Liu 2015 0.279 0.051 5 0.641 0.079 5 20.6% -4.92 [7.95, -1.88] Subtotal (95% CD 29 33 100.0% -5.28 [-7.09, -2.87] Heterogeneity: Tau ² = 4.96; Ch ² = 1.4.28, df = 4 (P = 0.006); P = 72% Test for overall effect Z = 4.29 (P < 0.0001) 1.2.2 mRNAs were transfected into OS cells with plasmid vectors Guoving Vu 2014 0.3123 0.0571 5 0.8884 0.0907 5 17.7% -4.48 [-7.29, -1.67] Jie 0ao 2012 1.2457 0.2466 5 1.7431 0.2984 5 5 41.2% -1.64 [-4.319, -0.10] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.381 6 41.0% -2.18 [-3.73, -0.63] Test for overall effect Z = 3.54 (P = 0.020); P = 34.% Test for overall effect Z = 3.54 (P = 0.020); P = 34.% Test for overall effect Z = 3.54 (P = 0.0004) 1.2.3 mRNAs were infected into OS cells by lenth/trus vectors Bayong Sun 2015 0.328 0.221 8 1.459 0.303 8 17.2% -4.03 [-5.92, -2.16] Heterogeneity: Tau ² = 0.46; Ch ² = 3.00, 2.16 = 2 (P = 0.22); P = 34% Test for overall effect Z = 3.54 (P = 0.0004) 1.2.3 mRNAs were infected into OS cells by lenth/trus vectors Bayong Sun 2015 0.328 0.221 8 1.459 0.302 5 6.3% -7.87 [-1.247, -3.27] Kang Han 2014 0.011 5 0.326 0.022 5 6.3% -6.43 [-1.33, -3.58] Heterogeneity: Tau ² = 2.17; Ch ² = 17.50, df = 7 (P = 0.01); P = 60% Test for overall effect Z = 7.09 (P < 0.0101) 5 0.326 0.029 5 7.44 [-3.60, -3.74] Heterogeneity: Tau ² = 2.17; Ch ² = 17.50, df = 7 (P = 0.01); P = 60% Test for overall effect Z = 7.70 (P < 0.00001) 1.2.4 mRNA were diheered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.36 0.59 10 3.96% -0.54 [+1.44, 0.38] Test for overall effect Z = 7.70 (P < 0.00001) 1.2.4 mRNA were diheered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.87 0.59 10 3.96% -0.54 [+1.44, 0.38] Test for overall effect Z = 7.70 (P < 0.00001) 1.2.4 mRNA were diheered by systematic administratio	1.2.1 miRNAs were transf	fected into	OS cells	direc	tly					
Lei Fan 2013 1 0.2 3 1.8 0.4 7 26.2% -200 [+3.77, -0.24] Liang Ge 2016 0.36 0.083 10 0.911 0.122 10 22.6.2% -5.06 [+7.01, -3.10] Wei Liu 2015 0.279 0.051 5 0.641 0.079 5 20.6% -4.92 [+7.95, -3.10] Wei Liu 2015 0.279 0.051 5 0.674 0.042 5 13.7% -8.30 [+1.91, 3.3, -3.46] Subtotal (95% C) 29 33 100.0% -5.28 [-7.69, -2.87] Test for overall effect $Z = 4.29$ ($P < 0.000$); $P = 72\%$ Test for overall effect $Z = 4.29$ ($P < 0.000$); $P = 72\%$ Test for overall effect $Z = 4.29$ ($P < 0.000$); $P = 72\%$ Test for overall effect $Z = 4.29$ ($P < 0.000$); $P = 72\%$ Test for overall effect $Z = 4.29$ ($P < 0.000$); $P = 72\%$ Test for overall effect $Z = 4.29$ ($P < 0.000$); $P = 72\%$ Test for overall effect $Z = 3.54$ ($P = 0.000$); $P = 72\%$ Test for overall effect $Z = 3.54$ ($P = 0.000$); $P = 72\%$ Test for overall effect $Z = 3.54$ ($P = 0.000$); $P = 72\%$ Test for overall effect $Z = 3.54$ ($P = 0.000$); $P = 72\%$ Test for overall effect $Z = 3.54$ ($P = 0.000$); $P = 72\%$ Test for overall effect $Z = 3.54$ ($P = 0.000$); $P = 72\%$ Test for overall effect $Z = 3.54$ ($P = 0.000$); $P = 72\%$ Test for overall effect $Z = 3.54$ ($P = 0.000$); $P = 72\%$ Test for overall effect $Z = 3.54$ ($P = 0.000$); $P = 72\%$ Test for overall effect $Z = 3.54$ ($P = 0.000$); $P = 72\%$ Test for overall effect $Z = 3.54$ ($P = 0.000$); $P = 72\%$ Test for overall effect $Z = 7.09$ ($P = 0.20$); $P = 34\%$ Test for overall effect $Z = 7.09$ ($P = 0.000$); $P = 60\%$ Test for overall effect $Z = 7.09$ ($P = 0.0000$) 1.2.3 miRNAs were infected into Oscells by lenthristration Misubiko 0.360 (201 3.10 5 0.367 0.59 10 3.96\% -0.54 [+1.44, 0.36] Tomobiro Fujkwara 2014 2.156 0.4665 15 3.09 0.6346 10 3.92\% -1.41 [+2.31, 0.50] Subtotal (69% C) -341 2.25 0.075 8 0.938 0.229 5 7.457 + 5.06 [+3.4, 1.38] 3.100.015 -5.74 [+8.06, -3.43] 4.29 [+7.05, -3.60 [+3.19] = 7.0005 Test for overall effect $Z = 7.19$ ($P = 0.000$)] $P = 60\%$ Test for overall effect $Z = 7.19$ ($P = 0.000$)] $P = 60\%$ Test for overall effect $Z $	Chi Cheng 2014	0.2737	0.0253	6	0.5516	0.0295	6	14.1%	-9.33 [-14.03, -4.64]	_
Liang Ge 2016 0.36 0.083 10 0.911 0.122 10 25.4% -5.06 [-7.01, -3.10] + Well Lu 2015 0.279 0.051 5 0.674 0.079 5 20.6% -4.92 [-7.65, -1.88] + .0006 $(-7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .0006 $(-7.7, -7.7, -3.10]$ + .00000 + .00000 + .00000 + .00000 + .00000 + .00000 + .00000 + .00000 + .00000 + .00000 + .00000 + .00000 + .00000	Lei Fan 2013	1	0.2	3	1.8	0.4	7	26.2%	-2.00 [-3.77, -0.24]	
We Liu 2015 0.279 0.051 5 0.641 0.079 5 20.6% $-4.92[+35,-1.88]$ Subtotal (95% C) 29 33 100.0% $-5.28[-7.69, -2.87]$ Test for overall effect $Z = 4.29 (P < 0.0001)$ 1.2.2 mRVNs were transfected into OS cells with plasmid vectors Guoding Xu 2014 0.312 0.0571 5 0.684 0.0907 5 17.7% $-4.48 [+7.29, -1.67]$ Jie Gao 2012 1.2457 0.2465 5 1.7431 0.2984 5 41.2% $-1.64 [+3.19, -0.10]$ Jie Gao 2012 1.137 0.174 2 6 1.7725 0.3381 6 41.0% $-2.37 [+3.36, -1.06]$ Heterogeneity. Tau ² = 0.6(; ChF = 30.2($\pm 2.2)$; F = 34% Test for overall effect $Z = 3.54 (P = 0.0004)$ 1.2.3 mRVNs were infected into OS cells by lentifyinus vectors Baoyong Sun 2015 0.328 0.221 8 1.459 0.303 8 17.2% $-4.03 [+5.92, -2.16]$ Jin Wang 2015 0.328 0.221 8 1.459 0.303 8 17.2% $-4.03 [+5.92, -2.16]$ Jin Wang 2015 0.328 0.221 8 1.459 0.303 8 17.2% $-4.03 [+5.92, -2.16]$ Jin Wang 2015 0.328 0.221 8 0.1459 0.0921 5 6.3% $-7.87 [+12.47, -3.27]$ Heterogeneity. Solution 10 0.0218 9 10.1561 0.019 0.1131 20 18.8% $-5.18 [+6.78, -3.58]$ Jin Wang 2015 0.024 5 0.4698 0.0302 5 6.3% $-8.43 [+3.33, -3.52]$ Heterogeneity. Tau ² = 0.47; ChF = 17.50, df = 7 (P = 0.01); P = 60% Test for overall effect $Z = 7.09 (P < 0.00001)$ 1.2.4 mRVNA were delevered by systematic administration Mitsuhko Cash 2011 3.32 0.65 10 3.87 0.59 10 39.6% $-0.54 [+1.44, 0.38]$ Tornohio Fujiwara 2014 2.155 0.6465 15 0.39 0.6346 10 39.2% $-1.57 [+1.80, -3.74]$ 4.159 [-5.81, -3.61] 5.100.0% $-5.77 [+8.00, -3.74]$ 5.100.0% $-1.25 [-2.13, -0.37]$ 4.12.17 cuff = 17.30 (-1.27 (P = 0.01); P = 60% Test for overall effect $Z = 2.77 (P = 0.000)$ 1.2.5 mRVNA were indected into tumor directly Jahau 2700 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% $-5.74 [+8.06, -3.43]$ 4.159 [-5.84, -3.34] Jahau 2700 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% $-5.74 [+8.06, -3.43]$ 4.159 [-5.84, -3.34] Heterogeneity. Tau ² = 0.16; ChF = 2.33, df = 2 (P = 0.31); F = 14% Test for overall effect $Z = 7.79 (P < 0.00001)$	Liang Ge 2016	0.36	0.083	10	0.911	0.122	10	25.4%	-5.06 [-7.01, -3.10]	
Xiaoji Luo 2014 0.369 0.021 5 0.674 0.042 5 13.7% -6.30 [13.13, 3.46] Heterogeneity, Tau ² = 4.96; Ch ² = 14.29, df = 4 (P = 0.006); P = 72% Test for overall effect $Z = 4.39$ (P < 0.0001) 1.22 miRNAs were transfected into OS cells with plasmid vectors Guoding Xiu 2014 0.3123 0.0571 5 0.6884 0.0907 5 17.7% -4.48 [7.29, -1.67] Xiaoji La 2012 1.2457 0.2465 5 17.431 0.2984 5 412% -1.64 [3.19, -0.10] Xiaoji La 2012 1.2457 0.2465 5 17.431 0.2984 5 412% -1.64 [3.19, -0.10] Xiaoji La 2012 1.2457 0.2465 5 17.431 0.2984 5 412% -1.64 [3.19, -0.10] Xiaoji La 2012 1.2457 0.2465 1 7.731 0.2984 5 412% -2.18 [3.73, -0.63] Xiatoti (95% C) 1.2.3 miRNAs were infected into OS cells by lentivirus vectors Bayong Su 2015 0.0328 0.221 8 1.459 0.303 8 17.2% -4.03 [-5.92, -2.15] Jin Wang 2014 0.5112 0.0599 5 1.1882 0.9921 5 6.9% -7.87 [+1.247, -3.27] Jin Wang 2014 0.5112 0.0599 5 1.1882 0.9921 5 6.9% -7.87 [+1.247, -3.27] Jin Wang 2014 0.512 0.0599 0 5 1.1892 0.9921 6 6.9% -2.59 [-3.84, -1.34] Lei Chen 2015 0.0256 0.0254 5 0.4680 0.0302 5 6.3% .64.2[+1.33, -3.52] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 11.6% -5.77 [+8.0, -2.73] Test for overall effect Z = 7.09 (P < 0.0001); P = 60% Test for overall effect Z = 7.09 (P < 0.0001); P = 60% Test for overall effect Z = 7.09 (P < 0.0001); P = 60% Test for overall effect Z = 7.79 (P < 0.0001) 1.2.4 miRNA were eleivered by systematic administration Misuhiko Casia 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [+1.44, 0.36] Tormohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.634 10 39.2% -0.54 [+1.44, 0.36] Tormohiro Fujiwara 2015 (3) 0.467 0.019 9 1.457 0.209 9 25.9% -5.74 [+8.06, -3.43] 4.2.2 miRNA were eleivered by systematic administration Misuhiko Casia 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [+1.44, 0.36] Tormohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6348 10 39.2% -0.54 [+1.44, 0.36] Tormohiro Fujiwara 2014 2.157 0.77 (P = 0.01); P = 60% Test for overall effect Z = 7.79 (P < 0.000) 1.2.5 miRNA swere infected into turnor directly Jahui Zhou 2015 (a) 0.467	Wei Liu 2015	0.279	0.051	5	0.641	0.079	5	20.6%	-4.92 [-7.95, -1.88]	
Subtat (95% C) 29 33 100.0% $.5.28$ [7.69, 2.87] Test for overall effect $Z = 4.29$ ($P < 0.0001$) 1.22 miRNAs were transfected into OS cells with plasmid vectors Guoxing $3u = 2012$ 1.2457 0.2465 5 1.7431 0.2984 5 41.2% -1.64 [5.319, 0.10] 1.6 Gao 2012 1.2457 0.2465 5 1.7431 0.2984 5 41.2% -1.64 [5.319, 0.10] 1.6 Gao 2012 1.1373 0.1742 6 1.7725 0.3381 6 41.0% -2.18 [5.37, 0.63] Subtata (95% C) 16 16 100.0% -2.37 [1.368, -1.06] Test for overall effect $Z = 3.64$ ($P = 0.0004$) 1.2.3 miRNAs were infected into OS cells by lentifying vectors Baoyong $3u = 2015$ 0.328 0.221 8 1.459 0.303 8 17.2% -4.03 [5.52, -2.16] 1.6 100.0% -2.57 [1.24, -3.27] 1.725 0.3281 6 0.291 0 1.585 0.198 10 2068 -2.59 [3.54, -1.34] Lei Chen 2013 0.2095 0.0254 5 0.4698 0.0302 5 6.3% -8.43 [+1.33, -3.52] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 11.8% -5.77 [+8.00, -2.73] Mit and 2014 0.144 0.011 5 0.326 0.029 5 7.4% -5.57 [+1.24, -3.27] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 11.8% -5.77 [+8.00, -3.74] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 11.8% -5.77 [+8.00, -3.74] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 11.6% -5.77 [+8.00, -3.74] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 11.8% -5.77 [+8.00, -3.74] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 11.8% -5.77 [+8.00, -3.74] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 10.8% -0.54 [+1.44, 0.36] The terogeneity. Tau" = 2.17, Chi [#] = 1.75, dif = 7 (P = 0.01); P = 60% Test for overall effect $Z = 7.09$ ($P < 0.00001$) 1.2.4 miRNA were delivered by systematic administration Misultiko 0.684 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [+1.44, 0.36] Tomohio Fujiwara 2014 2.155 0.6451 5 3.09 0.6364 10 3.92% -1.41 [+2.31, -0.50] Test for overall effect $Z = 2.77$ ($P = 0.000$) 1.2.5 miRNA sever einfected into tumor directly Jahui Zhou 2015 (a) 0.467 0.010 9 1.457 0.209 9 25.9% -5.74 [+8.06, -3.43] Jahui Zhou 2015 (a) 0.467 0.010 9 1.457 0.209 9 25.9% -5.74 [+8.06, -3.43] Jahui Zhou 2015 (a) 0.467 0.010 9 1.457 0.209 9 25.9% -5.74 [+8.06,	Xiaoji Luo 2014	0.369	0.021	5	0.674	0.042	5	13.7%	-8.30 [-13.13, -3.46]	
Heterogeneity: Tau" = 4.96; Ch" = 14.29; df = 4 (P = 0.006); P = 72% Test for overall effect Z = 4.29 (P < 0.0001) 1.2.2 miRNA were transfected into OS cells with plasmid vectors Guoxing Xu 2014 0.3123 0.0571 5 0.6884 0.0907 5 17.7% - 4.48 [7.29, -1.67] Jie Gao 2012 1.2457 0.2466 5 17.431 0.2384 5 41.2% Jie Gao 2012 1.2457 0.2466 5 17.431 0.2384 5 41.2% Jie Gao 2012 1.2457 0.2466 5 17.431 0.2384 5 41.2% Jie Gao 2012 1.2457 0.2466 5 17.431 0.2384 6 41.0% - 2.18 [-5.37, -0.63] Heterogeneity: Tau" = 0.46; Chi" = 3.02, df = 2 (P = 0.22); P = 34% Test for overall effect Z = 3.54 (P = 0.0004) 1.2.3 miRNAs were infected into OS cells by lentiMirus vectors Baoyong Su 2015 0.228 0.221 8 1.459 0.303 8 17.2% -4.03 [-5.92, -2.15] Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 6.5% -7.87 [-1.247, -3.27] Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 6.3% -5.18 [-6.78, -3.58] Heterogeneity: Tau" = 0.46; Chi" = 3.02, df = 2 (P = 0.22); P = 34% Test for overall effect Z = 7.09 (P < 0.0004) 1.2.3 miRNA were infected into OS cells by lentiMirus vectors Baoyong Sun 2014 0.444 0.011 5 0.326 0.029 5 7.4% -5.18 [-6.78, -3.48] Jin Wang 2015 0.429 100.72 6 0.97 0.099 6 11.6% -5.17 [-6.60, -3.74] Heterogeneity: Tau" = 2.17; Chi" = 17.50, df = 7 (P = 0.01); P = 60% Test for overall effect Z = 7.09 (P < 0.00001) 1.2.4 miRNA were delivered by systematic administration Mitsuhko Osaki 2011 3.32 0.065 10 3.67 0.59 10 39.6% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.0465 15 3.09 0.0344 10 39.2% -1.44 [-2.13, 0.60] 3.10 Cool 5 1.138 0.398 6 2.227 0.422 6 100.0% -1.25 [-2.13, -0.03] 4.25 Field Field C = 2.77 (P = 0.006) 1.2.5 miRNA swere delivered by systematic administration Mitsuhko Osaki 2011 3.32 0.67 2 0 (P = 0.13); P = 50% Test for overall effect Z = 2.77 (P = 0.006) 1.2.5 miRNA swere injected into turor directy Jahui Zhou 2015 (a) 0.47 0.17 9 1.457 0.208 9 25.9% -5.74 [-8.06, -3.43] Jahui Zhou 2015 (a) 0.375 0.075 8 0.998 0.219 8 42.2% Subtotal (95% Ch) -3.10; P = 2.33, df = 2 (P	Subtotal (95% CI)			29			33	100.0%	-5.28 [-7.69, -2.87]	◆
Test for overall effect Z = 4.29 ($P < 0.0001$) 1.2.2 miRNAs were transfected into OS cells with plasmid vectors Guoding XU 2014 0.3123 0.057 5 0.6884 0.0907 5 17.7% -4.48 [$7.29, -1.67$] Jle Gao 2012 1.2457 0.2465 5 1.7431 0.2984 5 41.2% -1.64 [$5.18, -0.01$] Subtotal (95% Ct) 1.1373 0.1742 6 1.7725 0.3381 6 41.0% -2.18 [$5.373, -0.63$] Subtotal (95% Ct) 1.1373 0.1742 7 ($P = 0.22$); $P = 34$ % Test for overall effect Z = 3.54 ($P = 0.000$) 1.2.3 miRNAs were infected into OS cells by lentivirus vectors Baoyong Sun 2015 0.328 0.221 8 1.459 0.303 8 17.2% -4.03 [$5.92, -2.16$] Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 6.9% -7.87 [$+12.47, -3.27$] Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 18.8% -5.18 [$6.78, -3.58$] Lei Chen 2013 0.2095 0.0254 5 0.4688 0.0302 5 6.3% -6.43 [$3.33, -3.52$] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 11.6% -5.77 [$+8.0, -2.73$] Xin Zhou 2013 0.3959 0.0607 6 0.9105 0.027 6 11.2% -6.03 [$+9.18, -2.88$] Zhengwen Sun 2014 0.144 0.011 5 0.326 0.029 5 7.4% -7.50 [$+1.24, 0.36$] Test for overall effect Z -7.19 ($P = -0.01$); $P = 60\%$ Test for overall effect Z -7.19 ($P = -0.01$); $P = 60\%$ Test for overall effect Z -7.10 ($P = -0.01$; $P = 60\%$ Test for overall effect Z -7.10 ($P = -0.01$; $P = 50\%$ Test for overall effect Z -2.77 ($P = -0.01$; $P = 50\%$ Test for overall effect Z -2.77 ($P = -0.01$; $P = 50\%$ Test for overall effect Z -2.77 ($P = -0.01$; $P = 50\%$ Test for overall effect Z -2.77 ($P = -0.01$; $P = 50\%$ Test for overall effect Z -2.77 ($P = -0.01$; $P = 50\%$ Test for overall effect Z -2.77 ($P = -0.01$; $P = 50\%$ Test for overall effect Z -2.77 ($P = -0.01$; $P = 50\%$ Test for overall effect Z -2.77 ($P = -0.01$; $P = 50\%$ Test for overall effect Z -2.77 ($P = -0.01$; $P = 50\%$ Test for overall effect Z -2.77 ($P = -0.01$; $P = 50\%$ Test for overall effect Z -2.77 ($P = -0.01$; $P = 50\%$ Test for overall effect Z -2.77 ($P = -0.000$) 1.2.5 miRNAs were injected into tumord iterety Jjahui Zhou 2015 ($a)$ 0.41 0.162 9 1.366 0.202 9 3	Heterogeneity: Tau ² = 4.96	i; Chi² = 14	4.28, df = 4	4 (P =	0.006); l²	= 72%				
1.2.2 miRNAs were transfected into OS cells with plasmid vectors Guoxing Xu 2014 0.3123 0.0571 5 0.6884 0.0907 5 17.7% -4.48 [7.29, -1.67] Je Gao 2012 1.2457 0.2465 5 1.7431 0.2984 5 41.2% Java 2012 1.1373 0.1742 6 1.7725 0.3381 6 41.0% -2.18 [-3.73, -0.63] 16 100.0% -2.37 [-3.68, -1.06] Heterogeneity: Tau ² = 0.46; Chi ² = 3.02, df = 2 (P = 0.22); P = 34% Test for overall effect: Z = 3.54 (P = 0.0004) 1.2.3 miRNAs were injected into OS cells by lentivirus vectors Bayong Sun 2015 0.328 0.221 8 1.4659 0.303 8 17.2% -4.03 [-5.92, -2.15] Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 6.9% -7.87 [-1.47, -3.27] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 20.6% -2.59 [-3.44, -1.34] Lei Chen 2013 0.2095 0.0254 5 0.4698 0.0302 5 6 1.5% -6.34 [-1.333, -3.52] Wei Wang 2015 0.429 0.007 6 0.9105 0.1027 6 11.2% -6.03 [-1.8, -3.63] Xin Zhou 2013 0.3995 0.0607 6 0.9105 0.1027 6 11.2% -7.50 [-1.188, -3.61] 3.21 cm 1 1.412 0.17 (chi ² C (P = 0.01); P = 60% Test for overall effect: Z = 7.09 (P < 0.00001) 1.2.4 miRNA were injected into tumor directy Jahu Zhou 2015 0.427 0.422 (P = 0.13); P = 50% Test for overall effect: Z = 2.77 (P = 0.006) 1.2.5 miRNAs were injected thu tumor directy Jahu Zhou 2015 (0) 0.41 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jahu Zhou 2015 (0) 0.41 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jahu Zhou 2015 (0) 0.41 0.102 9 1.366 0.202 9 3 31.9% -4.37 [-7.3, 2.42] 1.2.5 miRNAs were injected into tumor directy Jahu Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jahu Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 3.1.9% -4.37 [-7.3, 2.42] Jahu Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 3 31.9% -4.37 [-7.3, 2.42] Jahu Zhou 2015 (c) 0.41 0.162 9 1.366 0.202 9 3 31.9% -4.37 [-7.3, 2.43] Jahu Zhou 2015 (c) 0.41 0.162 9 1.366 0.202 9 3 31.9% -4.37 [-7.3, 2.43] Jahu Zhou 2015 (c) 0.41 0.162 9 1.366 0.202 9 3 31.9% -4.37 [-7.3, 2.43] Jahu Zhou 2015 (c) 0.41 0.162 9 1.366 0.202 9 3 31.9% -4.37 [-7.3, 2.43] Jahu Zhou 2015 (c) 0.41	Test for overall effect: Z = 4	4.29 (P < 0	.0001)							
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Constant (3) Constant (3) <td< td=""><td>Guoving Xu 2014</td><td>0 31 23</td><td>0.0571</td><td>5</td><td>1 8994</td><td>0 0007</td><td>5</td><td>17 7%</td><td>-4.491-7.29 -1.671</td><td>_-</td></td<>	Guoving Xu 2014	0 31 23	0.0571	5	1 8994	0 0007	5	17 7%	-4.491-7.29 -1.671	_ -
$\begin{array}{c} \text{All or 2012} & 1.2373 \ 0.1405 \ 0.5204 \ 0.1772 \ 0.2304 \ 0.1772 \ 0.2304 \ 0.1772 \ 0.237 \ 0.163 \ 0.163 \ 0.1772 \ 0.237 \ 0.163 \ 0.163 \ 0.163 \ 0.1772 \ 0.237 \ 0.163 \ 0.1773 \ 0.1772 \ 0.237 \ 0.163 \ 0.$	lie Geo 2012	1 2457	0.2465	5	1 7/21	0.0307	5	41 206	-1.64[-2.19]-0.10]	
Test for suborou differences: Ch ² = 3.67, df = 4 (P < 0.0001), P = 88.4% Test for suborou differences: Ch ² = 3.67, df = 4 (P < 0.0001), P = 88.4% Test for suborou differences: Ch ² = 3.67, df = 4 (P < 0.0001), P = 88.4% Test for suborou differences: Ch ² = 3.67, df = 4 (P < 0.0001), P = 88.4% Test for suborou differences: Ch ² = 3.67, df = 4 (P < 0.0001), P = 88.4%	Vong Von 2012	1.2407	0.2400	6	1.7431	0.2304	6	41.2 %	210[272_062]	
$\begin{aligned} \begin{array}{c} \text{Lab. Relations} & \text{Lab. F} \left[\text{Lab. S} & \text{Lab. S} \right] \right] \right] \right] \right\right) \right\right) \\ \end{array}{} \\ $	Subtotal (95% CI)	1.1373	0.1742	16	1.7725	0.3301	16	100 0%	-2.10 [-3.73, -0.03]	
Test for overall effect Z = 3.54 ($P = 0.0004$) 1.2.3 miRNAs were infected into OS cells by lentivirus vectors Baoyong Sun 2015 0.328 0.221 8 1.459 0.303 8 17.2% -4.03 [5.92, -2.15] Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 6.9% -7.87 [-12.47, -3.27] Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 18.8% -5.18 [6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.565 0.198 10 20.6% -5.29 [-3.84, -1.34] Lei Chen 2013 0.2095 0.0224 5 0.4698 0.0302 5 6 3.% -8.43 [-13.3, -3.52] Wei Wang 2015 0.429 0.072 6 0.9105 0.1027 6 11.2% -6.03 [-9.18, -2.73] Wei Wang 2015 0.429 0.072 6 0.9105 0.1027 6 11.2% -6.03 [-9.18, -2.73] Wei Wang 2013 0.3595 0.0607 6 0.9105 0.1027 6 11.2% -6.03 [-9.18, -2.88] Zhengwen Sun 2014 0.144 0.011 5 55 65 65 100.0% -5.17 [-6.60, -3.74] Heterogeneity: Tau ² = 2.17; Chi ² = 17.50; df = 7 ($P = 0.01$); $P = 60\%$ Test for overall effect Z = 7.09 ($P < 0.00001$) 1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [-1.44, 0.36] Tomohiro Fujkwara 2014 2.155 0.6465 15 3.09 0.6346 10 39.2% -1.41 [-3.31, -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.86, -0.69] Subtotal (95% C) 31 26 100.0% -1.25 [-2.13, -0.37] Heterogeneity: Tau ² = 0.30; Chi ² = 4.02, df = 2 ($P = 0.13$); $P = 50\%$ Test for overall effect Z = 2.77 ($P = 0.006$) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.44 0.152 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Jiahui Zhou 2015 (b) 0.47 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.47 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.47 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.47 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.47 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.47 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.47	Heterogeneity: Tau ² - 0.46	Chiz - 3	02 df - 2	/P - 0	22) 12-	24%	10	100.072	-2.57 [-5.00, - 1.00]	•
