Antitumor activity of fucoidan: a systematic review and meta-analysis

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Objective: To systematically review the antitumor activity of fucoidan based on the results of animal experimental studies.

Methods: The databases of the Cochrane Library, PubMed, Ovid MEDLINE, Web of Science, Embase, China National Knowledge Infrastructure (CNKI), Sino Med, Wanfang, and Chinese Science and Technology Periodicals (CQVIP) were searched for randomized and controlled animal experiments on the antitumor activity of fucoidan. The search included studies published up to 31 December 2020, and there was no limit to the start date. Endnote X9 software was employed to manage and screen the literature, Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) was used for assessment of risk of bias, and RevMan 5.3 software was used for meta-analysis.

Results: A total of 23 articles were included in the study. The results showed that compared with the control group, the fucoidan intervention group had significantly inhibited tumor weight, volume, and number. The combined effect values were mean difference (MD) =−0.94, 95% confidence interval (CI): −1.10 to −0.79; MD =−0.78, 95% CI: −1.06 to −0.50; and standardized mean difference (SMD) =−3.27, 95% CI: −4.30 to −2.23, respectively. The results of subgroup analysis showed that low-dose and intragastric administration of fucoidan had the best effect on breast cancer in controlling tumor weight, low-dose and intraperitoneal injection had the best effect on multiple myeloma in controlling tumor volume, and high-dose and intraperitoneal injection of fucoidan had the best effect on melanoma in controlling the number of tumors.

Conclusions: The existing evidence shows that fucoidan inhibits the growth and spontaneous metastasis of tumors in numerous animal models. The tumor type, dosage, and administration method have been shown to influence the effect of fucoidan, and thus its mechanism warrants further research. As the design quality of the included studies was not high, heterogeneity and bias may have affected the accuracy of the results.

Keywords: Fucoidan; animal tumor model; inhibition of tumor; meta-analysis

Introduction

Cancer is the one of the leading causes of death in developing countries, second only to cardiovascular and cerebrovascular diseases (1). Cancer prevention and treatment have become the focus of researchers all over the world. At present, chemotherapy and radiotherapy are the most common cancer treatments; however, their adverse side effects should not be underestimated (2,3). Cumulative researches of novel cancer treatments have shown that natural polysaccharide plays an important antitumor role due to its remarkable results, low toxicity, and minimal side effects (4). As a result, natural compounds with antitumor effects isolated from marine resources have been attracting considerable attention from the healthcare and pharmaceutical industries (5).

Fucoidan, also known as sulfated fucans, fucoidin, and fucan sulfate, was first extracted from palmate kelp by Kylin in 1913 (6). Fucoidan is a macromolecular polysaccharide rich in sulfate groups widely found in brown algae, such as *Fucus vesiculosus*, kelp, and *Sargassum* (5). Due to its range of biological effects, including antitumor, antioxidative, antiviral, anti-inflammatory, anticoagulation, and immunoregulatory activities (7), fucoidan has been considered to have great clinical potential.

To date, many animal experiments (8-30) and *in vitro* experiments (31-33) have been conducted in exploration of the antitumor effect of fucoidan. Tumor types have included colorectal cancer (8,12,16), breast cancer (9,14,15,23,28), prostate cancer (10,18), ovarian cancer (11), lung cancer (13,19-21,24,27), liver cancer (17,22), melanoma (21,25), multiple myeloma (26), and sarcoma (29,30). Among the animal experiments, we found that there were differences in the design, intervention protocols, and results. To the best of our knowledge, there has been only 1 clinical trial of the antitumor effect of fucoidan involving human participants. Hsiang *et al.* were the first to conduct a clinical trial of low molecular weight fucoidan as a first-line treatment for patients with metastatic colorectal cancer (34). In their study, the disease control rate was significantly improved by low-molecular weight fucoidan combined with chemotherapy plus target agents.

There are 3 main anticancer mechanisms of fucoidan. First, fucoidan can induce apoptosis, affect the normal mitosis of cancer cells, and inhibit cancer cell proliferation by regulating the growth cycle of cancer cells. Second, fucoidan can inhibit the formation of vascular endothelial growth factor and tumor angiogenesis, cut off the source of nutrient supply to the tumor, and starve the tumor so as to block the diffusion and metastasis of cancer cells. Third, fucoidan can activate the immune system and enhance the ability of natural killer (NK) cells and T cells to kill tumor cells (35).

By conducting a review of the existing evidence for the antitumor activities of fucoidan, this study aimed to reduce the risk of transposing animal experiment results to clinical practice, enhance the guiding value of animal experiments to clinical research, reduce repetitive and excessive animal experiments, and facilitate implementation of the principle of "reduction, substitution, and optimization". Based on the results of animal experiments, we systematically reviewed the antitumor activity of fucoidan so as to provide comprehensive guidance for clinical trials. To the best of our knowledge, no systematic review on the antitumor activity of fucoidan has been reported previously.

We present the following article in accordance with the PRISMA reporting checklist (available at https://dx.doi.org/10.21037/tcr-21-1733).

Methods

Inclusion criteria

(I)   Type of study: randomized and controlled trials involving animals.
(II) Subjects: cancer model mice, without limitation to the specific disease model or modelling methods.
(III) Intervention and control measures: intervention measure was fucoidan alone, excluding fucoidan combined with other drugs, the control group was treated with either physiological saline or phosphate-buffered saline (PBS).
(IV) Outcome indicators: tumor weight, tumor volume, and tumor number.
(V)   Literature language: language was restricted to articles published in either the Chinese or English language.