1.2.3 miRNAs were infected into OS cells by lent/virus vectors Bayong Sun 2015 0.328 0.221 8 1.459 0.303 8 17.2% -4.03 [-5.92, -2.15] Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 6.9% -7.87 [-12.47, -3.27] Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 18.8% -5.18 [-6.78, -5.80] Kang Han 2015 1.006 0.229 10 1.565 0.198 10 20.6% -2.59 [-3.84, -1.34] Lei Chen 2013 0.2095 0.0254 5 0.4698 0.0302 5 6.3% -6.43 [-13.8, -3.52] Wei Wang 2015 0.429 0.072 6 0.9105 0.1027 6 11.2% -6.03 [-9.18, -2.68] Zhengwen Sun 2014 0.144 0.011 5 0.326 0.029 5 7.4% -5.01 [-1.89, -3.10] Sultotai (05% C) 5 65 100.0% -5.517 [-6.60, -3.74] Heterogeneilty: Tau ² = 2.17; Chi ² = 17.50, df = 7 (P = 0.01); P = 60% Test for overall effect Z = 7.09 (P < 0.00001) 1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 39.2% -1.41 [-2.31, -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.86, -0.69] Sultotai (05% C) 31 26 100.0% -1.25 [-2.13, 0.37] Heterogeneilty: Tau ² = 0.30; Chi ² = 4 (02, df = 2 (P = 0.13); P = 50% Test for overall effect Z = 2.77 (P = 0.006) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.1457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Sultotai (05% C) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneilty: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); P = 14% Test for overall effect: Z = 7.19 (P < 0.00001) Test for overall effect: Z = 7.19 (P < 0.00001)	Test for overall effect: Z = 3	3.54 (P = 0	.0004)	(1 - 0	.22),1 =	54,0				
The subtroat given interference interferenc	4.2.2 miDNAe wore infect	od into OS	collo bu	lontini	rue voet	010				
badyoing solid 2015 0.328 0.221 6 1.483 0.303 6 17.29 $-4.05[+32, 2.15]$ Xang Han 2014 1.0437 0.086 10 1.6019 0.1131 20 18.8% $-5.18[+6.78, -3.58]$ Xang Han 2015 1.006 0.229 10 1.585 0.198 10 20.6% $-2.59[+3.84, -1.34]$ Lei Chen 2013 0.2095 0.0254 5 0.4698 0.0302 5 $6.3\% -6.34[+13.33, -3.52]$ Wel Wang 2015 0.429 0.072 6 0.97 0.099 6 11.6% $-5.77[+8.80, -2.73]$ Xin Zhou 2013 0.3595 0.067 6 0.9105 0.1027 6 11.2% $-6.03[+9.18, -3.80]$ Zhengwen Sun 2014 0.144 0.011 5 0.326 0.029 5 $7.4\% -7.50[+1.88], -3.10]$ Subtotal (95% CI) 5 0.4656 15 0.367 0.59 10 39.6% $-0.54[+1.44, 0.36]$ Test for overall effect $Z = 7.09$ (P < 0.00001) 1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% $-0.54[+1.44, 0.36]$ Tomohiro Fujiwara 2014 2.155 0.6466 15 3.09 0.6346 10 39.2% $-1.41[+2.31, -0.50]$ 1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% $-0.54[+1.44, 0.36]$ Tomohiro Fujiwara 2014 2.155 0.6466 15 3.09 0.6346 10 39.2% $-1.41[+2.31, -0.50]$ 1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% $-1.25[-2.13, -0.37]$ Heterogeneity. Tau ² = 0.30; Chi ² = 4.02, df = 2 (P = 0.13); P = 50% Test for overall effect $Z = 2.77$ (P = 0.006) 1.2.5 miRNAs were injected into tumor directly Jahui Zhou 2015 (b) 0.41 0.162 9 1.456 0.202 9 31.9% $-4.97[+7.03, -2.92]$ Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% $-3.60[+5.34, -1.86]$ Heterogeneity. Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); P = 14% Test for overall effect $Z = 7.19$ (P < 0.00001) Test for overall effect $Z = 7.19$ (P < 0.00001) Test for overall effect $Z = 7.19$ (P < 0.00001) Test for overall effect $Z = 7.19$ (P < 0.00001) Test for overall effect $Z = 7.19$ (P < 0.00001) Test for overall effect $Z = 7.19$ (P < 0.00001) Test for overall effect $Z = 7.19$ (P < 0.00001) Test for overall effect $Z = 7.19$ (P < 0.00001) Test for overall effect $Z = 7.19$ (P < 0.00001)	Paguang Cup 2015	0 220	0 224		1 450	0.000		17.204	4 0 2 1 5 0 2 1 5 1	
Sin Wang 2014 0.3112 0.0059 5 1.182 0.0921 5 0.0921 5 0.397 1.24, -3.27] Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 18.8% -5.18 [6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 20.6% -2.59 [-3.84, -1.34] Lei Chen 2013 0.2095 0.0254 5 0.4698 0.0302 5 6.3% -8.43 [-1.33, -3.52] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 11.6% -5.77 [-8.80, -2.73] Xin Zhou 2013 0.3595 0.0607 6 0.9105 0.1027 6 11.2% -6.03 [-9.18, -2.88] Zhengwen Sun 2014 0.144 0.011 5 0.326 0.029 5 7.4% -7.50 [-11.88, -3.10] Subtotal (95% CI) 55 665 10 0.01% -5.17 [-6.60, -3.74] Heterogeneity: Tau ² = 2.17; Chi ² = 17.50, df = 7 (P = 0.01); P = 60% Test for overall effect Z = 7.09 (P < 0.00001) 1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.6465 15 3.09 0.6346 10 39.2% -1.41 [-2.31, -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.86, -0.69] Subtotal (95% CI) 31 26 (100.0% -1.25 [-2.13, -0.37] Heterogeneity: Tau ² = 0.30; Ch ² = 4.02, df = 2 (P = 0.13); P = 50% Test for overall effect Z = 2.77 (P = 0.006) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.00 [-5.34, -1.86] Subtotal (95% CI) 26 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Ch ² = 2.33, df = 2 (P = 0.31); P = 14% Test for overall effect Z = 7.19 (P < 0.00001) Test for overall effect Z = 7.19 (P < 0.00001)	Baoyong Sun 2015	0.328	0.221	8 5	1.459	0.303	8	17.2%	-4.03 [-5.92, -2.15]	
Rang Han 20141.04370.085101.00190.11312018.%-5.18 [+0.76, -3.58]Kang Han 20151.0800.229101.5850.1981020.6%-2.59 [-3.84, -1.34]Lei Chen 20130.20950.025450.46980.030256.3%-8.43 [-13.33, -3.52]Wei Wang 20150.4290.07260.970.099611.6%-5.77 [-8.80, -2.73]Xin Zhou 20130.35950.060760.91050.11276611.2%-6.03 [+0.18, -2.88]Zhengwen Sun 20140.1440.01150.3260.02957.4%-7.50 [-11.89, -3.10]Subtotal (95% CI)550.665103.670.591039.6%-0.54 [-1.44, 0.36]Test for overall effect: Z = 7.09 (P < 0.00001)	Jin Wang 2014	0.5112	0.0599	10	1.1882	0.0921	20	0.9%	-7.87 [-12.47, -3.27]	
Kang Han 2015 1.006 0.229 10 1.585 0.198 10 2.05% -2.59 [-3.84, -1.34] Lei Chen 2013 0.2095 0.0254 5 0.469 0.0302 5 6.3% -8.43 [-1.33, -3.52] Wei Wang 2015 0.429 0.072 6 0.9105 0.1027 6 11.2% -6.03 [-9.18, -2.88] Thengwen Sun 2014 0.144 0.011 5 0.326 0.029 5 7.4% -5.77 [-8.80, -2.73] Subtotal (95% CI) 55 65 100.0% -5.17 [-6.60, -3.74] Heterogeneity: Tau ² = 2.17; Chi ² = 17.50, df = 7 (P = 0.01); P = 60% Test for overall effect: $Z = 7.09$ (P < 0.00001) 1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [-1.44, 0.36] Tormohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 39.2% -1.41 [-2.31, -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.86, -0.69] Subtotal (95% CI) 31 26 100.0% -1.25 [-2.13, -0.57] Heterogeneity: Tau ² = 0.30; Chi ² = 4.02, df = 2 (P = 0.13); P = 50% Test for overall effect: $Z = 2.77$ (P = 0.006) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.80 [-5.34, -1.86] Heterogeneity: Tau ² = 0.18; Chi ² = 2.3, df = 2 (P = 0.31); P = 14% Test for overall effect: $Z = 7.71 (P < 0.00001)$ Test for overall effect: $Z = 7.19 (P < 0.00001)$	Kang Han 2014	1.0437	0.085	10	1.6019	0.1131	20	18.8%	-5.18 [-6.78, -3.58]	
Let Chen 2013 0.2055 0.0224 5 0.4895 0.0302 5 0.3% -8.43 [13.3, -3.3, -3.2] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 11.6% -5.77 [-8.0, -2.73] Xin Zhou 2013 0.3595 0.0607 6 0.9105 0.1027 6 11.2% -6.03 [-9.18, -2.88] Zhengwen Sun 2014 0.144 0.011 5 0.326 0.029 5 7.4% -7.50 [-11.89, -3.10] Subtotal (95% C) 55 65 100.0% -5.17 [-6.60, -3.74] Heterogeneity: Tau ² = 2.17; Chi ² = 17.50, df = 7 ($P = 0.01$); $P = 60\%$ Test for overall effect: $Z = 7.09$ ($P < 0.00001$) 1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 39.2% -1.41 [-2.31, -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.86, -0.69] Subtotal (95% C) 31 26 100.0% -1.25 [-2.13, -0.37] Heterogeneity: Tau ² = 0.30; Chi ² = 4.02, df = 2 ($P = 0.13$); $P = 50\%$ Test for overall effect: $Z = 2.77$ ($P = 0.006$) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% C) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 ($P = 0.31$); $P = 14\%$ Test for overall effect: $Z = 7.19$ ($P < 0.00001$) Test for overall effect: $Z = 7.19$ ($P < 0.00001$)	Kang Han 2015	1.006	0.229	10	1.585	0.198	10	20.6%	-2.59 [-3.84, -1.34]	
We wang 2015 0.429 0.072 b 0.97 0.099 b 11.6% -5.77 [8.80, -2.73] Xin Zhou 2013 0.3595 0.0607 6 0.9105 0.1027 6 11.2% -6.03 [9.18, 2.88] Zhengwen Sun 2014 0.144 0.011 5 0.326 0.029 5 7.4% -7.50 [-11.89, -3.10] Subtotal (95% CI) 55 65 100.0% -5.17 [-6.60, -3.74] Heterogeneity: Tau ² = 2.17; Ch ² = 17.50, df = 7 (P = 0.01); I ² = 60% Test for overall effect Z = 7.09 (P < 0.00001) 1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 39.2% -1.41 [-2.31, -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.86, -0.69] Subtotal (95% CI) 31 26 100.0% -1.25 [-2.13, -0.37] Heterogeneity: Tau ² = 0.30; Ch ² = 4.02, df = 2 (P = 0.13); I ² = 50% Test for overall effect Z = 2.77 (P = 0.008) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% CI) 26 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Ch ² = 2.33, df = 2 (P = 0.31); I ² = 14% Test for overall effect Z = 7.19 (P < 0.00001) Test for suboroup differences: Chi ² = 34.57. df = 4 (P < 0.00001), I ² = 88.4%	Lei Chen 2013	0.2095	0.0254	5	0.4698	0.0302	5	6.3%	-8.43 [-13.33, -3.52]	
$\begin{aligned} & \text{Xin } 2 \text{ for } 2013 & 0.3955 \ 0.0407 & 6 \ 0.9105 \ 0.1027 & 6 \ 11.2\% & -6.03 \ P.318, -2.88 \ 2 \text{ hengwen } Sun 2014 & 0.144 & 0.011 & 5 & 0.326 & 0.029 & 5 & 7.4\% & -7.50 \ [-11.89, -3.10] \\ & \text{Subtotal } (95\% \text{ C}) & 55 & 65 & 100.0\% & -5.17 \ [-6.60, -3.74] \\ & \text{Heterogeneity: } \text{Tau}^2 = 2.17; \ \text{Ch}^2 = 17.50, \ \text{df} = 7 \ (P = 0.01); \ P = 60\% \\ & \text{Test for overall effect } Z = 7.09 \ (P < 0.00001) \end{aligned}$	Wei Wang 2015	0.429	0.072	6	0.97	0.099	6	11.6%	-5.77 [-8.80, -2.73]	
Zhengwen Sun 2014 0.144 0.011 5 0.326 0.029 5 7.4% -7.50 [$+1.38$, -3.10] Subtotal (95% CI) 55 65 100.0% -5.17 [-6.60, -3.74] Heterogeneity: Tau ² = 2.17; Chi ² = 17.50, df = 7 (P = 0.01); I ² = 60% Test for overall effect: $Z = 7.09$ (P < 0.00001) 1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 39.2% -1.41 [-2.31, -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.86, -0.69] Subtotal (95% CI) 31 26 100.0% -1.25 [-2.13, -0.37] Heterogeneity: Tau ² = 0.30; Chi ² = 4.02, df = 2 (P = 0.13); I ² = 50% Test for overall effect: $Z = 2.77$ (P = 0.006) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% CI) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); I ² = 14% Test for overall effect: $Z = 7.19$ (P < 0.00001) Test for overall effect: $Z = 7.19$ (P < 0.00001) Test for overall effect: $Z = 7.19$ (P < 0.00001). I ² = 88.4%	Xin Zhou 2013	0.3595	0.0607	6	0.9105	0.1027	6	11.2%	-6.03 [-9.18, -2.88]	
Subtrait (95% CI) = 33 = 35 = 35 = 35 = 35 = 35 = 35 = 100.0% = -3.17 [$-0.00, -3.74$] Heterogeneity: Tau ² = 2.17 ; Chi ² = 17.50 , df = 7 (P = 0.01); P = 60% Test for overall effect: Z = 7.09 (P < 0.00001) 1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% - 0.54 [$1.44, 0.36$] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 39.2% - 1.41 [$2.31, -0.50$] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% - 2.27 [$3.86, -0.69$] Subtotal (95% CI) 31 26 100.0% - 1.25 [$2.13, -0.37$] Heterogeneity: Tau ² = 0.30 ; Chi ² = 4.02 , df = 2 (P = 0.13); P = 50% Test for overall effect: Z = 2.77 (P = 0.006) 1.2.5 miRNAs were injected into tumor directy Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% - 5.74 [$8.06, -3.43$] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% - 4.97 [$-7.03, -2.92$] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% - 3.60 [$5.34, -1.86$] Subtotal (95% CI) 26 26 26 100.0% - 4.59 [$-5.84, -3.34$] Heterogeneity: Tau ² = 0.18 ; Chi ² = 2.33 , df = 2 (P = 0.31); P = 14% Test for overall effect: Z = 7.19 (P < 0.00001) Test for overall effect: Z = 7.19 (P < 0.00001) Test for subdroup differences: Chi ² = 34.57 . df = 4 (P < 0.00001). P = 88.4%	Znengwen Sun 2014	0.144	0.011	C	0.326	0.029	5	100.0%	-7.50 [-11.89, -3.10]	
Heterogeneity: $Tau^2 = 2.17$, $Chi^2 = 1.30$, $di = 7 (P = 0.01)$, $P = 80\%$ Test for overall effect: $Z = 7.09 (P < 0.00001)$ 1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 39.2% -1.41 [-2.31, -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.86, -0.69] Subtotal (95% CI) 31 26 100.0% -1.25 [-2.13, -0.37] Heterogeneity: Tau ² = 0.30; Chi ² = 4.02, df = 2 (P = 0.13); P = 50% Test for overall effect: $Z = 2.77 (P = 0.006)$ 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% CI) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); P = 14% Test for overall effect: $Z = 7.19 (P < 0.00001)$ Test for subgroup differences: Chi ² = 34.57. df = 4 (P < 0.00001). P = 88.4%	Sublotar (95% CI)	0.00	2 6 0 46 - 1	30	0.041.17	0.000	00	100.0%	-5.17 [-0.00, -5.74]	•
1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 39.2% -1.41 [-2.31, -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.66, -0.69] Subtotal (95% CI) 31 26 100.0% -1.25 [-2.13, -0.37] - Heterogeneity: Tau ² = 0.30; Chi ² = 4.02, df = 2 (P = 0.13); P = 50% Test for overall effect: Z = 2.77 (P = 0.006) -	Teet for everall effect: 7 = 2	; Chi= 17	.50, 01=	/ (P =	0.01); 1-=	= 60%				
1.2.4 miRNA were delivered by systematic administration Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 39.2% -1.41 [2.31 , -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.86 , $-0.69]$] Subtoal (95% CI) 31 26 100.0% -1.25 [-2.13 , -0.37] -1.25 [-2.13 , -0.37] Heterogeneity: Tau ² = 0.30; Chi ² = 4.02, df = 2 (P = 0.13); I ² = 50% -5.74 [-8.06 , -3.43] -5.74 [-8.06 , -3.43] Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06 , -3.43] Jiahui Zhou 2015 (b) 0.411 0.162 9 31.9% -4.97 [-7.03 , 2.92] -4.97 [-7.03 , 2.92] -4.97 [-7.03 , 2.92] -10 -5 0 5 10 Yu He 2014 0.375 0.998 0.219 8 42.2% -3.60 [-5.84 , -3.34]	Test for overall effect. $Z = 7$.09 (F < 0	.00001)							
Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 39.6% -0.54 [-1.44, 0.36] Tormohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 39.2% -1.41 [-2.31, -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.86, -0.69] Subtotal (95% CI) 31 26 100.0% -1.25 [-2.13, -0.37] Heterogeneity: Tau ² = 0.30; Chi ² = 4.02, df = 2 (P = 0.13); I ² = 50% Test for overall effect: $Z = 2.77$ (P = 0.006) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% CI) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); I ² = 14% Test for overall effect: $Z = 7.19$ (P < 0.00001) Test for subdroup differences: Chi ² = 34.57. df = 4 (P < 0.00001), I ² = 88.4%	1.2.4 miRNA were deliver	ed by syst	ematic a	dminis	stration					
Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 39.2% -1.41 [2.31, -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.86, -0.69] Subtoal (95% CI) 31 26 100.0% -1.25 [-2.13, -0.37] Heterogeneity: Tau ² = 0.30; Chi ² = 4.02, df = 2 (P = 0.13); I ² = 50% Test for overall effect: Z = 2.77 (P = 0.006) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% CI) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); I ² = 14% Test for overall effect: Z = 7.19 (P < 0.00001) Test for subdroup differences: Chi ² = 34.57. df = 4 (P < 0.00001). I ² = 88.4%	Mitsuhiko Osaki 2011	3.32	0.65	10	3.67	0.59	10	39.6%	-0.54 [-1.44, 0.36]	1
Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 21.2% -2.27 [-3.86, -0.69] Subtotal (95% CI) 31 26 100.0% -1.25 [-2.13, -0.37] Heterogeneity: Tau ² = 0.30; Chi ² = 4.02, df = 2 (P = 0.13); I ² = 50% Test for overall effect: $Z = 2.77$ (P = 0.006) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% CI) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); I ² = 14% Test for overall effect: $Z = 7.19$ (P < 0.00001) Test for subaroup differences: Chi ² = 34.57. df = 4 (P < 0.00001). I ² = 88.4%	Tomohiro Fujiwara 2014	2.155	0.6465	15	3.09	0.6346	10	39.2%	-1.41 [-2.31, -0.50]	-
Subtotal (95% Cl) 31 26 100.0% -1.25 [-2.13, -0.37] Heterogeneity: Tau ² = 0.30; Chi ² = 4.02, df = 2 (P = 0.13); I ² = 50% Test for overall effect: $Z = 2.77$ (P = 0.006) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% Cl) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); I ² = 14% Test for overall effect: $Z = 7.19$ (P < 0.00001) Test for subaroup differences: Chi ² = 34.57. df = 4 (P < 0.00001). I ² = 88.4%	Yong Zhao 2015	1.738	0.398	6	2.827	0.482	6	21.2%	-2.27 [-3.86, -0.69]	
Heterogeneity: Tau ² = 0.30; Chi ² = 4.02, df = 2 (P = 0.13); P = 50% Test for overall effect: $Z = 2.77$ (P = 0.006) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% Cl) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); P = 14% Test for overall effect: $Z = 7.19$ (P < 0.00001) Test for subaroup differences: Chi ² = 34.57. df = 4 (P < 0.00001). P = 88.4%	Subtotal (95% CI)			31			26	100.0%	-1.25 [-2.13, -0.37]	•
Test for overall effect: $Z = 2.77$ (P = 0.006) 1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% Cl) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); I ² = 14% Test for overall effect: $Z = 7.19$ (P < 0.00001) Test for subdroup differences: Chi ² = 34.57. df = 4 (P < 0.00001). I ² = 88.4%	Heterogeneity: Tau ² = 0.30	$Chi^2 = 4.$	02, df = 2	(P = 0	.13); l² =	50%				
1.2.5 miRNAs were injected into tumor directly Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 $[-8.06, -3.43]$ Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 $[-7.03, -2.92]$ Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 $[-5.34, -1.86]$ Subtotal (95% Cl) 26 26 100.0% -4.59 $[-5.84, -3.34]$ Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); I ² = 14% Test for overall effect: Z = 7.19 (P < 0.00001)	Test for overall effect: $Z = 2$	2.77 (P = 0	.006)							
Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 25.9% -5.74 [-8.06, -3.43] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% CI) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); I ² = 14% Test for overall effect: Z = 7.19 (P < 0.00001) Test for subaroup differences: Chi ² = 34.57. df = 4 (P < 0.00001). I ² = 88.4%	1.2.5 miRNAs were inject	ed into tur	nor direc	tly						
Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 31.9% -4.97 [-7.03, -2.92] Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% Cl) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); I ² = 14% Test for overall effect: Z = 7.19 (P < 0.00001) Test for subaroup differences: Chi ² = 34.57. df = 4 (P < 0.00001). I ² = 88.4%	Jiahui Zhou 2015 (a)	0.467	0.101	9	1.457	0.209	9	25.9%	-5.74 [-8.06, -3.43]	
Yu He 2014 0.375 0.075 8 0.998 0.219 8 42.2% -3.60 [-5.34, -1.86] Subtotal (95% Cl) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); l ² = 14% Test for overall effect: Z = 7.19 (P < 0.00001) Test for subaroup differences: Chi ² = 34.57. df = 4 (P < 0.00001). l ² = 88.4%	Jiahui Zhou 2015 (b)	0.41	0.162	9	1.366	0.202	9	31.9%	-4.97 [-7.03, -2.92]	
Subtotal (95% CI) 26 26 100.0% -4.59 [-5.84, -3.34] Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); I ² = 14% Test for overall effect: Z = 7.19 (P < 0.00001)	Yu He 2014	0.375	0.075	8	0.998	0.219	8	42.2%	-3.60 [-5.34, -1.86]	
Heterogeneity: Tau ² = 0.18; Chi ² = 2.33, df = 2 (P = 0.31); l ² = 14% Test for overall effect: Z = 7.19 (P < 0.00001)	Subtotal (95% CI)			26			26	100.0%	-4.59 [-5.84, -3.34]	●
Test for overall effect: Z = 7.19 (P < 0.00001) -10 -5 0 5 10 Favours miRNA Favours control	Heterogeneity: Tau² = 0.18	; Chi² = 2.	33, df = 2	(P = 0	.31); I² =	14%				
Test for subaroup differences: Chi ² = 34.57. df = 4 (P < 0.00001). I ² = 88.4%	Test for overall effect: Z = 7	7.19 (P < 0	.00001)							
-10 -5 0 5 10 Test for subaroup differences: Chi ² = 34.57. df = 4 (P < 0.00001). I ² = 88.4% Favours miRNA Favours control										
Test for subaroup differences: Chi² = 34.57. df = 4 (P < 0.00001). I² = 88.4%										-10 -5 0 5 10
	Test for subaroup differen	ces: Chi² =	= 34.57. di	f = 4 (F	o < 0.000	01). I ² = 8	38.4%			Favours mirking Favours control

3B

Stu. Wear Difference	Stu. Mean	stu. mean Dinerence		Control				ILUNA		
IV, Random, 95% Cl	IV, Rando	IV, Random, 95% Cl	Weight	Total	SD	Mean	Total	SD	Mean	Study or Subgroup
				s	d vectors	h plasmi	ells with	nto OS ce	nsfected ir	1.3.1 miRNA were tra
- -		1.72 [0.83, 2.61]	87.6%	20	0.1999	1.1885	10	0.103	1.497	K Tian 2015
		3.13 [0.57, 5.69]	12.4%	4	15.244	100	4	15.244	154.878	Xuming Wang 2014
		1.89 [0.98, 2.81]	100.0 %	24			14			Subtotal (95% CI)
					²= 4%	= 0.31); P	= 1 (P	= 1.04, df	0.04; Chi ² :	Heterogeneity: Tau ² =
)	< 0.0001	Z = 4.07 (P	Test for overall effect: J
-4 -2 0 2 4	-4 -2									
voure miRNA Eavoure cont	Eavoure miRNA									
/0	Favo						ble	ot applica	erences: No	Test for subaroup diffe

Figure 3: Meta-analysis of included studies evaluating the inhibitory effects on tumor weight after the aberrantly expressed miRNAs were corrected, when studies, reported miRNAs as tumor suppressors **A**. or oncogenes **B**. and used tumor weight as the major outcome measure, were stratified respectively by the miRNA delivery method. SD, standard deviation; CI, confidence interval.

together for a meta-analysis. This resulted 4 different miRNAs were anylyzed, including 3 tumor suppressor miRNAs(miR-195, miR-143 and miR-34a) and 1 oncogene(miR-214). As shown in Figure 4, part 1, there were 15 mice in both the interventionand the control arms. Tumor weight significantly decreased when the expression of down-regulated miR-195 was recovered (pooled MD = [-2.21]; 95% confidence interval [CI]: [-3.19]- [-1.24]; p = 0.35;) [62, 64]; there were 28 mice in the intervention arm and 28 in the control arm. Tumor weight significantly decreased when the expression of down-regulated miR-143 was recovered (pooled MD = [-3.64]; 95% confidence interval [CI]: [-7.35]- [0.06]; p < 0.00001; Figure 4, part 2) [17, 46]; there were 12 mice in the intervention arm and 12 in the control arm. Tumor weight significantly decreased when the expression of down-regulated miR-34a was rescued (pooled MD = [-2.23]; 95% confidence interval [CI]: [-3.34]- [-1.12]; *p* = 0.93; Figure 4, part 3) [33, 47]; there were 10 mice in the intervention arm and 10 in the control arm. Tumor weight significantly decreased when the expression of up-regulated miR-214 was downregulated (pooled MD = [4.88]; 95% confidence interval [CI]: [1.05]- [8.70]; *p* = 0.08; Figure 4, part 4) [34, 50].

As we could see in Figure 4A, the effect on inhibiting tumor weight was most significant when the aberrantly expressed oncogene miR-214 was corrected, and followed by rescuing miR-143, then miR-195 or miR-34a. The efficacy due to up-regulating miR-195 or miR-34a was comparable.

Inoculation sites of osteosarcoma cells

There were 21 studies which reported that tumor weight as the major outcome measure and miRNAs as tumor suppressors.

Fifteen of them applied OS xenograft models produced by subcutaneous inoculation of OS cells [25, 32, 35, 37, 44-47, 53-55, 58, 59, 61, 65]. These 15 studies were combined together for the meta-analysis, and there were 101 mice in the intervention arm and 105 in the control arm. Given that the heterogeneity was high among the studies, a random-effects model was selected, and the tumor weight considerably decreased when the decreased tumor suppressor miRNAs were corrected (pooled MD = [-4.89]; 95% confidence interval [CI]: [-5.86]- [-3.93]; p = 0.006; Figure 5A, upper part).

	n	irna		(Control			Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% Cl
1.4.1 miR-195									
Jie Gao 2012	1.2457	0.2465	5	1.7431	0.2984	5	39.7%	-1.64 [-3.19, -0.10]	
Kang Han 2015	1.006	0.229	10	1.585	0.198	10	60.3%	-2.59 [-3.84, -1.34]	
Subtotal (95% CI)			15			15	100.0 %	-2.21 [-3.19, -1.24]	◆
Heterogeneity: Tau ² = 0.0	00; Chi² =	0.87, df=	1 (P =	0.35); I ² :	= 0%				
Test for overall effect: Z =	= 4.46 (P <	0.00001)						
1.4.2 miR-143									
Jiahui Zhou 2015 (a)	0.467	0.101	9	1.457	0.209	9	31.8%	-5.74 [-8.06, -3.43]	_ _ _
Jiahui Zhou 2015 (b)	0.41	0.162	ğ	1.366	0.202	ğ	32.7%	-4.97 [-7.03, -2.92]	
Mitsuhiko Osaki 2011	3.32	0.65	10	3.67	0.59	10	35.6%	-0.54 [-1.44_0.36]	
Subtotal (95% CI)	0.02	0.00	28	0.01	0.00	28	100.0%	-3.64 [-7.35, 0.06]	
Heterogeneity: $Tau^2 = 9.8$	84: Chi ² = 1	27.86 df	= 2 (P	< 0.0000	1): P = 93	%			
Test for overall effect: Z =	1.93 (P =	0.05)			.,,				
1.4.3 miP.34a									
Kang Van 2012	1 1 2 7 2	0 1 7 4 2	8	1 7725	0 2201	6	61.0%	2 10 1 2 72 -0 621	
Vong Zhao 2015	1.1373	0.1742	0	2 0 2 7	0.3301	0	10.0%	-2.10[-3.73,-0.03]	
Subtotal (95% CI)	1.730	0.550	12	2.027	0.402	12	100.0%	-2.27 [-3.80, -0.03]	
Heterogeneity: Tau ² – 0.0	10: Chiž –	0 01 df-	1 (P -	0.03)-12-	- 0%	12	100.074	-2.20[-0.04, -1.12]	•
Test for overall effect: Z =	: 3.93 (P <	0.0001)		0.33),1 -	- 0 /0				
1.4.4 miR-214									
Yuming Wang 2014	154 879	15 244	4	100	15 244	4	55.6%	3131057560	_
Zhengyu Yu 2014	1 1 2 1	0 1 2 0 3	،	0.4522	0.0299	4 6	44.4%	7 06 (3.43, 10, 69)	
Subtotal (95% Cl)	1.121	0.1203	10	0.4522	0.0203	10	100.0%	4.88 [1.05, 8.70]	
Hotorogeneity: Tour = 6.1	M: Chiž –	2 01 df-	1 (P -	0.00\-12-	- 67%	10	100.074	4.00 [1.00, 0.1 0]	
Test for overall effect: 7 =	2 50 (P =	0.01,01-		0.00),1 -	- 07 /0				
100,101 0101011 01100L Z =	2.00 () -	0.017							
									-10 -5 0 5 10
Test for subaroup differe	nces: Chi	² = 13.51	. df = 3	(P = 0.00)	(4), $ ^2 = 77$	7.8%			Favours miRNA Favours co

Figure 4: Meta-analysis of included studies evaluating the inhibitory effects on tumor weight after the aberrantly expressed miRNAs were corrected, when all included studies used tumor weight as the major outcome measure were stratified by the names of miRNAs. SD, standard deviation; CI, confidence interval.

OS xenograft models induced by intratibial injection of OS cells were used in other 6 studies [17, 33, 42, 43, 62, 64]. Fifty-six mice in the intervention arm and 61 mice in the control arm were included. A random-effects model was used also due to the high heterogeneity among the included studies, and the tumor weight significantly decreased after the decreased tumor suppressor miRNAs were corrected (pooled MD = [-2.17]; 95% confidence interval [CI]: [-3.34]- [-1.00]; p < 0.0001; Figure 5A, lower part).

As we could see in Figure 5A, the overall effects on reducing the tumor weight were more significant when

	r	niRNA		0	Control			Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% Cl
1.5.1 Subcutaneous inocu	lation								
Baoyong Sun 2015	0.328	0.221	8	1.459	0.303	8	8.7%	-4.03 [-5.92, -2.15]	
Chi Cheng 2014	0.2737	0.0253	6	0.5516	0.0295	6	3.2%	-9.33 [-14.03, -4.64]	<u> </u>
Guoxing Xu 2014	0.3123	0.0571	5	0.6884	0.0907	5	6.2%	-4.48 [-7.29, -1.67]	
Jiahui Zhou 2015 (a)	0.467	0.101	9	1.457	0.209	9	7.5%	-5.74 [-8.06, -3.43]	
Jiahui Zhou 2015 (b)	0.41	0.162	9	1.366	0.202	9	8.2%	-4.97 [-7.03, -2.92]	
Jin Wang 2014	0.5112	0.0599	5	1.1882	0.0921	5	3.3%	-7.87 [-12.47, -3.27]	
Lei Chen 2013	0.2095	0.0254	5	0.4698	0.0302	5	3.0%	-8.43 [-13.33, -3.52]	
Lei Fan 2013	1	0.2	3	1.8	0.4	7	9.1%	-2.00 [-3.77, -0.24]	
Liang Ge 2016	0.36	0.083	10	0.911	0.122	10	8.5%	-5.06 [-7.01, -3.10]	
Wei Liu 2015	0.279	0.051	5	0.641	0.079	5	5.7%	-4.92 [-7.95, -1.88]	
Wei Wang 2015	0.429	0.072	6	0.97	0.099	6	5.7%	-5.77 [-8.80, -2.73]	_ —
Xiaoji Luo 2014	0.369	0.021	5	0.674	0.042	5	3.1%	-8.30 [-13.13, -3.46]	
Xin Zhou 2013	0.3595	0.0607	6	0.9105	0.1027	6	5.5%	-6.03 [-9.18, -2.88]	
Yong Zhao 2015	1.738	0.398	6	2.827	0.482	6	9.7%	-2.27 [-3.86, -0.69]	
Yu He 2014	0.375	0.075	8	0.998	0.219	8	9.2%	-3.60 [-5.34, -1.86]	
Zhengwen Sun 2014	0.144	0.011	5	0.326	0.029	5	3.5%	-7.50 [-11.89, -3.10]	
Subtotal (95% CI)			101			105	100.0 %	-4.89 [-5.86, -3.93]	♦
Heterogeneity: Tau ² = 1.85	; Chi ² = 33	2.31, df =	15 (P =	: 0.006);	I² = 54%				
Test for overall effect: $Z = 9$	9.95 (P < 0	.00001)							
1.5.2 Intratibial inoculation	n								
Jie Gao 2012	1.2457	0.2465	5	1.7431	0.2984	5	15.4%	-1.64 [-3.19, -0.10]	
Kang Han 2014	1.0437	0.085	10	1.6019	0.1131	20	15.1%	-5.18 [-6.78, -3.58]	
Kang Han 2015	1.006	0.229	10	1.585	0.198	10	16.9%	-2.59 [-3.84, -1.34]	-
Kang Yan 2012	1.1373	0.1742	6	1.7725	0.3381	6	15.3%	-2.18 [-3.73, -0.63]	
Mitsuhiko Osaki 2011	3.32	0.65	10	3.67	0.59	10	18.7%	-0.54 [-1.44, 0.36]	+
Tomohiro Fujiwara 2014	2.155	0.6465	15	3.09	0.6346	10	18.6%	-1.41 [-2.31, -0.50]	
Subtotal (95% CI)			56			61	100.0 %	-2.17 [-3.34, -1.00]	•
Heterogeneity: Tau ² = 1.70	; Chi² = 23	7.27, df =	5 (P <	0.0001);	l² = 82%				
Test for overall effect: Z = 3	8.63 (P = 0	.0003)							
									-20 -10 0 10

5B

<u>Study or Subgroup</u> 1.6.1 Subcutaneous ino 1/ Tian 2015	Mean oculation	SD	Total	Mean	SD	Total			
1.6.1 Subcutaneous ino	oculation				50	TULA	weight	IV, Random, 95% Cl	IV, Random, 95% Cl
V Tion 2015									
N Hall 2015	1.497	0.103	10	1.1885	0.1999	20	31.9%	1.72 [0.83, 2.61]	=
Xiuhui Wang 2014	0.753	0.064	6	0.349	0.03	6	20.7%	7.46 [3.65, 11.28]	_- -
Xuming Wang 2014	154.878	15.244	4	100	15.244	4	26.0%	3.13 [0.57, 5.69]	- - -
Zhengyu Xu 2014	1.121	0.1203	6	0.4522	0.0289	6	21.4%	7.06 [3.43, 10.68]	
Subtotal (95% CI)			26			36	100.0%	4.42 [1.57, 7.26]	
Heterogeneity: Tau ² = 6.	.38; Chi² =	= 15.66, d	df = 3 (F	P = 0.001); l ² = 81 9	%			
Test for overall effect: Z =	= 3.05 (P	= 0.002)							
									-20 -10 0 10
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Figure 5: Meta-analysis of included studies evaluating the inhibitory effects on tumor weight after the aberrantly expressed miRNAs were corrected, when studies reported miRNAs as tumor suppressors **A**. or oncogenes **B**. used tumor weight as the major outcome measure were stratified by injection sites of osteosarcoma cells. SD, standard deviation; CI, confidence interval.

the OS xenograft models were produced by subcutaneous injection than by intratibial injection.

There were 4 studies reported tumor weight as the major outcome measure and miRNAs as oncogenes, which used OS xenograft models produced by subcutaneous inoculation of OS cells [34, 49-51]. There were 26 mice in the intervention arm and 36 in the control arm. Given that the heterogeneity was high among the studies, a random-effects model was selected, and the tumor weight considerably decreased when the expressions of the oncogene miRNAs were corrected (pooled MD = [4.42]; 95% confidence interval [CI]: [1.57]- [7.26]; p = 0.001; Figure 5B).

Inoculated osteosarcoma cell lines

The delaminating analysis based on the 5 different OS cell lines that were used to produce OS xenograft models in the included studies, were performed. Given that the heterogeneity was high across the studies, a random-effects model was chosen. Among studies reported tumor weight as the major outcome measure and miRNAs as tumor suppressors, 5 studies [53, 54, 58, 59, 65], used MG-63 for OS xenograft model, were combined together for the meta-analysis, and there were 34 mice in the intervention and 38 mice in the control arm. Tumor weight significantly decreased when the aberrantly expressed miRNAs were corrected (pooled MD = [-3.97]; 95% confidence interval [CI]: [-5.39]- [-2.55]; *p* = 0.06; Figure 6A, part 1);data from the 3 studies [44-46] used U2 OS for OS xenograft model, were combined together for the meta-analysis, and there were 20 mice in both the intervention and control arms. Tumor weight significantly decreased when the aberrantly expressed miRNAs were corrected (pooled MD = [-6.90]; 95% confidence interval [CI]: [-9.88]- [-3.91]; p = 0.15; Figure 6A,part 2);data from the 3 studies [32, 46, 55] used Saos-2 for OS xenograft model, were combined together for the metaanalysis, and there were 20 mice in both the intervention and control arms. Tumor weight significantly decreased when the aberrantly expressed miRNAs were corrected (pooled MD = [-5.59]; 95% confidence interval [CI]: [-7.18]-[-4.00]; p = 0.87; Figure 6A, part 3); data from the 5 studies [17, 37, 43, 47, 61] used 143B for OS xenograft model, were combined together for the meta-analysis, and there were 42 mice in the intervention arm and 37 in the control arm. Tumor weight noticeably decreased when the aberrantly expressed miRNAs were corrected (pooled MD = [-2.53]; 95% confidence interval [CI]: [-4.11]- [-0.96]; p=0.0004; Figure 6A, part 4); data from the 3 studies [33, 42, 62] used SOSP-9607 for OS xenograft model, were combined together for the meta-analysis, and there were 26 mice in the intervention arm and 36 in the control arm. Tumor weight significantly decreased when the aberrantly expressed miRNAs were corrected (pooled MD = [-3.28]; 95% confidence interval [CI]: [-5.02]-[-1.55]; p = 0.01; Figure 6A, part 5).

As we could see in Figure 6A, the overall effects on reducing the tumor weight were most significant when the OS xenograft models were produced by injection of U2 OS cells, then by injection of Saos-2, and followed by injection of MG-63, then143B or SOSP-9607.

There were 3 studies reported tumor weight as the major outcome measure and miRNAs as oncogenes, used Saos-2 for OS xenograft model, were combined together for the meta-analysis, and there were 16 mice in both the intervention and control arms [34, 49, 50]. Tumor weight significantly decreased when the aberrantly expressed miRNAs were corrected (pooled MD = [5.61]; 95% confidence interval [CI]: [2.64]- [8.58]; p = 0.09; Figure 6B).

When all the included studies used tumor volume as the major outcome measure were stratified by whether the abnormally expressed miRNAs function as oncogenes or tumor suppressors in the pathogenesis of osteosarcoma

Thirty-one studies that included measurements of tumor volume were divided into 2 subgroups according to the function of abnormally expressed miRNAs.

One subgroup included 28 studies, which reported that miRNAs function as the tumor suppressors, and thus the data were combined for a meta-analysis [25, 33, 35-42, 44, 45, 47, 48, 52-65] A total of 195 mice in the intervention arm and 242 in the control arm were included. The tumor volume was considerably suppressed after the decreased miRNAs were restored in a random-effects model. And the pooled MD = [-4.65]; 95% confidence interval [CI]: [-5.43]- [-3.88]; p < 0.00001; Figure 7, upper part).

The 3 studies reported that miRNAs function as oncogenes in OS as described above, and thus the data were combined for a meta-analysis [34, 50, 51]. A total of 20 mice in the intervention arm and 30 in the control arm were included. The tumor volume was considerably suppressed after the aberrantly expressed miRNAs were restored in a random-effects model. And the pooled MD = [3.88]; 95% confidence interval [CI]: [0.48]- [7.27]; p = 0.005 (Figure 7, lower part).

When above included studies that reported miRNAs as tumor suppressors or oncogenes were further stratified respectively by the following factors:

The miRNA delivery method

There were 28 studies which reported that tumor volume as the major outcome measure and miRNAs as tumor suppressors. MiRNAs were directly transfected into the OS cells in 10 studies, and thus the data were combined for a meta-analysis [40, 41, 44, 48, 54-57, 59, 65] There were 76 mice in the intervention arm and 90 mice in the control arm. The tumor volume showed a statistically

Study or Subgroup Mean SD Total Mean SD Total Weight N. Random, 95% CI M. Random, 95% CI 1.7.1 M6.63 Baryong Sun 2015 0.328 0.221 8 1.459 0.303 8 2.2.8% -4.03 [5 92, -2.15] Image 2016 0.36 0.083 0.021 10 0.21 10 2.2.10 -5.0.67 (1), -3.10] Image 2016 0.368 0.098 0.021 5 0.67 4 0.042 5 7.0% -6.30 [13.13, -3.46] Image 2016 0.368 0.001 -3.97 [-5.39, -2.55] Image 2016 0.36 4 0.042 5 7.0% -6.30 [13.13, -3.46] Image 2016 0.5516 0.0295 6 25.2% -3.31 [14.03, -4.64] Image 2014 0.2737 0.0253 6 0.5516 0.0295 6 25.2% -9.33 [14.03, -4.64] Image 2014 1.659 (-0 20 100.0% -5.74 [+8.06, -3.43] Image 2016 Image 2017		n	niRNA		(Control			Std. Mean Difference	Std. Mean Difference
17.1 MG-63 Baryong Su 2015 0.328 0.221 8 1.459 0.303 8 2.2.8% -4.03 [5.92, -2.15] Lei Fan 2013 1 0.2 3 1.8 0.4 7 24.0% -2.201 [5.77, 0.2.16] Vanipi Luo 2014 0.369 0.021 5 0.674 0.042 5 7.0% +3.01 [3.3, 3.46] Vanipi Luo 2014 0.369 0.021 5 0.674 0.042 5 7.0% +3.01 [5.3, 3.46] Vanipi Luo 2014 0.377 0.075 8 0.999 0.219 8 24.2% -3.301 [5.3, 4.64] Test for overall effect Z = 5.48 (P < 0.0001) 1.467 0.202 9 5.03 [-7.03, -2.92] - Jahui Zhou 2015 (b) 0.410 0.623 9 1.366 0.202 9 5.04 [-7.03, -2.92] - Jahui Zhou 2015 (b) 0.209 0.0254 5 0.498 -4.37 [-7.03, -2.92] - - - - - - - - - - - - - - - - - -	Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% Cl
Bacyonic Sun 2015 0.328 0.221 8 1.459 0.303 8 2.28% -4.03[+592,-215] Lei Fan 2013 1 0.2 3 1.8 0.4 7 24.0% -2.00[+3.77, -0.24] Lang Ge 2016 0.36 0.083 10 0.911 0.121 10 2.21% -5.06[+7.01, -3.10] Xiaoji Luo 2014 0.395 0.075 8 0.988 2.18 8.24% -3.60[+5.34, -1.86] Subtotal (95% C) 34 38 100.0% -3.397[-5.39, -2.55] • Attercogeneity, Tau ² = 1.38, Ch ² = 9.01, df= 4 (P = 0.06); P = 56% 7.0% +3.397[-5.39, -2.55] • Test for overall effect Z = 5.48 (P < 0.00001)	1.7.1 MG-63									
Lei Fan 2013 1 1 0.2 3 18 0.4 7 24 0% -200 [3.77, 0.24] Liang Ge 2016 0.36 0.083 10 0.911 0.122 10 22.1% -5.06 [-7.01, -3.10] Xiaoji Luo 2014 0.368 0.021 5 0.674 0.042 5 7.0% -6.30 [-7.31, -3.46] Yu He 2014 0.375 0.075 8 0.988 0.218 8 24.2% -3.60 [-5.34, -1.86] Subtotal (95% C) 34 38 100.0% -3.397 [-5.38, -2.55] Heterogeneity. Tau ² = 1.38; Ch ² = 0.10; J. P = 65% Test for overall effect Z = 5.48 (P = 0.06); P = 56% Test for overall effect Z = 4.53 (P = 0.06); P = 66% Subtotal (95% C) 20 20 20 9 50.0254 5 0.4668 0.0302 5 23.9% -6.43 [-1.33, -3.52] Subtotal (95% C) -20 20 20 100.0% -6.90 [-3.68, -3.43] Heterogeneity. Tau ² = 3.44; Ch ² = 0.15; J. P = 48% Test for overall effect Z = 4.53 (P = 0.0001) 1.7.3 Saos-2 Jahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 47.1% -5.74 [-8.06, -3.43] Wei Liu 2015 0 0.369 5 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [-9.16; J. F. 88] Subtotal (95% C) 20 20 20 100.0% -5.59 [-7.18, -4.00] Heterogeneity. Tau ² = 0.00; Ch ² = 0.28, df = 2 (P = 0.87); P = 0% Test for overall effect Z = 6.89 (P = 0.0001) 1.7.4 1438 Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [-1.247, -3.27] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 1.42 -25 [-3.64, 1.44, 0.36] Tomohior Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 27.2% -0.64 [-1.44, 0.36] Test for overall effect Z = 6.89 (P = 0.0001) 1.7.4 1438 Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [-1.247, -3.27] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 1.42 -27 2% -0.54 [-1.44, 0.36] Tomohior Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 27.2% -0.54 [-1.44, 0.36] Tomohior Fujiwara 2014 2.150 0.6465 11 3.67 0.99 10 27.2% -0.54 [-1.44, -3.60] 4.142 -3.55 [-4.11, -0.56] 4.142 -3.55 [-4.11, -3.56] 5.18 [-6.78, -3.58] 5.100.0% -3.28 [-5.02, -1.55] 4.141 [-2.31,	Baovong Sun 2015	0.328	0.221	8	1.459	0.303	8	22.8%	-4.03 [-5.92, -2.15]	
$\begin{aligned} & ang 0e 2016 & 0.36 & 0.083 & 10 & 0.911 & 0.122 & 10 & -5.06 7.01, -3.10 \\ & Aa 0 Lu 0 2014 & 0.369 & 0.021 & 5 & 0.674 & 0.042 & 5 & 7.06 & -6.30 -3.13, -3.46 \\ & Aa 0 Lu 0 2014 & 0.375 & 0.075 & 8 & 0.980 & 0.218 & 8 & 2.428 & -3.80 -5.34, -1.86 \\ & Subtotal (95% C) & -34 & -388 & 0.000 \\ & Aetrogonelity, Tau2 = 1.38; Ch2 = 9.01, df = 4 (P = 0.06); P = 56% \\ Test for overall effect Z = 5.48 (P < 0.00001) \\ & Jau 12hou 2015 (b) & 0.41 & 0.162 & 9 & 1.366 & 0.202 & 9 & 50.96 & -4.97 -7.03, -2.82 \\ Jahu 12hou 2015 (b) & 0.41 & 0.162 & 9 & 1.366 & 0.202 & 9 & 50.96 & -4.97 -7.03, -2.82 \\ Jahu 12hou 2015 (b) & 0.41 & 0.162 & 9 & 1.366 & 0.202 & 9 & 50.96 & -4.97 -7.03, -2.82 \\ Jahu 12hou 2015 (b) & 0.41 & 0.162 & 9 & 1.366 & 0.202 & 9 & 20 & 100.09 \\ & -8.43 -1.33, -3.52 \\ Jahui 12hou 2015 (a) & 0.467 & 0.101 & 9 & 1.457 & 0.209 & 9 & 47.1\% & -5.74 -8.06, -3.43 \\ & +etorogenelity, Tau2 = 3.44; Ch2 = 3.04; df = 2 (P = 0.87); P = 0\% \\ Test for overall effect Z = 4.53 (P < 0.00001) \\ & 1.73 Saos 2 \\ Jahui 22015 & 0.279 & 0.051 & 5 & 0.644 & 0.079 & 5 & 2.75\% & -4.92 -9.87, 1-88 -9.87 \\ & +etorogenelity, Tau2 = 0.00; Ch2 = 0.28, df = 2 (P = 0.87); P = 0\% \\ Test for overall effect Z = 6.89 (P < 0.00001) \\ & 1.74 1438 \\ Jin Wang 2014 & 0.5112 & 0.0599 & 5 & 1.1882 & 0.0921 & 5 & 8.4\% & -7.87 -2.47, -3.27 \\ & Mitsuhko Ceak 2011 & 3.32 & 0.65 & 10 & 3.67 & 0.59 & 10 & 27.2\% & -0.54 -1.44, 0.36 -9.87 \\ & Feotogenelity, Tau2 = 0.10; Ch2 = 0.26, df = 4 (P = 0.0004); P = 80\% \\ & Test for overall effect Z = 3.15 (P = 0.002) \\ & 1.75 SOSP-9007 \\ & Kang 4ha 2015 & 1.0437 & 0.085 & 10 & 1.6019 & 0.113 & 20 & 31.9\% & -5.18 -6.78, -3.58 -7.87 -1.44 -3.31, -0.39 -9.87 -1.44 -2.31, -0.39 -9.87 -1.44 -2.31, -0.39 -9.87 -7.46 -2.73 -7.46 -2.73 -7.46 -2.73 -7.46 -2.75 -3.84 -3.38 -3.28 -5.02 -1.55 -7.46 -2.75 -2.47 -2.86 -2.75 -3.86 -1.93 -3.28 -5.02 -5.48 -4.24 -3.56 -3.48 -3.56 -3.56 -3.56 -3.56$	Lei Fan 2013	1	0.2	3	1.8	0.4	7	24.0%	-2.00 [-3.77, -0.24]	
Xiaoji (uo 2014) 0.366 0.021 5 0.674 0.042 5 7.0% -8.30 [+3.13, -3.46] Yu He 2014 0.375 0.075 8 0.998 0.219 8 2.42.2% -3.60 [-5.34, -3.66] Heterogeneity, Tau" = 1.38; Ch" = 9.01, df = 4 (P = 0.06); P = 56%, Test for overall effect Z = 5.48 (P < 0.00001)	Liang Ge 2016	0.36	0.083	10	0.911	0.122	10	22.1%	-5.06 [-7.01, -3.10]	-
Yu He 2014 0.375 0.075 8 0.998 0.219 8 24.2% -3.80 (F 5.34, -1.86) Subtotal (95% C) 34 38 100.0% -3.97 (E.3.9, -2.55) -3.80 (F 5.34, -1.86) Chi Cheng 2014 0.2737 0.0253 6 0.5516 0.0295 6 25.2% -9.33 (-14.03, -4.64) Jiahu Zhou 2015 (b) 0.41 0.182 9 1.366 0.022 5 23.9% -4.43 (-17.03, -2.92) Lei Chen 2013 0.2095 0.0254 5 0.4698 0.0302 5 23.9% -4.43 (-13.3, -4.64) Test for overall effect Z = 4.53 (P < 0.0001)	Xiaoji Luo 2014	0.369	0.021	5	0.674	0.042	5	7.0%	-8.30 [-13.13, -3.46]	
Subtotal (95% C) 200 0000) Heterogeneity: Tau" = 1.38; Chi" = 9.01, df = 4 (P = 0.000; P = 56% Test for overall effect Z = 5.48 (P < 0.00001) 1.72 U2 OS Chi Cheng 2014 0.2737 0.0253 6 0.5516 0.0295 6 25.2% -9.33 [+14.03, -4.64] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 50.9% -4.97 [-7.03, -2.92] Lei Chen 2013 0.2095 0.0224 5 0.4688 0.3020 5 2.33.9% -4.93 [-13.33, -3.52] Subtotal (95% C) 20 100.0% -6.90 [-9.88, -3.91] Heterogeneity: Tau" = 3.44; (Chi" = 3.84; df = 2 (P = 0.15); P = 48% Test for overall effect Z = 4.53 (P < 0.0001) 1.7.3 Saos-2 Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 47.1% -5.74 [+8.06, -3.43] Wei Liu 2015 0.279 0.051 5 0.641 0.079 5 27.5% -4.92 [-7.55, -1.88] Wei Liu 2015 0.279 0.051 5 0.641 0.079 5 27.5% -4.92 [-7.55, -1.88] Wei Liu 2015 0.279 0.051 5 0.641 0.079 5 27.5% -4.92 [-7.55, -1.88] Wei Liu 2015 0.279 0.051 5 0.641 0.079 5 27.5% -4.92 [-7.55, -1.88] Wei Liu 2015 0.279 0.051 5 0.641 0.079 5 27.5% -4.92 [-7.55, -1.88] Wei Liu 2015 0.279 0.051 5 0.641 0.079 5 27.5% -5.59 [-7.18, 4.00] Heterogeneity: Tau" = 2.4%; df = 2 (P = 0.87); P = 0% Test for overall effect Z = 8.89 (P < 0.00001) 1.7.4 1438 Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [-12.47, -3.27] Mitsuhko 0.saki 2011 3.32 0.65 10 3.67 0.599 10 2.72% -0.54 [-1.44, 0.36] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 14.2% -5.77 [-8.80, -2.73] Witsuhko 0.528, df = 4 (P = 0.0004); P = 80% Test for overall effect Z = 3.15 (P = 0.02); df = 4 (P = 0.0004); P = 80% Test for overall effect Z = 3.15 (P = 0.002) 1.75 SOSP.9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 Kang Han 2014 1.0437 0.0	Yu He 2014	0.375	0.075	8	0.998	0.219	8	24.2%	-3 60 [-5 34 -1 86]	
Heterogeneity, Tau ² = 1.38; Chi ² = 9.01, df = 4 ($P = 0.06$); $P = 56\%$ Test for overall effect. Z = 5.48 ($P < 0.00001$) 1.7.2 U2 OS Chi Cheng 2014 0.2737 0.0253 6 0.5516 0.0295 6 25.2% -9.33 [+1.03, -4.84] Jiahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.0202 9 50.9% -4.37 [-7.03, 2.92] Lei Chen 2013 0.2095 0.0254 5 0.4698 0.0302 20 100.0% -4.39 [-7.03, 2.92] Lei Chen 2013 0.2095 0.0254 5 0.4698 0.0302 20 100.0% -6.90 [-9.88, -3.91] Heterogeneity, Tau ² = 3.44; Chi ² = 3.84, df = 2 ($P = 0.15$); $P = 48\%$ Test for overall effect. Z = 4.53 ($P < 0.00001$) 1.7.3 Saos-2 Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 47.1% -5.74 [+8.06, -3.43] Wei Liu 2013 0.3695 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [-9.18, -2.88] Xin Zhou 2013 0.3695 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [-9.18, -2.88] Xin Zhou 2013 0.3695 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [-9.18, -2.88] Xin Zhou 2013 0.3695 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [-9.18, -2.88] Xin Zhou 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [+12.47, -3.27] Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 27.2% -0.54 [+1.44, 0.36] Torronitor Fujiwara 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [+12.47, -3.27] Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 27.2% -0.54 [+1.44, 0.36] Torronitor Fujiwara 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [+12.47, -3.27] Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 27.2% -0.54 [+1.44, 0.36] Torronitor Fujiwara 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [+12.47, -3.27] Mitsuhiko Osaki 2011 3.32 0.65 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.036 0.229 10 1.585 0.198 10 3.56% -2.29 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 2 61.775 0.3381 6 22.4% -2.18 [-3.73, -0.03] Heterogeneity, Tau ² = 1.78 (chi ² = 4.67 = 0.002) Test for overall effect. Z = 3.71 ($P = 0.0002$) Test for overall effect. Z = 3.71 ($P = 0.0002$) Test for overall effect. Z = 3.71 ($P = 0.0002$)	Subtotal (95% CI)	0.010	0.010	34	0.000	0.210	38	100.0%	-3.97 [-5.39, -2.55]	◆
Test for overall effect Z = 5.46 (P = 0.00001) 1.7.2 U2 OS Chi Cheng 2014 0.2737 0.0253 6 0.5516 0.0295 9 50.9% -4.97 [-7.03, -2.92] Jahui Zhou 2015 (b) 0.41 0.162 9 1.366 0.202 9 50.9% -4.97 [-7.03, -2.92] Lei Chen 2013 0.2095 0.0254 5 0.4698 0.0302 5 23.9% -8.43 [+13.33, -3.52] Subtotal (95% CI) 20 100.0% -6.90 [-9.86, -3.43] -6.90 [-9.86, -3.43] -6.90 [-9.86, -3.43] Jahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 47.1% -5.74 [+9.06, -3.43] Vei Liu 2015 0.279 0.051 5 0.641 0.079 5 27.5% -4.92 [-7.95, -1.88] Xin Zhou 2013 0.3595 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [+9.18, -2.88] -5.59 [-7.18, -4.00] Heterogeneity: Tau" = 0.00; Chi" = 0.28, df = 2 (P = 0.87); P = 0% 20 100.0% -5.57 [+8.40, 0.36] -5.59 [-7.18, -4.00] -5.59 [-7.18, -4.00] -5.59 [-7.18, -4.00] -2.63 [+4.14, 0.36]	Heterogeneity Tau ² = 1.38	Chi ² = 9	01 $df = 4$	(P = 0	$(16) \cdot 1^2 = 1$	56%				-
1.72 U2 OS Chi Cheng 2014 0.2737 0.0253 6 0.5516 0.0229 9 50.9% -4.37 (7.03, 2.92) 1.61 Cheng 2014 0.2095 0.0244 5 0.4698 0.0302 5 23.9% -4.37 (7.03, 2.92) 1.61 Cheng 2013 0.2095 0.0244 5 0.4698 0.0302 5 23.9% -4.37 (7.03, 2.92) 1.61 Cheng 2014 0.2095 0.0244 5 0.4698 0.0302 5 23.9% -4.38 (1-33, -4.54) 1.61 Cheng 2014 0.2095 0.0244 0.79 20 100.0% -6.90 (-9.86, -3.43) 1.61 Cheng 2015 0.279 0.051 5 0.641 0.079 5 27.5% -4.92 (-5.1.88) Via Izhou 2015 0.279 0.051 5 0.641 0.079 5 27.5% -4.92 (-7.9.27) Subtotal (95% Ct) 20 20 20 100.0% -5.59 (-7.18, -4.00) -5.59 (-7.18, -4.00) -5.57 (-1.88) Via Izhou 2015 0.329 5 1.1882 0.921 5 8.4% -7.87 (-12.47, -3.27) Mitsuhiko Osaki 2011 <t< td=""><td>Test for overall effect: 7 = 5</td><td>48 (P < 0</td><td>00001</td><td>, - ·</td><td></td><td></td><td></td><td></td><td></td><td></td></t<>	Test for overall effect: 7 = 5	48 (P < 0	00001	, - ·						
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Lei Chen 2013 0.2095 0.0254 5 0.4698 0.0302 5 23.9% $-8.431[13.33, -3.52]$ Subtotal (95% C) 20 20 100.0% $-8.09[-9.88, -3.91]$ Heterogeneity: Tau" = 3.44, Ch = 2 (P = 0.15); P = 48% Test for overall effect: Z = 4.53 (P < 0.00001) 1.7.3 Saos-2 Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 47.1% $-5.74 [8.06, -3.43]$ Wei Liu 2015 0.279 0.051 5 0.641 0.079 5 27.5% $-6.03 [-9.18, -2.88]$ Subtotal (95% C) 20 20 100.0% $-5.59 [-7.18, -4.00]$ Heterogeneity: Tau" = 0.00; Chi" = 0.29, df = 2 (P = 0.87); P = 0% Test for overall effect: Z = 6.88 (P < 0.00001) 1.7.4 143B Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% $-7.87 [-12.47, -3.27]$ Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 27.2% $-1.41 [+23.1, 0.50]$ Wei Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% $-7.87 [-12.47, -3.27]$ Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 27.2% $-1.41 [+23.1, 0.50]$ Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 14.2% $-5.77 [-8.80, -2.73]$ Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 22.9% $-2.27 [-3.86, -0.68]$ Subtotal (95% C) 42 37 100.0% $-2.53 [-4.11, -0.96]$ Heterogeneity: Tau" = 2.17; Chi" = 20.26, df = 4 (P = 0.0004); P = 80% Test for overall effect: Z = 3.15 (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% $-5.18 [-6.76, -3.58]$ Kang Han 2015 1.036 0.229 10 1.585 0.198 10 36.6% $-2.59 [-3.24, -1.34]$ Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% $-2.18 [-5.73, -0.63]$ Subtotal (95% C) 26 36 100.0% $-3.28 [-5.02, -1.55]$ Heterogeneity: Tau" = 1.79; Chi" = 8.45, df = 2 (P = 0.01); P = 76% Test for overall effect: Z = 3.71 (P = 0.002) Test for overall effect: Z = 3.71 (P = 0.002) Test for overall effect: Z = 3.71 (P = 0.002).	liahui Zhou 2015 (h)	n 41	0 162	ğ	1 366	0.202	ğ	50.9%	-4 97 [-7 03 -2 92]	
Subtotal (95% C) 20 100.0 20 20 100.0 4 -6.90 [-9.88, -3.91] Heterogeneity: Tau" = 3.44; Chi"= 3.84; df = 2 (P = 0.15); P = 48% Test for overall effect: Z = 4.53 (P < 0.00001) 1.7.3 Saos-2 Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 47.1% -5.74 [+8.06, -3.43] Wei Liu 2015 0.279 0.051 5 0.641 0.079 5 27.5% -4.92 [-7.55, -1.88] Wei Liu 2015 0.3595 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [+9.18, -2.88] Subtotal (95% C) 20 20 100.0% -5.59 [-7.18, -4.00] Heterogeneity: Tau" = 0.00; Chi" = 0.28, df = 2 (P = 0.87); P = 0% Test for overall effect: Z = 6.89 (P < 0.00001) 1.7.4 143B Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [-12.47, -3.27] Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 27.2% -0.64 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 27.2% -0.64 [-1.44, 0.56] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 27.2% -0.64 [-1.44, 0.56] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 14.2% -5.77 [-8.80, -2.73] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 22.9% -2.27 [-3.86, -0.69] Subtotal (95% C) 42 37 100.0% -2.53 [-4.11, -0.96] Heterogeneity: Tau" = 2.17; Chi" = 20.26, df = 4 (P = 0.0004); P = 80% Test for overall effect: Z = 3.15 (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% C) 26 36 100.0% -3.28 [-5.02, -1.55] Heterogeneity: Tau" = 1.79; Chi" = 8.45, df = 2 (P = 0.01); P = 76% Test for overall effect: Z = 3.71 (P = 0.002) Test for overall effect: Z = 3.71 (P = 0.002) Test for overall effect: Z = 3.71 (P = 0.002) Test for overall effect: Z = 3.71 (P = 0.002)	Lei Chen 2013	0 2095	0.0254	5	0 4698	0.202	5	23.9%	-8 43 [-13 33 -3 52]	_ _
Heterogeneily: Tau ² = 3.44; Ch ² = 3.84, df = 2 (P = 0.15); P = 48% Test for overall effect Z = 4.53 (P < 0.00001) 1.7.3 Saos-2 Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 47.1% -5.74 [8.06, -3.43] Wei Liu 2015 (a) 0.3595 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [-9.18, -2.88] Xin Zhou 2013 0.3595 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [-9.18, -2.88] Xin Zhou 2013 0.3595 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [-9.18, -2.88] Xin Zhou 2013 0.3595 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [-9.18, -2.88] Wei Liu 2011 3.32 0.65 10 3.67 0.59 10 27.2% -6.054 [-1.44, 0.36] Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [-12.47, -3.27] Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 27.2% -1.41 [-2.31, -0.50] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 14.2% -5.77 [-8.80, -2.73] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 22.9% -2.27 [-3.86, -0.69] Subtotal (95% Ch) 42 37 100.0% -2.25 [-4.141, -0.96] Heterogeneity: Tau ² = 2.17; Ch ² = 20.26, df = 4 (P = 0.0004); P = 80% Test for overall effect Z = 3.15 (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Heterogeneity: Tau ² = 1.79; Ch ² = 8.45, df = 2 (P = 0.01); P = 76% Test for overall effect Z = 3.71 (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-3.73, -0.63] Heterogeneity: Tau ² = 1.79; Ch ² = 8.45, df = 2 (P = 0.01); P = 76% Test for overall effect Z = 3.71 (P = 0.002) 1.7.5 SOSP-9607 Heterogeneity: Tau ² = 1.79; Ch ² = 8.45, df = 2 (P = 0.01); P = 76% Test for overall effect Z = 3.71 (P = 0.002) 1.7.5 SOSP-9607 Test for overall effect Z = 3.71 (P = 0.002)	Subtotal (95% CI)	0.2000	0.0204	20	0.4000	0.0002	20	100.0%	-6.90[-9.88, -3.91]	◆
Test for versal effect $Z = 4.53 (P < 0.0001)$ 1.7.3 Saos-2 Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 47.1% -5.74 [-8.06, -3.43] Wei Liu 2015 0.279 0.051 5 0.641 0.079 5 27.5% -4.92 [-7.95, -1.88] Vin Zhou 2013 0.3595 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [-9.18, -2.88] Subtotal (95% CI) 20 20 100.0% -5.59 [-7.18, -4.00] Heterogeneity: Tau ² = 0.00; Ch ² = 0.28, df = 2 (P = 0.87); P = 0% Test for overall effect Z = 6.89 (P < 0.00001) 1.7.4 143B Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [-12.47, -3.27] Witsuniko 0saki 2011 3.32 0.65 10 3.67 0.59 10 27.2% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 27.2% -1.41 [-2.31, -0.50] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 14.2% -5.77 [-8.80, -2.77] Yong Zhao 2015 1.738 0.38 6 2.827 0.482 6 2.29% -2.27 [-3.86, -0.69] Heterogeneity: Tau ² = 2.17; Ch ² = 20.26, df = 4 (P = 0.0004); P = 80% Test for overall effect Z = 3.15 (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% CI) 26 36 100.00% -2.18 [-3.73, -0.63] Subtotal (95% C) 26 36 10.00% -2.18 [-3.73, -0.63] Fest for overall effect Z = 3.71 (P = 0.002) Test for overall effect Z = 3.71 (P = 0.002) Test for overall effect Z = 3.71 (P = 0.002) Test for overall effect Z = 3.71 (P = 0.002)	Heterogeneity $Tau^2 = 3.44$	Chi ² = 3	84 df= 2	P = 0	15): I ² = .	48%	20	1001010	0100 [-0100, 010 1]	•
1.7.3 Saos-2 Jiahui Zhou 2015 (a) 0.467 0.101 9 1.457 0.209 9 47.1% -5.74 [-8.06, -3.43] Wei Liu 2015 0.279 0.051 5 0.641 0.079 5 27.5% -4.92 [-7.95, -1.88] Xin Zhou 2013 0.3595 0.0607 6 0.9105 0.1027 6 25.4% -6.03 [-9.18, -2.88] Subtotal (95% Cl) 20 20 100.0% -5.59 [-7.18, -4.00] + 1.7.4 143B Jin Wang 2014 0.5112 0.0599 5 1.8.4% -7.87 [-12.47, -3.27] Mitsuhiko Osaki 2011 3.2 0.65 10 3.67 0.59 10 27.2% -1.41 [-2.31, -0.50] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 2.2.9% -2.27 [-3.86, -0.69] + Subtotal (95% Cl) 42 37 100.0% -2.53 [-4.11, -0.96] + Heterogeneity: Tau" = 2.17; Chi" = 20.26, df = 4 (P = 0.0004); P = 80% -5.18 [-6.78, -3.58] + + Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 </td <td>Test for overall effect: 7 - 4</td> <td>, OIII = 0. 53 /P < 0</td> <td>04,01-2</td> <td>. (1 - 0</td> <td>.15),1 =</td> <td>40 /0</td> <td></td> <td></td> <td></td> <td></td>	Test for overall effect: 7 - 4	, OIII = 0. 53 /P < 0	04,01-2	. (1 - 0	.15),1 =	40 /0				
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$\begin{array}{cccccccccccccccccccccccccccccccccccc$	1.7.3 Sans-2									
$\begin{array}{c} \text{Mitchick 2015} & 0.761 & 0.161 & 0.161 & 0.173 & 0.54 & 1.761 & 0.713 & 0.54 & 1.781 & 0.713 & 0.4.92 \ [r.7, 55, -1.8] \\ \text{Xin Zhou 2013} & 0.3595 & 0.0607 & 6 & 0.9105 & 0.1027 & 6 & 25.4\% & -6.03 \ [r.9, 18, -2.88] \\ \text{Subtotal (95% CI)} & 20 & 20 & 100.0\% & -5.59 \ [r.7, 18, -4.00] \\ \text{Heterogeneity: Tau2 = 0.00; Chi2 = 0.28, df = 2 (P = 0.87); P = 0\% \\ \text{Test for overall effect: Z = 6.89 (P < 0.00001)} \\ \hline \textbf{1.7.4 \ 1438} \\ \text{Jin Wang 2014} & 0.5112 & 0.0599 & 5 & 1.1882 & 0.0921 & 5 & 8.4\% & -7.87 \ [r.12.47, -3.27] \\ \text{Mitsuhiko Osaki 2011} & 3.32 & 0.65 & 10 & 3.67 & 0.59 & 10 & 27.2\% & -0.54 \ [r.144, 0.36] \\ \text{Tomohiro Fujiwara 2014} & 2.155 & 0.6465 & 15 & 3.09 & 0.6346 & 10 & 27.2\% & -0.54 \ [r.144, 0.36] \\ \text{Tomohiro Fujiwara 2015} & 0.429 & 0.072 & 6 & 0.97 & 0.099 & 6 & 14.2\% & -5.77 \ [r.8.0, -2.73] \\ \text{Vei Wang 2015} & 0.429 & 0.072 & 6 & 0.97 & 0.099 & 6 & 14.2\% & -5.77 \ [r.8.0, -2.73] \\ \text{Vei Wag 2015} & 1.738 & 0.398 & 6 & 2.827 & 0.482 & 6 & 22.9\% & -2.27 \ [r.3.86, -0.69] \\ \text{Subtotal (95% CI)} & 42 & 37 & 100.0\% & -2.53 \ [r.4.11, -0.96] \\ \text{Heterogeneity: Tau2 = 2.17; Chi2 = 20.26, df = 4 (P = 0.0004); P = 80\% \\ \text{Test for overall effect: Z = 3.15 (P = 0.002)} \\ \text{I.7.5 SOSP-9607} \\ \text{Kang Han 2014} & 1.0437 & 0.085 & 10 & 1.6019 & 0.1131 & 20 & 31.9\% & -5.18 \ [r.6.78, -3.58] \\ \text{Kang Han 2014} & 1.0437 & 0.085 & 10 & 1.6019 & 0.1131 & 20 & 31.9\% & -5.18 \ [r.6.78, -3.58] \\ \text{Kang Han 2014} & 1.0437 & 0.085 & 10 & 1.6019 & 0.1131 & 20 & 31.9\% & -5.18 \ [r.6.78, -3.58] \\ \text{Kang Han 2014} & 1.0437 & 0.085 & 10 & 1.6019 & 0.1131 & 20 & 31.9\% & -5.18 \ [r.6.78, -3.58] \\ \text{Kang Han 2014} & 1.0437 & 0.085 & 10 & 1.6019 & 0.1131 & 20 & 31.9\% & -5.18 \ [r.6.78, -3.58] \\ \text{Kang Han 2014} & 1.0437 & 0.085 & 10 & 1.6019 & 0.1585 & 0.28 & -2.59 \ [r.3.84, -1.34] \\ \text{Heterogeneity: Tau2 = 1.79; Chi2 = 8.45, df = 2 (P = 0.01); P = 76\% \\ \text{Test for overall effect: Z = 3.71 (P = 0.0002)} \\ \hline Test for overall effect: Z = 3.71 (P = 0.0002) \\ \hline \textbf{Test for overall effect: Z = 3.71 ($	liabui Zhou 2015 (a)	0.467	0 1 0 1	q	1 4 5 7	0.209	q	471%	-5 74 1-8 06 -3 431	
The final field of the field o	Weiliu 2015	0.401	0.051	5	0.641	0.200	5	27 5%	-4 92 [-7 95 -1 88]	
The terogeneity: Tau ² = 0.00; Chi ² = 0.28, df = 2 (P = 0.87); P = 0% Test for overall effect Z = 6.89 (P < 0.00001) 1.7.4 143B Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [-12.47, -3.27] Mitsuhko Osaki 2011 3.32 0.65 10 3.67 0.59 10 27.2% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 27.2% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2015 0.429 0.072 6 0.97 0.099 6 14.2% -5.77 [-8.80, -2.73] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 22.9% -2.27 [-3.86, -0.69] Subtotal (95% Cl) 42 37 100.0% -2.53 [-4.11, -0.96] Heterogeneity: Tau ² = 2.17; Chi ² = 20.26, df = 4 (P = 0.0004); P = 80% Test for overall effect Z = 3.15 (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2014 1.0437 0.085 10 1.565 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% Cl) 26 36 100.0% -3.28 [-5.02, -1.55] Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); P = 76% Test for overall effect Z = 3.71 (P = 0.0002) Test for overall effect Z = 3.71 (P = 0.002)	Xin Zhou 2013	0.275	0.001	6	0.041	0.073	6	25.4%	-6.03[-0.18 -7.88]	_ _
$\begin{array}{c} \text{Let or geneily: Tau^2} = 0.00; \ \text{Ch}^{\mu} = 0.28, \ \text{df} = 2 \ (P = 0.87); \ P = 0\% \\ \text{Test for overall effect: } Z = 6.89 \ (P < 0.00001) \\ \hline 1.7.4 \ 143B \\ \text{Jin Wang 2014} & 0.5112 \ 0.0599 \ 5 \ 1.1882 \ 0.0921 \ 5 \ 8.4\% \ -7.87 \ [+12.47, -3.27] \\ \text{Mitsuhiko Osaki 2011} & 3.32 \ 0.65 \ 10 \ 3.67 \ 0.59 \ 10 \ 27.2\% \ -0.54 \ [+1.44, 0.36] \\ \hline \text{Tormohiro Fujiwara 2014} \ 2.155 \ 0.6465 \ 15 \ 3.09 \ 0.6346 \ 10 \ 27.2\% \ -1.41 \ [+2.31, -0.50] \\ \text{Wei Wang 2015} \ 0.429 \ 0.072 \ 6 \ 0.97 \ 0.099 \ 6 \ 22.9\% \ -2.27 \ [+3.80, -0.69] \\ \text{Subtotal (95% Cl)} & 42 \ 37 \ 100.0\% \ -2.53 \ [-4.11, -0.96] \\ \text{Heterogeneily: Tau^2 = 2.17; \ Chi^2 = 20.26, \ df = 4 \ (P = 0.0004); \ P = 80\% \\ \text{Test for overall effect: } Z = 3.15 \ (P = 0.002) \\ \text{Kang Han 2014} & 1.0437 \ 0.085 \ 10 \ 1.6019 \ 0.1131 \ 20 \ 31.9\% \ -2.58 \ [-6.78, -3.58] \\ \text{Kang Han 2014} & 1.0437 \ 0.085 \ 10 \ 1.6019 \ 0.1131 \ 20 \ 31.9\% \ -2.59 \ [+3.84, -1.34] \\ \text{Kang Yan 2012} & 1.1373 \ 0.1742 \ 6 \ 1.7725 \ 0.3381 \ 6 \ 32.4\% \ -2.18 \ [-6.78, -3.68] \\ \text{Subtotal (95\% Cl)} & 26 \ 36 \ 100.0\% \ -3.28 \ [-5.02, -1.55] \\ \text{Heterogeneily: Tau^2 = 1.79; \ Chi^2 = 8.45, \ df = 2 \ (P = 0.01); \ P = 76\% \\ \text{Test for overall effect: } Z = 3.71 \ (P = 0.0002) \\ \end{array}$	Subtotal (95% CI)	0.0000	0.0007	20	0.3103	0.1027	20	100.0%	-5.59 [-7.18 -4.00]	•
Test for overall effect: $Z = 6.89$ (P < 0.00001) 1.7.4 143B Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [-12.47, -3.27] Milsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 27.2% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 27.2% -1.41 [-2.31, -0.50] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 14.2% -5.77 [-8.80, -2.73] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 22.9% -2.27 [-3.86, -0.69] Subtotal (95% CI) 42 37 100.0% -2.53 [-4.11, -0.96] Heterogeneity: Tau ² = 2.17; Chi ² = 20.26, df = 4 (P = 0.0004); I ² = 80% Test for overall effect: $Z = 3.15$ (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% CI) 26 36 100.0% -3.28 [-5.02, -1.55] Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); I ² = 76% Test for overall effect: $Z = 3.71$ (P = 0.002) Test for subbroup differences: Chi ² = 11.42, df = 4 (P = 0.02), I ² = 65.0%	Hotorogeneity: Tou ² - 0.00	Chiž – O	28 df - 2	(P - 0	87): I ² –	n%	20	100.074	-5.55 [-1.10, -4.00]	Ŧ
1.7.4 143B Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [-12.47, -3.27] Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 27.2% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 27.2% -1.41 [-2.31, -0.50] Wei Wang 2015 0.429 0.072 6 0.97 0.99 6 14.2% -5.77 [-8.80, -2.73] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 22.9% -2.27 [-3.86, -0.69] Subtotal (95% CI) 42 37 100.0% -2.53 [-4.11, -0.96] • Heterogeneity: Tau ² = 2.17; Chi ² = 20.26, df = 4 (P = 0.0004); I ² = 80% -5.18 [-6.78, -3.58] • • Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] • Kang Yan 2012 1.1373 0.1742 6 1.7725 0.381 6 32.4% -2.18 [-3.73, -0.63] •	Test for overall effect: 7 = 6	, CIII = 0. : 00 / D ~ 0	20, ui – 2 00001\	(F = 0	.07),1 =	0.70				
1.7.4 143B Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [-12.47, -3.27] Misuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 27.2% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 27.2% -1.41 [-2.31, -0.50] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 14.2% -5.77 [-8.80, -2.73] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 2.29% -2.27 [-3.86, -0.69] Subtotal (95% CI) 42 37 100.0% -2.53 [-4.11, -0.96] Heterogeneity: Tau ² = 2.17; Chi ² = 20.26, df = 4 (P = 0.0004); I ² = 80% Test for overall effect: $Z = 3.15$ (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% CI) 26 36 100.0% -3.28 [-5.02, -1.55] Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); I ² = 76% Test for overall effect: $Z = 3.71$ (P = 0.0002) Test for overall effect: $Z = 3.71$ (P = 0.0002) Test for subproup differences: Chi ² = 11.42, df = 4 (P = 0.02), I ² = 65.0%	restion overall ellect. Z = 0	1.09 (F < 0	.00001)							
Jin Wang 2014 0.5112 0.0599 5 1.1882 0.0921 5 8.4% -7.87 [-12.47, -3.27] Mitsuhiko Osaki 2011 3.32 0.65 10 3.67 0.59 10 27.2% -0.54 [-1.44, 0.36] Tomohiro Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 27.2% -1.41 [-2.31, -0.50] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 14.2% -5.77 [-8.80, -2.73] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 22.9% -2.27 [-3.86, -0.69] Subtotal (95% CI) 42 37 100.0% -2.53 [-4.11, -0.96] Heterogeneity: Tau ² = 2.17; Chi ² = 20.26, df = 4 (P = 0.0004); P = 80% Test for overall effect: $Z = 3.15$ (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-5.73, -0.63] Subtotal (95% CI) 26 36 100.0% -3.28 [-5.02, -1.55] Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); P = 76% Test for overall effect: $Z = 3.71$ (P = 0.0002) Test for overall effect: $Z = 3.71$ (P = 0.0002)	1.7.4 143B									
$\begin{array}{c} \mbox{Mitsuhiko Osaki 2011} & 3.32 & 0.65 & 10 & 3.67 & 0.59 & 10 & 27.2\% & -0.54 [-1.44, 0.36] \\ \mbox{Tomohiro Fujiwara 2014} & 2.155 & 0.6465 & 15 & 3.09 & 0.6346 & 10 & 27.2\% & -1.41 [-2.31, -0.50] \\ \mbox{Wei Wang 2015} & 0.429 & 0.072 & 6 & 0.97 & 0.099 & 6 & 14.2\% & -5.77 [-8.80, -2.73] \\ \mbox{Yong Zhao 2015} & 1.738 & 0.398 & 6 & 2.827 & 0.482 & 6 & 22.9\% & -2.27 [-3.86, -0.69] \\ \mbox{Subtoal (95\% CI)} & 42 & 37 & 100.0\% & -2.53 [-4.11, -0.96] \\ \mbox{Heterogeneity: Tau2 = 2.17; Chi2 = 20.26, df = 4 (P = 0.0004); I2 = 80\% \\ \mbox{Test for overall effect: Z = 3.15 (P = 0.002)} \\ \mbox{I.7.5 SOSP-9607} \\ \mbox{Kang Han 2014} & 1.0437 & 0.085 & 10 & 1.6019 & 0.1131 & 20 & 31.9\% & -5.18 [-6.78, -3.58] \\ \mbox{Kang Han 2015} & 1.006 & 0.229 & 10 & 1.585 & 0.198 & 10 & 32.6\% & -2.59 [-3.84, -1.34] \\ \mbox{Kang Yan 2012} & 1.1373 & 0.1742 & 6 & 1.7725 & 0.381 & 6 & 32.4\% & -2.18 [-3.73, -0.63] \\ \mbox{Subtoal (95% CI)} & 26 & 36 & 100.0\% & -3.28 [-5.02, -1.55] \\ \mbox{Heterogeneity: Tau2 = 1.79; Chi2 = 8.45, df = 2 (P = 0.01); I2 = 76\% \\ \mbox{Test for overall effect: Z = 3.71 (P = 0.0002)} \\ \mbox{Test for overall effect: Z = 3.71 (P = 0.002)} \\ Test for subdroup differences: Chi2 = 11.42, df = 4 (P = 0.02), I2 = 65.0\% \\ \mbox{Test for subdroup differences: Chi2 = 11.42, df = 4 (P = 0.02), I2 = 65.0\% \\ \mbox{Test for subdroup differences: Chi2 = 11.42, df = 4 (P = 0.02), I2 = 65.0\% \\ \mbox{Test for overall effect: Z = 3.71 (P = 0.02), I2 = 65.0\% \\ \mbox{Test for overall effect: Z = 3.71 (P = 0.02), I2 = 65.0\% \\ \mbox{Test for subdroup differences: Chi2 = 11.42, df = 4 (P = 0.02), I2 = 65.0\% \\ \mbox{Test for overall effect: Z = 3.71 (P = 0.02), I2 = 65.0\% \\ \mbox{Test for overall effect: Z = 3.71 (P = 0.02), I2 = 65.0\% \\ \mbox{Test for overall effect: Z = 3.71 (P = 0.02), I2 = 65.0\% \\ \mbox{Test for overall effect: Z = 3.71 (P = 0.02), I2 = 65.0\% \\ \mbox{Test for overall effect: Z = 3.71 (P = 0.02), I2 = 65.0\% \\ \mbox{Test for overall effect: Z = 3.71 (P$	Jin Wang 2014	0.5112	0.0599	5	1.1882	0.0921	5	8.4%	-7.87 [-12.473.27]	
Tomohino Fujiwara 2014 2.155 0.6465 15 3.09 0.6346 10 27.2% -1.41 [-2.31, -0.50] Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 14.2% -5.77 [-8.80, -2.73] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 22.9% -2.27 [-3.86, -0.69] Subtotal (95% Cl) 42 37 100.0% -2.53 [-4.11, -0.96] Heterogeneity: Tau ² = 2.17; Chi ² = 20.26, df = 4 (P = 0.0004); I ² = 80% Test for overall effect: Z = 3.15 (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% Cl) 26 36 100.0% -3.28 [-5.02, -1.55] Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); I ² = 76% Test for overall effect: Z = 3.71 (P = 0.002) Test for subaroup differences: Chi ² = 11.42, df = 4 (P = 0.02), I ² = 65.0%	Mitsuhiko Osaki 2011	3.32	0.65	10	3.67	0.59	10	27.2%	-0.54 [-1.44, 0.36]	-
Wei Wang 2015 0.429 0.072 6 0.97 0.099 6 14.2% -5.77 [-8.80, -2.73] Yong Zhao 2015 1.738 0.398 6 2.827 0.482 6 22.9% -2.27 [-3.86, -0.69] Subtotal (95% CI) 42 37 100.0% -2.53 [-4.11, -0.96] Heterogeneity: Tau ² = 2.17; Chi ² = 20.26, df = 4 (P = 0.0004); ² = 80% Test for overall effect: $Z = 3.15$ (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% CI) 26 36 100.0% -3.28 [-5.02, -1.55] Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); ² = 76% Test for overall effect: $Z = 3.71$ (P = 0.002) Test for subaroup differences: Chi ² = 11.42, df = 4 (P = 0.02), ² = 65.0%	Tomohiro Euliwara 2014	2 1 5 5	0.6465	15	3.09	0.6346	10	27.2%	-1 41 [-2 31 -0 50]	-
The form of the f	Wei Wang 2015	0.429	0.072	6	0.97	0.099	6	14.2%	-5 77 (-8 80 -2 73)	- -
Subtotal (95% CI) 42 37 100.0% -2.53 [-4.11, -0.96] Heterogeneity: Tau ² = 2.17; Chi ² = 20.26, df = 4 (P = 0.0004); I ² = 80% 37 100.0% -2.53 [-4.11, -0.96] Heterogeneity: Tau ² = 2.17; Chi ² = 20.26, df = 4 (P = 0.0004); I ² = 80% -5.18 [-6.78, -3.58] - 1.7.5 SOSP-9607 - - - Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] - Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] - Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-3.73, -0.63] - Subtotal (95% CI) 26 36 100.0% -3.28 [-5.02, -1.55] - - Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); I ² = 76% - 0 10 <td>Yong Zhao 2015</td> <td>1,738</td> <td>0.398</td> <td>ñ</td> <td>2 827</td> <td>0.000</td> <td>6</td> <td>22.9%</td> <td>-2.27 [-3.86 -0.69]</td> <td></td>	Yong Zhao 2015	1,738	0.398	ñ	2 827	0.000	6	22.9%	-2.27 [-3.86 -0.69]	
Heterogeneity: Tau ² = 2.17; Chi ² = 20.26, df = 4 (P = 0.0004); P = 80% Test for overall effect: Z = 3.15 (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% Cl) 26 36 100.0% -3.28 [-5.02, -1.55] Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); I ² = 76% Test for overall effect: Z = 3.71 (P = 0.0002) Test for subgroup differences: Chi ² = 11.42, df = 4 (P = 0.02), I ² = 65.0%	Subtotal (95% CI)	1.100	0.000	42	2.021	0.102	37	100.0%	-2.53 [-4.11, -0.96]	◆
Test for overall effect: $Z = 3.15$ (P = 0.002) 1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% Cl) 26 36 100.0% -3.28 [-5.02, -1.55] • Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); l ² = 76% Test for overall effect: Z = 3.71 (P = 0.0002) • • • Test for subgroup differences: Chi ² = 11.42, df = 4 (P = 0.02). l ² = 65.0% Favours miRNA Favours control	Heterogeneity: Tau ² = 2.17	: Chi ² = 20	126 df=	4 (P =	0.0004):	l ² = 80%				
1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% Cl) 26 36 100.0% -3.28 [-5.02, -1.55] • Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); I ² = 76% • -20 -10 0 10 20 Test for overall effect: Z = 3.71 (P = 0.0002) Test for subgroup differences: Chi ² = 11.42, df = 4 (P = 0.02), I ² = 65.0% Favours miRNA Favours control	Test for overall effect: $Z = 3$	15(P = 0	002)							
1.7.5 SOSP-9607 Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% Cl) 26 36 100.0% -3.28 [-5.02, -1.55] • Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); I ² = 76% • -10 0 10 20 Test for overall effect: Z = 3.71 (P = 0.0002) Test for subaroup differences: Chi ² = 11.42. df = 4 (P = 0.02). I ² = 65.0% Favours miRNA Favours control										
Kang Han 2014 1.0437 0.085 10 1.6019 0.1131 20 31.9% -5.18 [-6.78, -3.58] Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% Cl) 26 36 100.0% -3.28 [-5.02, -1.55] -20 -10 0 10 20 Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); i ² = 76% Test for overall effect: Z = 3.71 (P = 0.0002) -10 0 10 20 Test for subaroup differences: Chi ² = 11.42. df = 4 (P = 0.02). i ² = 65.0% -50.0% -10 0 10 20	1.7.5 SOSP-9607									
Kang Han 2015 1.006 0.229 10 1.585 0.198 10 35.6% -2.59 [-3.84, -1.34] Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% CI) 26 36 100.0% -3.28 [-5.02, -1.55] • Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); I ² = 76% Test for overall effect: Z = 3.71 (P = 0.0002) • • • Test for subaroup differences: Chi ² = 11.42, df = 4 (P = 0.02), I ² = 65.0% • • • •	Kang Han 2014	1.0437	0.085	10	1.6019	0.1131	20	31.9%	-5.18 [-6.78, -3.58]	-
Kang Yan 2012 1.1373 0.1742 6 1.7725 0.3381 6 32.4% -2.18 [-3.73, -0.63] Subtotal (95% CI) 26 36 100.0% -3.28 [-5.02, -1.55] Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); l ² = 76% Test for overall effect: $Z = 3.71$ (P = 0.0002) Test for subaroup differences: Chi ² = 11.42, df = 4 (P = 0.02), l ² = 65.0%	Kang Han 2015	1.006	0.229	10	1.585	0.198	10	35.6%	-2.59 [-3.84, -1.34]	+
Subtotal (95% Cl) 26 36 100.0% -3.28 [-5.02, -1.55] Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); I ² = 76% -3.28 [-5.02, -1.55] -10 0 10 20 Test for overall effect: Z = 3.71 (P = 0.0002) -10 0 10 20 -10 0 10 20 Test for subaroup differences: Chi ² = 11.42. df = 4 (P = 0.02). I ² = 65.0% Favours miRNA Favours control	Kang Yan 2012	1.1373	0.1742	6	1.7725	0.3381	6	32.4%	-2.18 [-3.73, -0.63]	
Heterogeneity: Tau ² = 1.79; Chi ² = 8.45, df = 2 (P = 0.01); l ² = 76% Test for overall effect: Z = 3.71 (P = 0.0002) Test for subgroup differences: Chi ² = 11.42, df = 4 (P = 0.02), l ² = 65.0%	Subtotal (95% CI)			26			36	100.0%	-3.28 [-5.02, -1.55]	◆
Test for overall effect: $Z = 3.71$ (P = 0.0002) -10 -20 -10	Heterogeneity: Tau ² = 1.79	; Chi² = 8.	45, df = 2	(P = 0	.01); I ² = 1	76%				
Test for subgroup differences: Chi ² = 11.42. df = 4 (P = 0.02). I ² = 65.0% Favours miRNA Favours control	Test for overall effect: Z = 3	.71 (P = 0	.0002)	·. ·	,, .					
Test for subgroup differences: Chi ² = 11.42. df = 4 (P = 0.02). I ² = 65.0% $1 + 1 + 1 + -20 - 10 = 0$ $-20 - 10 = 0$ $-20 - 10 = 0$ $10 = 20$ Favours miRNA Favours control Favours control			,							
-20 -10 0 10 20 Test for subaroup differences: Chi ² = 11.42, df = 4 (P = 0.02), I ² = 65.0%										
Test for subaroup differences: Chi#= 11.42, df = 4 (P = 0.02), I#= 65.0% Favours miRNA Favours control										-20 -10 0 10 20
	Test for subaroup difference	ces: Chi²=	= 11.42. 0	if = 4 (F	e = 0.02).	I ² = 65.0	%			Favours miRNA Favours control

6**B**

	m	NRNA		Control				Std. Mean Difference	Std. Mean Difference		
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% Cl		
1.8.1 Saos-2											
Xiuhui Wang 2014	0.753	0.064	6	0.349	0.03	6	29.3%	7.46 [3.65, 11.28]	_ ■		
Xuming Wang 2014	154.878	15.244	4	100	15.244	4	39.9%	3.13 [0.57, 5.69]			
Zhengyu Xu 2014	1.121	0.1203	6	0.4522	0.0289	6	30.8%	7.06 [3.43, 10.68]			
Subtotal (95% CI)			16			16	100.0%	5.61 [2.64, 8.58]	•		
Heterogeneity: Tau ² =	4.06; Chi2:	= 4.86, dt	f = 2 (P	= 0.09); I	²= 59%						
Test for overall effect:	Z= 3.70 (P	= 0.0002	2)								
									-20 -10 0 10		
									Equation PNA Equation of		

Figure 6: Meta-analysis of included studies evaluating the inhibitory effects on tumor weight after the aberrantly expressed miRNAs were corrected, when studies reported miRNAs as tumor suppressors **A.** or oncogenes **B.** and used tumor weight as the major outcome measure, were stratified by osteosarcoma cell lines used to produce osteosarcoma xenograft models . SD, standard deviation; CI, confidence interval.

significant difference when the decreased miRNAs were corrected (pooled MD = [-4.48]; 95% confidence interval [CI]: [-5.60]- [-3.36]; p = 0.001; Figure 8A, part 1) in a random-effects model. MiRNAs with vectors of plasmids were transfected into the OS cells in 6 studies, and were combined for a meta-analysis. There were 40 mice in the intervention arm and 57 in the control arm. The tumor volume significantly reduced when the decreased tumor suppressor miRNAs were corrected (pooled MD = [-4.01]; 95% confidence interval [CI]: [-5.89]- [-2.13]; p < 0.00001; Figure 8A, part 2)in a random-effects model [33, 35, 36, 60, 63, 64]. MiRNAs were infected into the OS cells by lentivirus vectors in 8 studies, and were combined for a meta-analysis. There were 53 mice in the intervention arm and 63 in the control arm. The tumor volume significantly decreased when the decreased tumor suppressor miRNAs were corrected (pooled MD = [-5.50]; 95% confidence interval [CI]: [-6.67]- [-4.32]; p = 0.21; Figure 8A, part 3)in a random-effects model [25, 37, 38, 42, 45, 53, 61, 62]. MiRNAs were injected into tumor directly in 3 studies, and were combined for a meta-analysis. There were 20 mice in the intervention arm and 26 in the control arm. The tumor volume significantly decreased when the aberrantly expressed miRNAs were corrected (pooled MD = [-5.40]; 95% confidence interval [CI]: [-8.80]- [-1.99]; p = 0.002; Figure 8A, part 4) in a random-effects model [39, 52, 58].

Only one study reported that miRNA was delivered

	miRNA Control Std. Mean Difference								Std. Mean Difference
Study or Subaroup	Mean	SD	Total	Mean	SD	Total	Weight	N. Random, 95% Cl	IV. Bandom, 95% Cl
2 1 1 Tumor sunnressor	Mean	50	Total	Medir	50	Total	TTOMIN	IV, Randolli, 55 / Cl	
Pagyong Sun 2015	59 667	14 667	0	210 667	22	0	2.2%	-9 57 1 1 2 52 -5 621	
Chi Chong 2014	220 7254	24 9705	0	470 7665	12 5222	0	2.2.70	-6.72[-10.10]-2.26]	
Eang li 2012 (a)	220.7234	24.0703	4	470.7505	43.3233	4	2.070	-0.72 [-10.19, -3.20]	-
Fang Ji 2013 (a)	0.09	0.0707	4	0.3004	0.0700	4	3.070	-2.50 [-4.71, -0.28]	-
Pang Ji 2013 (b)	0.0699	0.0554	4	0.2313	0.059	4	3.0%	-2.45 [-4.04, -0.27]	+
Guodong Li 2013	0.1558	0.0183	0	0.2602	46.260	12	3.9%	-4.22 [-0.00, -2.38]	
Guoding Duan 2015	284.71	40 5075		310.825	40.209		4.0%	-0.70[-1.79, 0.39]	
	3/3.1343	48.5075	5	813.4328	97.0149	5	2.1%	-5.19[-8.30, -2.01]	-
Hao Zhang 2010 (a)	0.04	0.0323	6	0.2892	0.0723	6	3.5%	-4.11 [-0.41, -1.81]	
Hao Zhang 2010 (b)	0.029	0.0243	6	0.2293	0.0579	6	3.5%	-4.16[-6.49, -1.84]	
Jie Gao 2012	110.3728	53.4846	5	226.094	59.8055	5	4.1%	-1.84 [-3.46, -0.23]	
Jie Jin 2013	584.0426	63.2405	5	1,449.6658	161.5369	10	3.2%	-5.87 [-8.53, -3.20]	
Jin Wang 2014	686.8421	94.7368	5	1,509.8684	159.8684	5	2.5%	-5.66 [-9.07, -2.24]	
Kang Han 2014	1,487.8049	195.122	10	2,560.9756	263.9395	20	4.3%	-4.28 [-5.67, -2.89]	-
Kang Han 2015	1,424.242	212.121	10	2,545.455	272.727	10	4.0%	-4.40 [-6.15, -2.64]	+
Kang Yan 2012	1,007.3382	110.2941	6	1,580.8676	102.9412	6	3.1%	-4.96 [-7.64, -2.29]	
Lei Chen 2013	269.906	32.9154	5	642.9467	57.0533	5	2.0%	-7.23 [-11.49, -2.98]	
Lei Fan 2013	1.1	0.2	8	1.6	0.3	8	4.5%	-1.85 [-3.08, -0.63]	-
Lei Song 2013	542.3744	82.0669	4	1,442.612	114.8936	4	1.4%	-7.84 [-13.41, -2.27]	
Liang Ge 2016	385.761	75.081	10	755.987	54.369	10	3.7%	-5.41 [-7.48, -3.34]	+
Masanori Kawano 2015	94.739	22.291	7	225.2355	20.0791	14	3.6%	-6.02 [-8.24, -3.81]	-
Meng Xu 2014	766.949	88.983	8	1,360.169	114.407	8	3.4%	-5.47 [-7.87, -3.08]	+
Tatsuya Iwasaki 2015	104.979	31.579	7	258.5315	19.4387	14	3.5%	-6.16 [-8.41, -3.90]	+
Wei Liu 2015	200	40.476	5	459.524	64.286	5	3.1%	-4.36 [-7.12, -1.61]	
Wei Wang 2015	0.718	0.102	6	1.426	0.141	6	3.0%	-5.31 [-8.14, -2.48]	
Xiaohui Sun 2015	1,356.522	513.043	6	2,695.652	373.913	6	4.0%	-2.75 [-4.51, -1.00]	+
Xiaoji Luo 2014	152.607	21.739	5	415.651	32.609	5	1.6%	-8.57 [-13.56, -3.59]	
Xinyu Wu 2013 (a)	755.0336	40.2685	6	1,147.651	76.2622	12	3.5%	-5.57 [-7.85, -3.29]	-
xinyu Wu 2013 (b)	785.2349	35.2349	6	1,218.1208	92.2791	12	3.6%	-5.22 [-7.38, -3.05]	-
Yong Zhao 2015	1,618.497	624.277	6	3,445.087	508.671	6	3.9%	-2.96 [-4.80, -1.13]	+
Yu He 2014	0.328	0.058	8	1.009	0.052	8	1.7%	-11.69 [-16.46, -6.92]	
Zhengwen Sun 2014	210.035	23,123	5	521.147	52,553	5	2.1%	-6.92 [-11.012.83]	
Subtotal (95% CI)			195			242	100.0%	-4.65 [-5.43, -3.88]	•
Heterogeneity: Tau ² = 3.13	: Chi ² = 106.6	9. df = 30 (f	P < 0.00	0001): I ² = 729	8				
Test for overall effect: Z = 1	1.74 (P < 0.00	0001)							
		,							
2.1.2 Oncogenesis									
K Tian 2015	2.019.231	384.615	10	1.557.692	274.316	20	41.2%	1.43 (0.58, 2.28)	
Xuming Wang 2014	1.211	0.073	4	0.766	0.078	4	27.7%	5 1 2 [1 34 8 91]	
Zhengyu Xu 2014	1 358 9041	142 4658	6	624 6575	71 2329	6	31.0%	6 0 2 [2 87, 9 17]	
Subtotal (95% CI)	. 1000.0041		20	027.0010		30	100.0%	3.88 [0.48, 7.27]	◆
Heterogeneity: $Tau^2 = 7.09$: Chi² = 10.52	df = 2 (P =	0.005	· I² = 81%					
Test for overall effect: $7 = 2$	24 (P = 0.03)		2.000/						
									-20 -10 0 10 20
Test for subgroup differen	ces: Chi ² = 23	.04. df = 1 (P < 0.0	0001), I ² = 95	.7%				Favours miRNA Favours control

Figure 7: Meta-analysis of studies evaluating the inhibitory effects on tumor volume a

Figure 7: Meta-analysis of studies evaluating the inhibitory effects on tumor volume after the aberrantly expressed miRNAs were corrected, when all included studies used tumor volume as the major outcome measure were stratified by the function of miRNAs in the pathogenesis of osteosarcoma. SD, standard deviation; CI, confidence interval.

by the tail vain [47].

As we could see in Figure 8A, the overall effects on reducing the tumor volume showed no significant difference among different miRNA delivery methods, with a slight better of miRNAs being infected with lentivirus vectors or injected into tumor directly, then being directly transfected into OS cells or transfected with plasmid vectors.

8A

There were 3 studies which reported that tumor volume as the major outcome measure and miRNAs as oncogenes. MiRNAs were transfected into OS cells with plasmid vectors in 2 studies, and the data were combined for a meta-analysis [49, 51]. There were 14 mice in the intervention arm and 24 in the control arm. Tumor volume was also significantly inhibited after correction of the oncogene miRNA expression (pooled MD = [2.80]; 95%

	m	IRNA		C	ontrol			Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% Cl
2.2.1 miRNAs were trans	fected into OS	s cells direc	:tly						
Chi Cheng 2014	220.7254	24.8705	6	478.7565	43.5233	6	5.9%	-6.72 [-10.19, -3.25]	
Fang Ji 2013 (a)	0.09	0.0707	4	0.3004	0.0755	4	9.0%	-2.50 [-4.71, -0.29]	
Fang Ji 2013 (b)	0.0699	0.0554	4	0.2313	0.059	4	9.0%	-2.45 [-4.64, -0.27]	
Hao Zhang 2010 (a)	0.04	0.0323	6	0.2892	0.0723	6	8.7%	-4.11 [-6.41, -1.81]	
Hao Zhang 2010 (b)	0.029	0.0243	6	0.2293	0.0579	6	8.6%	-4.16 [-6.49, -1.84]	
Lei Fan 2013	1.1	0.2	8	1.6	0.3	8	11.8%	-1.85 [-3.08, -0.63]	
Liang Ge 2016	385.761	75.081	10	755.987	54.369	10	9.4%	-5.41 [-7.48, -3.34]	
Masanori Kawano 2015	94.739	22.291	7	225.2355	20.0791	14	9.0%	-6.02 [-8.24, -3.81]	
Mena Xu 2014	766.949	88.983	8	1,360,169	114.407	8	8.5%	-5.47 [-7.87, -3.08]	
Tatsuva Iwasaki 2015	104.979	31.579	7	258.5315	19,4387	14	8.8%	-6.16 [-8.41, -3.90]	
Wei Liu 2015	200	40.476	5	459.524	64.286	5	7.5%	-4.36 [-7.12, -1.61]	
Xianii Lun 2014	152.607	21,739	5	415.651	32,609	5	3.7%	-8 57 [-13 56 -3 59]	_ -
Subtotal (95% CI)		2111-0-0	76		02.000	90	100.0%	-4.48 [-5.60, -3.36]	◆
Heterogeneity Tau ² = 2.3	7: Chi² = 31.05	df = 11 (P :	= 0.001	1): I ² = 65%					-
Test for overall effect: Z =	7.84 (P < 0.000	101)	0.00	.,,. = 00.0					
	1.04 (1 - 0.000	,01,7							
2.2.2 miRNAs were trans	fected into O	S cells with	plasn	nid vectors					
Gunging Duan 2015	284.71	17.91	7	310.825	46 269	7	17.0%	-0.70 (-1.79, 0.39)	-
Guoving Xu 2014	373 1343	48 5075	5	813 4328	97 0149	5	11.9%	-5 19 [-8 36 -2 01]	
lie Gan 2012	110 3728	53 4846	5	226 094	59,8055	5	15.9%	-1 84 [-3 46 -0 23]	
lie lln 2013	584.0426	63 2405	5	1 449 6659	161 5369	10	13.2%	-5.87 [-8.53 -3.20]	—
Kang Van 2012	1 007 2292	110 2041	6	1 590 9676	102.0412	6	12.2%	-4.96 [-7.64 -2.29]	
Vipuu Mu 2012 (a)	766.0226	40.2695	6	1 1 4 7 6 5 1	76 2622	12	14.2%	667 [7 96 2 20]	
vipyu Vitu 2013 (a)	705 2240	26 2240	6	1 210 1200	02 2701	12	14.2%	-5.22 [-7.85] -3.28]	
Subtotal (05% CI)	703.2345	33.2345	40	1,210.1200	32.2731	57	100.0%	4 04 [5 90 2 13]	•
Hotorogonoity Tou2 - 5.1	1. 0.68 - 25.02	df = 6 /D -	0 0000	243:12 - 0.200		37	100.074	-4.01[-3.03, -2.13]	•
Tect for overall effect: 7 -	1, 0 = 35.82, 1.000	, ui = 0 (F <	0.0000	51),1 = 05 %					
restion overall ellect. Z =	+.10 (P < 0.000	,,,							
2.2.3 miRNAs were infect	ted into OS cel	lls by lentivi	irus ve	ectors					
Baoyong Sun 2015	58 667	14 667	8	310 667	32	8	7.5%	-9 57 [-13 52 -5 63]	_ —
Jin Wang 2014	686 8421	94 7368	5	1 509 8684	159 8684	5	9.5%	-5.66 [-9.07 -2.24]	_ —
Kang Han 2014	1 497 9049	195 1 2 2	10	2 560 9756	263 0305	20	20.0%	-4 28 [-5 67 -2 89]	+
Kang Han 2014	1 424 242	212 121	10	2,500.5750	203.33333	10	23.0%	-4.20 [-5.07, -2.03]	+
Lei Chen 2013	269 906	22 0154	6	642 9467	57 0522	5	6.6%	-7 22 1 1 49 -2 99	
Lei Cong 2012	542 2744	02.0104	4	1 442 612	114 9026		4 1 96	-7.25[-11.45,-2.30]	
Wei Weng 2015	0 710	0 1 0 2	- -	1,442.012	0.1.41	- -	4.1 20	-7.04 [-13.41, -2.27]	
Zhongwon Qun 2014	210.025	22 1 22	6	521 1 4 2 0	62.662	6	7 1 96	-5.51 [-0.14, -2.40]	
Subtotal (05% CI)	210.035	23.125	- 63	521.147	52.555	63	100.0%	550[667 432]	•
Listeregeneity Tou2 - 0.2	1. ONR - 0.00	4f = 7 (D = 0	0.011	- 200		05	100.0%	-5.50[-0.07, -4.52]	•
Test for everall effect: 7 - 1	+; Chi= 9.68, i	ui = 7 (P = 0	1.21), 1-	= 28%					
Test for overall effect. $Z =$	a. 15 (P < 0.000	501)							
2.2.4 miRNAs were inject	ed into tumor	directly							
Guodona Li 2013	0 1660	0.0192	2	0.2602	0.0266	12	20.2%	-4 22 6 06 -2 201	-
Viaobui Que 2015	1 266 622	612.042	6	2 606 652	272 04 2	12	20.2%	-9.22 [0.00, -2.30]	
Alaonul Sun 2015	1,356.522	0.043	6	2,095.052	3/3.913	6	38.5%	-2.75 [-4.51, -1.00]	
10 He 2014	0.328	0.058	20	1.009	0.052	26	23.3%	- 11.09 [-10.40, -0.92] 5 40 [9 90 4 90]	-
Subtotal (05% CI)	0.068-11.00	46-0.05	20	18-020		20	100.0%	-5.40 [-6.60, -1.99]	•
Subtotal (95% CI)		. ur = 2 (P =	0.002)	1 = 83%					
Subtotal (95% CI) Heterogeneity: Tau ² = 7.0: Teet for overall effect: 7 = 7	2; Chir = 11.98,								
Subtotal (95% CI) Heterogeneity: Tau² = 7.0: Test for overall effect: Z = 3	3.11 (P = 0.002	2)							
Subtotal (95% Cl) Heterogeneity: Tau² = 7.0: Test for overall effect: Z = 1	3.11 (P = 0.002	2)							
Subtotal (95% Cl) Heterogeneity: Tau ² = 7.0: Test for overall effect: Z = 1	2, CHP = 11.98 3.11 (P = 0.002	2)							-20 -10 0 10



Std. Mean Difference Std. Mean Difference miRNA Control Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI IV, Random, 95% Cl 2.3.1 miRNA were transfected into OS cells with plasmid vectors K Tian 2015 63.0% 1.43 [0.58, 2.28] 2,019.231 384.615 10 1,557.692 274.316 20 Xuming Wang 2014 0.073 0.766 0.078 37.0% 5.12 [1.34, 8.91] 1.211 4 4 Subtotal (95% CI) 14 24 100.0% 2.80 [-0.70, 6.29] Heterogeneity: Tau² = 4.86; Chi² = 3.48, df = 1 (P = 0.06); l² = 71% Test for overall effect: Z = 1.57 (P = 0.12) -20 -10 10 20 ń Favours miRNA Favours control Test for subgroup differences: Not applicable

Figure 8: Meta-analysis of included studies evaluating the inhibitory effects on tumor volume after the aberrantly expressed miRNAs were corrected, when studies reported miRNAs as tumor suppressors **A.** or oncogenes **B.** and used tumor volume as the major outcome measure were stratified by the miRNA delivery method. SD, standard deviation; CI, confidence interval.

confidence interval [CI]: [-0.70]- [6.29]; p = 0.06; Figure 8B) in a random-effects model.

Only 1 study reported that miRNA was infected into the OS cells by lentivirus vectors as being described above [34].

The names of miRNAs

In order to find out if different miRNA has different influence on OS growth, data of same miRNA from more than 2 studies (if have), which reported that tumor volume was the major outcome measure, were combined together for a meta-analysis. This resulted 5 different miRNAs were analyzed, including 4 tumor suppressor miRNAs(miR-195, miR-143, miR-34a and miR-133) and 1oncogene(miR-214). There were 15 mice in the intervention arm and 15 in the control arm. Tumor volume significantly decreased when the expression of down-regulated miR-195 was recovered (pooled MD = [

-3.10]; 95% confidence interval [CI]: [-5.60]- [-0.59]; p = 0.04; Figure 9, part 1) [62, 64]. There were 12 mice in the intervention arm and 12 in the control arm. Tumor volume significantly decreased when the expression of down-regulated miR-143 was recovered (pooled MD = [-4.14]; 95% confidence interval [CI]: [-5.77]- [-2.50]; p= 0.97; Figure 9, part 2) [41] .There were 24 mice in the intervention arm and 36 in the control arm. Tumor volume significantly decreased when the expression of downregulated miR-34a was recovered (pooled MD = [-4.53]; 95% confidence interval [CI]: [-5.81]-[-3.24]; p = 0.25; Figure 9, part 3) [33, 36, 47]. There were 8 mice in the intervention arm and 8 in the control arm. Tumor volume there no significantly decreased when the miR-133a was recovered (pooled MD = [-2.48]; 95% confidence interval [CI]: [-4.03]- [-0.92]; p = 0.92; Figure 9, part 4) [40]. There were 10 mice in the intervention arm and 10 in the

	m	iRNA		C	ontrol			Std. Mean Difference	Std. Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% Cl
2.4.1 miR-195									
Jie Gao 2012	110.3728	53.4846	5	226.094	59.8055	5	50.9%	-1.84 [-3.46, -0.23]	-
Kang Han 2015	1,424.242	212.121	10	2,545.455	272.727	10	49.1%	-4.40 [-6.15, -2.64]	•
Subtotal (95% CI)			15			15	100.0%	-3.10 [-5.60, -0.59]	•
Heterogeneity: Tau ² = 2	2.52; Chi² = 4.	40, df = 1 (P	r = 0.04); I² = 77%					
Test for overall effect: Z	C= 2.43 (P = 0	.02)							
2.4.2 miR-143									
Hao Zhang 2010 (a)	0.04	0.0323	6	0.2892	0.0723	6	50.5%	-4.11 [-6.411.81]	-
Hao Zhang 2010 (b)	0.029	0.0243	6	0.2293	0.0579	6	49.5%	-4.16 [-6.491.84]	
Subtotal (95% CI)	0.010	0.02.00	12	0.2200	0.0010	12	100.0%	-4.14 [-5.772.50]	◆
Heterogeneity: Tau ² = (0.00; Chi ² = 0.1	00. df = 1 (P	= 0.97); ² = 0%					-
Test for overall effect: Z	(= 4.95 (P < 0	.00001)	0.01	,,, ,,,					
2.4.3 miR-34a									
Kang Yan 2012	1 007 3382	110 2941	6	1 580 8676	102 9412	6	18.5%	-4 96 [-7 64 -2 29]	
Xinvu Wu 2013 (a)	755 0336	40 2685	8	1 147 651	76 2622	12	23.7%	-5 57 [-7 85 -3 20]	
vinyu 18(u 2013 (b)	785 2349	35 2349	a a	1 218 1208	92 2791	12	25.6%	-5 22 [-7 38 -3 05]	-
Yong 7hao 2015 (b)	1 618 497	624 277	a a	3 4 4 5 0 8 7	508 671	6	32.0%	-2.96 [-4.90 -1.13]	-
Subtotal (95% CI)	1,010.437	024.277	24	5,445.007	500.071	36	100.0%	-4.53 [-5.81, -3.24]	•
Heterogeneity: Tau ² - (1.46: Chi≅ – 4	09 df - 3 (P	- 0.25)· IZ = 26%		00	1001074	-400 [-00 1, -0124]	
Test for overall effect: Z	(= 6.91 (P < 0	.000001)	- 0.23	7,1 - 20,0					
2.4.4 miD 422a									
2.4.4 milk-1338 Fang li 2012 (a)	0.00	0 0707	4	0.2004	0.0765	4	10.1%	-2.50 [.4.71 -0.20]	-
Fang Ji 2013 (a)	0.09	0.0707	4	0.3004	0.0755	4	49.470	-2.50 [-4.71, -0.28]	
Subtotal (05% CI)	0.0099	0.0554	4	0.2313	0.009	4	100.0%	-2.40 [-4.04, -0.27]	▲
Hotorogonoity Tou? - (0.00: 068-01	00 df = 1 /P	- 0.00	0.13 - 0.04		0	100.0%	-2.40 [-4.03, -0.32]	•
Tect for everall effect: 7	1.00, CHP = 0. (= 2.12/P = 0	00, ui = 1 (F	= 0.90	0,12 = 0.90					
restion overall effect. 2	. = 3.13 (F = 0	.002)							
2.4.5 miR-214									
Xuming Wang 2014	1.211	0.073	4	0.766	0.078	4	40.9%	5.12 [1.34, 8.91]	
Zhengyu Xu 2014	1,358.9041	142.4658	6	624.6575	71.2329	6	59.1%	6.02 [2.87, 9.17]	
Subtotal (95% CI)			10			10	100.0%	5.65 [3.23, 8.07]	
Heterogeneity: Tau ² = (0.00; Chi² = 0.	13, df = 1 (P	= 0.72); I ² = 0%					
Test for overall effect: Z	(= 4.58 (P < 0	.00001)							
									-20 -10 0 10 2
Test for subaroup diffe	rences: Chi² =	= 56.58. df =	4 (P <	0.00001). I ² =	92.9%				Favours mireina Favours con

Figure 9: Meta-analysis of included studies evaluating the inhibitory effects on tumor volume after the aberrantly expressed miRNAs were corrected, when all included studies used tumor volume as the major outcome measure were stratified by the names of miRNAs. SD, standard deviation; CI, confidence interval.

control arm. Tumor volume significantly decreased when the expression of up-regulated miR-214 was recovered (pooled MD = [5.65]; 95% confidence interval [CI]: [3.23]-[8.07]; p = 0.72; Figure 9, part 5) [34, 50].

As we could see in Figure 9, the effects on inhibiting tumor volume were most significant when the aberrantly

expressed miR-34a, miR-143 and miR-214 were corrected, and then followed by miR-195 and miR-133a

Inoculation sites of osteosarcoma cells

28 studies reported tumor volume as the major outcome measure and miRNAs as tumor suppressors, were divided into 2 subgroups according to the inoculation sites

	m	irna		C	ontrol			Std. Mean Difference	Std. Mean Differen
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% (
2.5.1 Subcutaneous inoc	culation								
Baoyong Sun 2015	58.667	14.667	8	310.667	32	8	2.7%	-9.57 [-13.52, -5.63]	
Chi Cheng 2014	220.7254	24.8705	6	478.7565	43.5233	6	3.1%	-6.72 [-10.19, -3.25]	
Fang Ji 2013 (a)	0.09	0.0707	4	0.3004	0.0755	4	4.2%	-2.50 [-4.71, -0.29]	
Fang Ji 2013 (b)	0.0699	0.0554	4	0.2313	0.059	4	4.2%	-2.45 [-4.64, -0.27]	
Guodong Li 2013	0.1558	0.0183	6	0.2602	0.0256	12	4.5%	-4.22 [-6.06, -2.38]	-
Guoging Duan 2015	284.71	17.91	7	310.825	46.269	7	5.2%	-0.70 [-1.79, 0.39]	-
Guoxing Xu 2014	373.1343	48.5075	5	813.4328	97.0149	5	3.3%	-5.19 [-8.36, -2.01]	
Hao Zhang 2010 (a)	0.04	0.0323	6	0.2892	0.0723	6	4.1%	-4.11 [-6.41, -1.81]	
Hao Zhang 2010 (b)	0.029	0.0243	6	0.2293	0.0579	6	4.1%	-4.16 [-6.49, -1.84]	
Jie Jln 2013	584.0426	63.2405	5	1.449.6658	161.5369	10	3.8%	-5.87 [-8.53, -3.20]	
Jin Wang 2014	686.8421	94,7368	5	1,509,8684	159.8684	5	3.1%	-5.66 [-9.07, -2.24]	
Lei Chen 2013	269,906	32,9154	5	642,9467	57.0533	5	2.5%	-7.23 [-11.492.98]	
Lei Fan 2013	1.1	0.2	8	1.6	0.3	8	5.1%	-1.85 [-3.08 -0.63]	-
Lei Song 2013	542 3744	82 0669	ă	1 442 612	114 8936	4	1.8%	-7 84 [-13 41 -2 27]	
Liang Ge 2016	385 761	75 081	10	755 987	54 369	10	4.3%	-5 41 [-7 48 -3 34]	—
Masanori Kawano 2015	94 739	22 291	7	225 2355	20 0791	14	4.2%	-6.02[-8.24]-3.81]	-
Meng Xu 2014	766 949	88 983		1 360 160	114 407	.4	4.2%	-5 47 (-7 87 -3 08)	
Tateuva Iwasaki 2015	104.979	31 579	7	258 5315	19 4387	14	4.0%	-616[-841 -390]	
Wei Liu 2015	200	40.476	5	459 524	64 286	5	3 7%	-4 36 [-7 17 -1 61]	_
Wei Wang 2015	0 719	0.102	a a	1 4 26	0 1 4 1	a a	3.6%	-5 31 [-9 14 -7 49]	_
Viaobui Que 2015	1 256 522	612 042	0 A	2 605 652	272 012	0 8	4 6%	-2.75 [-4.61 -1.00]	
Viacii Luo 2014	152 607	21 720	5	415 651	22 600	6	2.070	-2.75 [-4.51, -1.00]	
Xiaoji Edo 2014 Vievu VAu 2012 (a)	766.0226	40.2606	6	413.031	76 2622	12	2.170	-6.57 [-13.50, -3.59]	
vinyu Wu 2013 (a)	705 2240	40.2000	6	1 210 1200	02 2701	12	4.170	-5.37 [-7.63, -3.29]	
Xinyu Wu 2013 (b)	1 610 407	00.2049	6	2 4 4 5 0 9 7	92.2791	12	4.270	-0.22 [-7.30, -3.00]	
TURIQ ZRIAU 2015 Vu Lio 2014	1,018.497	024.277	0	3,445.087	0.052	0	4.3%	-2.90 [-4.60, -1.13]	
Turne 2014	0.320	0.000	°	521.147	63,663	° 2	2.270	-11.09[-10.40,-0.92]	
Subtotal (05% CI)	210.035	23.123	164	3Z1.147	52.553	204	2.0%	-0.92 [-11.01, -2.03]	•
Subtotal (95% CI)	0.00.00.45	46 - 00 (D	104	041-12-240		201	100.0%	-4.00 [-5.77, -5.90]	•
Test for everall effect: 7 =	10,011-= 99.45	, ui = 20 (P	< 0.000	101), 1 = 74%					
restion overall ellect. Z -	10.55 (F < 0.00	5001)							
2.5.2 Intratibial inoculation	on								
Jie Gao 2012	110.3728	53.4846	5	226.094	59.8055	5	27.4%	-1.84 [-3.46, -0.23]	
Kang Han 2014	1,487.8049	195.122	10	2,560.9756	263.9395	20	30.4%	-4.28 [-5.67, -2.89]	+
Kang Han 2015	1,424.242	212.121	10	2,545.455	272.727	10	25.7%	-4.40 [-6.15, -2.64]	
Kang Yan 2012	1,007,3382	110.2941	6	1,580.8676	102.9412	6	16.6%	-4.96 [-7.64, -2.29]	
Subtotal (95% CI)			31	.,		41	100.0%	-3.76 [-5.13, -2.38]	◆
Heterogeneity: Tau ² = 1.1	3; Chi ² = 7.21,	df = 3 (P = 0	0.07); l²	= 58%					
Test for overall effect: Z =	5.34 (P < 0.00	001)							
									-20 -10 0 10
To al face and second second									Favours miRNA Favours
Test for subaroup differen	nces: $Chi^2 = 1.7$	74. df = 1 (P	= 0.19	$I^2 = 42.4\%$. alouio mintra i tavouro



Study or Subgroup Mean SD Total Mean SD Total Weight W, Random, 95% Cl IV, Random, 95% Cl 2.6.1 Subcutaneous inoculation K Tian 2015 2,019.231 384.615 10 1,557.692 274.316 20 41.2% 1.43 [0.58, 2.28] IV IV <th></th> <th colspan="3">miRNA</th> <th>C</th> <th>ontrol</th> <th></th> <th></th> <th>Std. Mean Difference</th> <th>Std. Mean Difference</th>		miRNA			C	ontrol			Std. Mean Difference	Std. Mean Difference
2.6.1 Subcutaneous inoculation K Tian 2015 2,019.231 384.615 10 1,557.692 274.316 20 41.2% 1.43 [0.58, 2.28] Xuming Wang 2014 1.211 0.073 4 0.766 0.078 4 27.7% 5.12 [1.34, 8.91] Zhengyu Xu 2014 1,358.9041 142.4658 6 624.6575 71.2329 6 31.0% 6.02 [2.87, 9.17] Subtotal (95% Cl) 20 30 100.0% 3.88 [0.48, 7.27] Heterogeneity: Tau² = 7.09; Chi² = 10.52, df = 2 (P = 0.005); l² = 81% 75.81% 70.000 70.000 Test for overall effect: Z = 2.24 (P = 0.03) 0 0 0 0	Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% Cl
K Tian 2015 2,019.231 384.615 10 1,557.692 274.316 20 41.2% 1.43 [0.58, 2.28] Xuming Wang 2014 1.211 0.073 4 0.766 0.078 4 27.7% 5.12 [1.34, 8.91] Zhengyu Xu 2014 1,358.9041 142.4658 6 624.6575 71.2329 6 31.0% 6.02 [2.87, 9.17] Subtotal (95% Cl) 20 30 100.0% 3.88 [0.48, 7.27] Heterogeneity: Tau ² = 7.09; Ch ² = 10.52, df = 2 (P = 0.005); l ² = 81% Test for overall effect: $Z = 2.24$ (P = 0.03)	2.6.1 Subcutaneous i	inoculation								
Xuming Wang 2014 1.211 0.073 4 0.766 0.078 4 27.7% 5.12 [1.34, 8.91] Zhengyu Xu 2014 1.358.9041 142.4658 6 624.6575 71.2329 6 31.0% 6.02 [2.87, 9.17] Subtotal (95% Cl) 20 30 100.0% 3.88 [0.48, 7.27] Heterogeneity: Tau ² = 7.09; Chi ² = 10.52, df = 2 (P = 0.005); l ² = 81% 7.09; Chi ² = 10.52, df = 2 (P = 0.005); l ² = 81% Test for overall effect: Z = 2.24 (P = 0.03) -20.005); l ² = 81% -20.005); l ² = 81%	K Tian 2015	2,019.231	384.615	10	1,557.692	274.316	20	41.2%	1.43 [0.58, 2.28]	-
Zhengyu Xu 2014 1,358.9041 142.4658 6 624.6575 71.2329 6 31.0% 6.02 [2.87, 9.17] Subtotal (95% Cl) 20 30 100.0% 3.88 [0.48, 7.27] Heterogeneity: Tau ² = 7.09; Chi ² = 10.52, df = 2 (P = 0.005); I ² = 81% Test for overall effect: Z = 2.24 (P = 0.03)	Xuming Wang 2014	1.211	0.073	4	0.766	0.078	4	27.7%	5.12 [1.34, 8.91]	
Subtotal (95% Cl) 20 30 100.0% 3.88 [0.48, 7.27] Heterogeneity: Tau ² = 7.09; Chi ² = 10.52, df = 2 (P = 0.005); l ² = 81% Test for overall effect: Z = 2.24 (P = 0.03)	Zhengyu Xu 2014	1,358.9041	142.4658	6	624.6575	71.2329	6	31.0%	6.02 [2.87, 9.17]	
Heterogeneity: Tau ² = 7.09; Chi ² = 10.52, df = 2 (P = 0.005); l ² = 81% Test for overall effect: Z = 2.24 (P = 0.03)	Subtotal (95% CI)			20			30	100.0 %	3.88 [0.48, 7.27]	◆
Test for overall effect: Z = 2.24 (P = 0.03) -20 -10 0	Heterogeneity: Tau ² =	: 7.09; Chi ² = 1	0.52, df = 2	(P = 0.0)	005); I ² = 81°	%				
-20 -10 0	Test for overall effect:	Z = 2.24 (P = 0	0.03)							
-20 -10 0										
										-20 -10 0 10
E SYNTHY FUELDE E SYNTH										Eavoure miRNA Eavoure co

Figure 10: Meta-analysis of included studies evaluating the inhibitory effects on tumor volume after the aberrantly expressed miRNAs were corrected, when studies reported miRNAs as tumor suppressors **A.** or oncogenes **B.** and used tumor volume as the major outcome measure were stratified by injection sites of osteosarcoma cells. SD, standard deviation; CI, confidence interval.

of OS cells.

One subgroup included 24 studies that compared the anti-osteosarcoma effects in OS xenograft models produced by subcutaneous injection, with the rectification of the abnormally expressed miRNAs [25, 35-41, 44, 45, 47, 48, 52-61, 63, 65]. There were164 mice in the intervention arm and 201 in the control arm. The tumor volume was significantly suppressed by correcting the abnormally expressed miRNAs (pooled MD = [-4.86]; 95% confidence interval [CI]: [-5.77- [-3.96]; p < 0.00001; Figure 10A, upper part) in a random-effects model.

Another subgroup had 4 studies that compared the anti-osteosarcoma effects in OS xenograft models produced by intratibial injection, with the rectification of the abnormally expressed tumor suppressor miRNAs [33, 42, 62, 64]. There were 31 mice in the intervention arm and 41 mice in the control arm. The tumor volume significantly decreased by correcting the abnormally expressed miRNAs (pooled MD = [-3.76]; 95% confidence interval [CI]: [-5.13]- [-2.38]; p = 0.07; Figure 10A, lower part) in a random-effects model.

As shown in Figure 10A, the overall effects on inhibiting tumor volume were better when the OS xenograft models were produced by subcutaneous injection than by intratibial injection.

There were 3 studies reported tumor volume as the major outcome measure and miRNAs as oncogenes, which used OS xenograft models produced by subcutaneous inoculation of OS cells [34, 50, 51]. There were 20 mice in the intervention arm and 30 in the control arm. Given that the heterogeneity was high among the studies, a random-effects model was selected, and the tumor weight considerably decreased when the expressions of the oncogene miRNAs were corrected (pooled MD = [3.88]; 95% confidence interval [CI]: [0.48]- [7.27]; p = 0.005; Figure 10B).

Inoculated osteosarcoma cell lines

The delaminating analysis of data from studies reported tumor volume as the major outcome measure was performed, based on the 5 different OS cell lines for OS xenograft models in the included studies. Among studies reported miRNAs as tumor suppressors, data from the 11 studies [36, 38-41, 53, 54, 57-59, 65], which used MG-63 for OS xenograft model, were combined together for the meta-analysis, and there were 72 mice in the intervention arm and 91 mice in the control arms. Tumor volume significantly decreased by rescuing the downregulated miRNAs (pooled MD = [-5.46]; 95% confidence interval [CI]: [-7.03]- [-3.90]; p < 0.00001; Figure 11A, part 1) in a random-effects model; data from the 6 studies [40, 41, 44, 45, 52, 60], which used U2 OS for OS xenograft model, were combined together for the meta-analysis, and there were 34 mice in both the intervention and control arms. Tumor volume significantly decreased by rescuing the downregulated miRNAs (pooled MD = [-3.49]; 95% confidence interval [CI]: [-5.36]- [-1.62]; p = 0.0004; Figure 11A, part 2) in a random-effects model; data from the 4 studies [36, 55, 56, 63], which used Saos-2 for OS xenograft model, were combined together for the meta-analysis, and there were 23 mice in the intervention arm and 41 mice in the control arm. Tumor volume significantly decreased by rescuing the downregulated miRNAs (pooled MD = [-5.42]; 95% confidence interval [CI]: [-6.63]- [-4.22]; *p* = 0.80; Figure 11A, part 3) in a random-effects model; data from the 3 studies [37, 47, 61], which used 143B for OS xenograft model, were combined together for the meta-analysis, and there were 17 mice in both the intervention and control arms. Tumor volume significantly decreased by rescuing the downregulated miRNAs (pooled MD = [-4.23]; 95% confidence interval [CI]: [-6.05]-[-2.42]; p = 0.23; Figure 11A, part 4) in a random-effects model; data from the 3 studies [33, 42, 62], which used SOSP-9607 for OS xenograft model, were combined together for the meta-analysis, and there were 26 mice in the intervention arm and 36 mice in the control arm. Tumor volume significantly decreased by rescuing the downregulated miRNAs (pooled MD = [-4.42]; 95% confidence interval [CI]: [-5.42]- [-3.41]; p = 0.91; Figure 11A, part5) in a random-effects model.

As we could see in Figure 11A, the effects on reducing the tumor volume was best when the OS xenograft models were produced by injection of MG-63, then Saos-2, and followed by 143B, U2 OS or SOSP-9607.

There were 2 studies reported tumor volume as the major outcome measure and miRNAs as oncogenes, used Saos-2 for OS xenograft model, were combined together for the meta-analysis, and there were 10 mice in both the intervention and control arms [34, 50]. Tumor volume significantly decreased when the aberrantly expressed miRNAs were corrected (pooled MD = [5.65]; 95% confidence interval [CI]: [3.23]- [8.07]; p = 0.72; Figure 11B).

DISCUSSION

The functional contributions of miRNAs in the development and progression of malignancies have resulted in the development of new therapeutic approaches. Strategies include blocking the up-regulated oncogenic miRNAs using antisense oligonucleotides, or rescuing the downregulated cancer suppressor miRNAs by miRNA mimics [66]. MiRNAs also could be injected into the systemic circulation or introduced into the body (such as into a limb or the peritoneal cavity) or directly injected into a tumor mass [66]. On the other hand, the therapeutic agent to correct the miRNAs being abnormally regulated could be introduced into progenitor or stem cells that would be transplanted subsequently [67].

Several papers have reported that in the *in vivo* models, miRNAs could delay tumor formation and resulted in significantly smaller tumors when transfected into OS cells, compared with non-transfected cells.

11A

	m	IRNA		C	ontrol			Std. Mean Difference	Std. Mean Difference
Study or Subaroup	Mean	SD	Total	Mean	SD	Total	Weight	IV. Random. 95% Cl	IV. Random, 95% Cl
2.7.1 MG-63									
Baovong Sun 2015	58.667	14.667	8	310.667	32	8	7.2%	-9.57 [-13.52, -5.63]	_ —
Fang Ji 2013 (a)	0.09	0.0707	4	0.3004	0.0755	4	10.5%	-2.50 [-4.71, -0.29]	
Guodong Li 2013	0.1558	0.0183	6	0.2602	0.0256	12	11.3%	-4.22 [-6.062.38]	
Hao Zhang 2010 (a)	0.04	0.0323	6	0.2892	0.0723	6	10.4%	-4.11 [-6.41, -1.81]	
Lei Fan 2013	1.1	0.0020	8	1.6	0.3	8	12.3%	-1.85[-3.08]-0.63]	-
Lei Song 2013	542.3744	82.0669	4	1.442.612	114,8936	4	5.0%	-7.84 [-13.41, -2.27]	
Liang Ge 2016	385 761	75.081	10	755.987	54,369	10	10.8%	-5 41 [-7 48 -3 34]	-
Tatsuva Iwasaki 2015	104 979	31 579	7	258 5315	19 4387	14	10.5%	-6 16 [-8 41 -3 90]	
Xiaoji Luo 2014	152 607	21 739	. 5	415 651	32 609	5	5.7%	-8 57 [-13 56 -3 59]	
Xinyu Wu 2013 (a)	755 0336	40 2685	ñ	1 147 651	76 2622	12	10.4%	-5 57 [-7 85 -3 29]	- - -
Yu He 2014	0.328	0.058	8	1 009	0.052	.2	6.0%	-11 69 [-16 46 -6 92]	_ _
Subtotal (95% CI)	0.520	0.000	72	1.005	0.002	91	100.0%	-5.46 [.7.03 .3.90]	◆
Heterogeneity Tau ² – 4 76	Chi2 - 4310	df = 10 /P	< 0.000	01\·IZ - 77%			1001078	-0.40 [-1.00, -0.00]	•
Test for overall effect: Z = 8	6.85 (P < 0.000	, ui = 10 (i)01)	~ 0.000	01),1 = 77.0	,				
27211205									
Chi Chong 2014	220 7254	24 0705	e	470 7605	12 6222		12.0%	6 72 140 40 - 2 251	
Eang li 2012 /b)	220.7254	24.8705	0	4/8./005	43.5233	0	17.0%	-0.72[-10.19,-3.25]	
Fang Ji 2013 (b)	0.0699	17.04	4	0.2313	46.060	4	17.8%	-2.45 [-4.04, -0.27]	-
Guoding Duan 2015	284.71	17.91		310.825	40.209		21.8%	-0.70[-1.79, 0.39]	
Hao Zhang 2010 (b)	0.029	0.0243	6	0.2293	0.0579	6	17.3%	-4.10[-0.49, -1.84]	
Lei Chen 2013 Viesbui Run 2015	269.906	32.9154	5	642.9467	57.0533	5	10.6%	-7.23 [-11.49, -2.98]	_
Xiaonui Sun 2015	1,356.522	513.043	24	2,695.652	373.913	5	19.5%	-2.75 [-4.51, -1.00]	▲
Subiotal (95% CI)			34			34	100.0%	-3.49 [-3.30, -1.02]	•
Test for overall effect: Z = 3	3.65 (P = 0.000	, ai = 5 (P =)3)	0.0004	i); i= / 8%					
273 5200 2									
2.7.J 3008-2	504.0406	62.2405	-	4 440 6660	404 5000	10	20.20	5 07 1 0 52 2 201	_ _
Jie Jiri 2013 Meseneri Keurene 2015	064.0420	03.2405	5	1,449.0008	101.0309	10	20.3%	-5.87 [-8.53, -3.20]	
Masahon Kawano 2015	94.739	22.291		220.2300	20.0791	14	29.5%	-0.02 [-8.24, -3.81]	
view Wei 2015	200	40.470	c c	409.024	04.280	10	19.1%	-4.30[-7.12,-1.01]	-
Subtotal (05% CI)	785.2349	35.2349	23	1,218.1208	92.2791	12	31.0%	-0.22 [-7.38, -3.00]	
Hotorogonoity Touit = 0.00	- Chiz - 0.00	df = 2 /P = 0	2J 100\-JR	- 0%		41	100.0%	-3.42 [-0.03, -4.22]	•
Test for overall effect: Z = 8	3.83 (P < 0.00)	ui – 3 (F – 0 001)	1.00), 1	- 0 %					
0.7.4.400		-							
2.7.4 143B	696 9421	94 7369	6	1 509 8694	159 8694	6	21 0%	-5 66 19 07 -2 241	
Mei Wang 2014	000.0421	0 102	8	1 426	0 1 / 1	6	20.0%	-5.31 [.9.142.24]	
Vong 7hog 2015	1 619 407	624 277	0	3 4 4 5 0 9 7	609.671	0	10 1 04	-3.31 [-0.14, -2.40]	-
Subtotal (95% Cl)	1,010.497	024.277	17	3,443.007	300.071	17	45.1%	-2.30 [*4.00, *1.13]	▲
Heterogeneity: Tou ² = 0.06	Chi ² = 2.96	df = 2 (P = 0	1 2 2 V IZ	= 37%		.,	.00.070	-420 [-0003, -2:42]	•
Test for overall effect: Z = 4	4.58 (P < 0.000	001)	.20),1	- 52.10					
275 5050 0607									
2.7.3 303P-9007	4 407 00 40	105 100	4.0	0.500.0350	000.0005		50.00	1001007 000	_
Kang Han 2014	1,487.8049	195.122	10	2,560.9756	263.9395	20	52.8%	-4.28 [-5.67, -2.89]	_
Kang Han 2015	1,424.242	212.121	10	2,545.455	272.727	10	33.0%	-4.40 [-6.15, -2.64]	
Kang Yan 2012 Subtotal (95% CI)	1,007.3382	110.2941	6 26	1,580.8676	102.9412	6 36	14.2% 100.0%	-4.96 [-7.64, -2.29] -4.42 [-5.42, -3.41]	•
Heterogeneity: Tau ² = 0.00); Chi² = 0.20.	df = 2 (P = 0).91); I ^z	= 0%					
Test for overall effect: Z = 8	8.58 (P < 0.000	001)							
									-20 -10 0 10 20
									Favours miRNA Favours control

Test for subaroup differences: $Chi^2 = 4.48$. df = 4 (P = 0.34). $I^2 = 10.7\%$

11B

	miRNA Control							Std. Mean Difference	Std. Mean Difference		
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% Cl	IV, Random, 95% Cl		
2.8.1 Saos-2											
Xuming Wang 2014	1.211	0.073	4	0.766	0.078	4	40.9%	5.12 [1.34, 8.91]	 − ∎ −		
Zhengyu Xu 2014	1,358.9041	142.4658	6	624.6575	71.2329	6	59.1%	6.02 [2.87, 9.17]	-∎ -		
Subtotal (95% CI)			10			10	100.0%	5.65 [3.23, 8.07]	•		
Heterogeneity: Tau ² =	0.00; Chi ² = 0	13, df = 1 (F	P = 0.73	2); I ² = 0%							
Test for overall effect:	Z = 4.58 (P < 0	0.00001)									
									-20 -10 0 10 20		
	Eavours miRNA Eavours control										
Test for subaroup diff	erences: Not a	eldesilaa									

Figure 11: Meta-analysis of included studies evaluating the inhibitory effects on tumor volume after the aberrantly expressed miRNAs were corrected, when studies reported miRNAs as tumor suppressors **A.** or oncogenes **B.** and used tumor volume as the major outcome measure were stratified by osteosarcoma cell lines used to produce OS xenograft models. SD, standard deviation; CI, confidence interval.

As well, systemic injection of miRNA/atelocollagen complexes could avoid spontaneous lung metastases in OS [17, 32-45, 63-65]. These results suggest the potential for miRNAs to be used as therapeutic targets for OS.

Since the understanding of the function of miRNAs in OS remains inadequate, we don't know if miRNAs could be directly used for the treatment of patients with OS.

In researches aimed at improving human health care, animal studies still play a crucial role in creating hypotheses that sheds light on the test in preventative or therapeutic clinical trials of new potential interventions. The underlying principle for use of animal studies is to minimize the risks to patients, since only interventions estimated prospectively safe and effective are eventually moved into clinical trials [68, 69].

However, the results usually vary from one study to the next, the conclusions and interpretation are not always straightforward, also no single study is executed flawlessly in all steps, the decisions about effectiveness of an intervention or validity of a hypothesis cannot be based on the results of a single animal study. Therefore, a mechanism is required to pool together the data across studies [68, 70-73].

This is the first meta-analysis to summarize the preclinical data and evaluate the potential value of miRNAs as therapeutic targets for OS. We carried out a systematic literature search that included both English and Chinese databases to make sure the comprehensiveness of the studies that were assessed. Two reviewers separately reviewed the studies, evaluated methodological quality, and extracted the data to evade the bias. This is not a comprehensive list of all therapies that has ever been tried in pre-clinical models of OS, but rather, a systematic review and meta-analysis of specific therapies that are being considered for human translation.

However, the reliability of experimental conclusions depends on the high quality experimental design, analysis and reporting. Bias occurs in the results of an animal study or the conclusions drawn from it when a systematic error exists. There are a large number of potential sources of bias, and the risks of selection bias and measurement bias, which are the most important bias, may be diminished through simple study design features, such as randomisation and blinded assessment of outcomes [70, 74]. Unfortunately, previous research has recognized a low popularity of reporting measures to reduce the risk of bias for specific animal disease models [75-80]. Failure to depict the research methods and report data properly consequently has potential scientific, ethical, and economic meanings for the entire research procedure and the reputation of those involved in it. This is particularly right for animal research. The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines were developed to promote high-quality, comprehensive reporting to allow an precise critical review of what was done and found in the animal researches, which includes a checklist of 20 items describing the minimum information that all scientific publications reporting research using animals should include, for example the number and specific animal characteristics (species, strain, sex, and genetic background); detail information of housing and husbandry; and methods of the experiments, statistics and analyses (including detail methods used to reduce the bias such as randomization and blinding)."

The limitations of the included original studies were also shared in this study as any other meta-analysis. Though we searched both English and Chinese databases, we cannot confirm that all the related studies have been found. Moreover, other important reasons of bias that must be considered are discriminating reporting and publishing [81], since positive data are more likely to be published, the estimations may be overstated due to the evidence for publication bias. Meanwhile, papers published in other languages beyond English and Chinese had not been included in this manuscript due to the language barrier.

We retrieved 36 studies which met the inclusion criteria in this paper, and methodological quality of these included papers was assessed with a component method like that recommended by The Cochrane Collaboration in assessing risk of bias [82]. As we could see in Table 2, all studies reported details on the experimental procedures and animal numbers and strains used were reported except which was not clear in 1 study. Animal ages were reported in most of the studies and animal genders were reported in almost half of the studies. 23 publications described the reporting potential conflicts of interest and study funding. While only six studies reported randomization, one study reported inclusion/exclusion criteria and blinded assessment of outcome; no included studies described allocation concealment, sample-size calculation and reporting of animals excluded from analysis. The absence of above information could be caused by the real flaws in the experimental design or reporting omissions. Therefore, efforts should be made in the future to improve bioscience research design and reporting, such as using the ARRIVE guidelines.

Heterogeneity is acceptable in a meta-analysis, it would be surprising if many studies were completed by different groups in different places with different methods, all of them ended up by measuring the same fundamental parameters. Furthermore, animal studies are usually small (with a sample size of about 10 in each group). Therefore, the challenge is to decide on the most fitting approach to evaluate heterogeneous studies. When heterogeneity cannot be ignored, one analytical technique is to integrate the data into a random-effects model, which involves a hypothesis that the effects being estimated in diverse studies are not equal, however follow some distribution [83, 84]". And more studies on a single micRNA intervention for OS are necessary in the future.

As the data of our meta-analyses were highly

heterogeneous in terms of different cell lines and injection sites of OS cells used for generating animal models, name and function of miRNAs in the pathogenesis of OS, and different vectors were used for microRNA delivery, delaminating analyses were performed based on each of these heterogeneities to allow for evaluation of the distribution of true effects.

By the delaminating analyses based on the factors above, we further explored the effects on reducing the tumor growth, by alteration of the aberrantly expressed miRNAs. Once tumor weight and tumor volume both were taken into account, our results demonstrated that the anti-osteosarcoma effects were the best when miRNAs were infected into OS cells with lentivirus vectors, the up-regulated oncogene miR-214 was corrected or OS xenograft models were produced by subcutaneous injection. However, the inhibitory effect on tumor growth was proved to be the most poor when the OS xenograft models were produced by injection of SOSP-9607 cells. These data indicated that the therapeutic effects, by correcting the aberrantly expressed miRNAs, on OS were closely associated with the route of miRNAs being interfered, which specific miRNA being involved and the original location of the OS, and also the specific pathological type of OS. This implies intervention effect of different miRNA may possess specificity in different pathological or different original OS. So as this work provides a theoretical basis for the future individualized treatment endeavor. Nevertheless, further studies on the inherent correlation between specific miRNA and OS pathological type are necessary.

The results of this meta-analysis suggest that miRNAs are potential therapeutic targets for OS. Our results illustrated 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. Moreover, more animal studies with the rigorous design must be carried out, wide-ranging preclinical safety and toxicity studies would be needed before a miRNAbased treatment could be translated from animal studies to human use.

MATERIALS AND METHODS

Literature search strategy and selection criteria

We systematically searched 7 databases including PubMed, Web of Science, Embase, Wan Fang Database, China Knowledge Resource Integrated Database, VIP Database, and Chinese BioMedical since their initiation date to May 10, 2016, without restrictions of the languages, publication status or publication dates. The search strategy included the following terms: (MicroRNA OR miRNA) AND osteosarcoma AND (mice OR mouse).

Two reviewers (J.L.C. and Y.M.L.) independently

selected the literatures by reviewing the titles, abstracts and full texts according to the eligibility criteria. Disagreements were determined by agreement with a third author (Y.P.Y.). Only studies satisfied the criteria were included in the meta-analysis.

Eligibility criteria

Types of studies

Controlled studies that estimate the therapeutic effects on OS in mouse models by correcting the abnormally expressed miRNAs were searched. All studies only having *in vitro* research data and clinical case reporters were excluded.

Types of participants

Any gender, any age, or any strain of laboratory mice inoculated with OS cells *via* subcutaneous or intratibial injection.

Types of intervention

Any method for correcting the altered miRNA expression of OS in mouse models was included.

Type of outcome measure

Xenograft models, derived by inoculation of human cancer cells including ectopic xenograft and orthotopic xenograft according to the transplant site, play critical roles in screening new anticancer agents, evaluation the therapeutic efficacy and toxicity. Standard animal models could save money and time, and afford evidence to support clinical trials for anticancer reagents discovery [85]. Tumor volume and tumor weight are indicators used for assessing the anticancer efficacy of anticancer reagents in cancer xenograft models. Xenograft models included in this meta-analysis were produced by subcutaneous or intratibial inoculation of OS cells.

Tumor volume

Tumor volume was calculated according to the digital vernier caliper measurements using the following formula: $0.5 \times a \times b$, where a is the largest dimension and b is the square of perpendicular diameter [86].

Tumor weight

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

Data extraction

The details were extracted from the included studies independently by two authors (J.L.C. and Y.M.L.) in this meta-analysis, which included first author name, publication year, information of mice (strain, age and gender), number of mice in each group, method used to produce OS mouse model, how the abnormally expressed miRNA was corrected, and the primary measured outcomes. Data were collected by mean outcome and standard deviation (SD) for each comparison. All data showed by graphs only without numbers were estimated by GetData Graph Digitizer 2.24. A third reviewer (Y.P.Y.) determined any disagreements between the two reviewers.

Evaluation of methodological quality in the individual study

There are no established dependable and valid tools for the judgment of the methodological quality in animal studies. STAIR (the initial Stroke Therapy Academic Industry Roundtable) was used to evaluate the reporting and design quality of the included studies, which 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 [87], and was updated by the STAIR group in 2009 based on the Recommendations for Ensuring Good Scientific Inquiry for America. Two authors (Q.S. and Y.J.W.) assessed the methodological qualities in all included studies and presented as a yes or no. The "unclear" signified that the methodological quality was not clear.

Statistical analysis

Data were pooled together for analysis if outcomes were reported by 2 or more studies. Two primary outcomes (tumor volume and tumor weight) were analyzed individually. We conducted pair-wise meta-analysis for studies, which directly compared the influence on tumor growth between corrected miRNAs expression and the control (abnormally expressed miRNAs),to determine the pooled relative effect of each intervention compared with the other effect for each measurement outcome of interest, and the mean differences (MDs) of the post-intervention values from the different interventions were determined. We adopted the post-intervention values in meta-analysis derived from the baseline values being comparable between target miRNA group and mimic miRNA or placebo control group, as specified by a Cochrane review [88].

Final consequences from the studies to evaluate differences between the intervention and control group were analyzed by the REVIEW MANAGER 5.1.2 software offered by the Cochrane Collaboration, and I² was calculated to evaluate the heterogeneity. Heterogeneity was existed if the *p* value was less than 0.10 by the chi-square (x^2) test. If the I² value was greater than 50%, the results were thought to have a high level of heterogeneity

[88]. Clinically and statistically homogeneous studies should be pooled using the fixed-effects model [88]. Clinically homogeneous and statistically heterogeneous studies should be pooled using the random-effects model [88]. When same outcomes were measured using different instruments across studies, we used a standardized mean difference (SMD) in the meta-analysis to combine continuous data.

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CONFLICT OF INTEREST

No conflict of interest was declared.

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