Exclusion criteria

Duplicate publication or research with the same data; reviews, conference abstracts, letters for articles, and editorials; and literature with incorrect data.

Retrieval strategy

The databases of the Cochrane Library, PubMed, Ovid MEDLINE, Web of Science, Embase, China National

Knowledge Infrastructure (CNKI), Sino Med, Wanfang Database, and Chinese Science and Technology Periodicals (CQVIP) were searched for studies on the antitumor effects of fucoidan. The search time was from the establishment of each database to 31 December 2020. In addition, applicable references from selected studies were also included. The search included combinations of medical subject heading (MeSH) terms and key words. Search terms included Fucoidan, sulfated fucans, Fucoidin, Fucan sulfate, Mekabu fucoidan, fucan sulfate Hor-1, Neoplasms, Tumor, Cancer, Malignancy, Mice, Mus, Mouse, Rats, Rat, Rattus, Animal Experimentation, Animal Research, and Animal Experiment.

Specific retrieval strategy (taken from the PubMed database as an example):

(I)   "fucoidan" [Supplementary Concept];
(II)   sulfated fucans OR fucan sulfate OR fucoidin OR Mekabu fucoidan OR meFucoidan OR fucan sulfate Hor-1;
(III)   #1 OR #2;
(IV)   "Neoplasms"[Mesh];
(V)   Neoplasia OR Neoplasias OR Neoplasm OR Tumors OR Tumor OR Cancer OR Cancers OR Malignancy OR Malignancies OR Malignant Neoplasms OR Malignant Neoplasm OR Benign Neoplasms OR Benign Neoplasm;
(VI)   #4 OR #5;
(VII)   "Mice"[Mesh];
(VIII) Mus OR Mouse OR Rats OR Rat OR Rattus OR Animal Experimentation OR Animal Research OR Animal Experiment;
(IX)   #7 OR #8;
(X)   #3 AND #6 AND #9 (Filters: from 1981–2020).

Literature screening and data extraction

The software Endnote X9 (Clarivate Analytics, London, UK) was used by 2 trained reviewers to screen the literature strictly according to the inclusion and exclusion criteria. First, on the basis of reading the titles and abstracts of the literature, any article that obviously did not meet the inclusion criteria was eliminated. The full text of the remaining literature was then screened again according to the inclusion and exclusion criteria. If any differences arose between the 2 reviewers, a resolution was sought through discussion and analysis. If a consensus was not reached, a third reviewer was consulted before a decision was made regarding inclusion.

The data extracted from the included studies included the following: (I) title, first author, publication year, and author nationality; (II) animal species, weight, age, sample size, and cancer type; (III) fucoidan source, extraction method, intervention components, purity and dosage, administration method, intervention time, and intervention measures of control group; and (IV) tumor weight, tumor volume, and tumor number.

Bias risk assessment

The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool (36) was used to evaluate the included literature. This tool lists 10 items concerning different forms of bias, including selectivity bias, implementation bias, measurement bias, loss of follow-up bias, reporting bias, and others (36).

Statistical analysis

In accordance with the Cochrane *Handbook* for *Systematic Reviews* of *Interventions* (37), the data (mean ± standard deviation) of multiple intervention groups were merged, and then the results were statistically analyzed using RevMan 5.3 software (Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2014). Heterogeneity analysis was carried out on the included literatures. If there was no obvious heterogeneity among the included studies ($I^2 \leq 50\%$, $P \geq 0.10$), a fixed effect model (FEM) was used for the meta-analysis. If there was heterogeneity between studies ($I^2 > 50\%$, $P < 0.10$), the random effect model (REM) was used for meta-analysis. Subgroup and sensitivity analyses were used to explore the potential sources of heterogeneity and evaluate the reliability of the results. Mean difference (MD) and 95% confidence interval (CI) were calculated for the continuous data. Standardized mean difference (SMD) and 95% CI were used to calculate the results with different measurement methods, different measurement units or large differences. When at least 10 studies were included, a funnel chart was used to evaluate publication bias.

Subgroup analysis was proposed for the following: (I) different dosages of fucoidan (low, medium, and high); (II) different administration methods of fucoidan (intraperitoneal injection, tail vein injection, intragastric administration, and oral administration); (III) different cancer types (lung cancer, liver cancer, breast cancer, melanoma, sarcoma, colorectal cancer, multiple myeloma, prostate cancer, and ovarian cancer); and (IV) different

mouse types (nude mice and non-nude mice).

Results

Literature retrieval results

A total of 1,187 papers were initially retrieved. Using Endnote X9 software and manual duplicate checking, 600 duplicate documents were eliminated. According to the inclusion and exclusion criteria, a further 564 studies were excluded after reading the title, abstract, and full text of the remaining literature. Finally, 11 English (8-18) and 12 Chinese (19-30) language studies were included. Among the 23 papers, there were 2 dissertations (21,30), and each dissertation involved 2 independent studies. After completion of the screening process, a total of 23 articles involving 25 studies were included in the meta-analysis. The literature screening process is shown in *Figure 1*.

Basic characteristics of the included studies

The basic characteristics of the included studies are shown in *Table 1*. Among them, 3 studies used BALB/c mice (9,15,28), 7 studies used nude mice (8,10,11,13,16-18), 3 studies used Sprague-Dawley (SD) rats (12,14,23), 7 studies used C57BL/6J mice (19-21,24,25,27), 1 used Institute of Cancer Research (ICR) mice (22), 1 used nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice (26), and 3 used Kunming mice (29,30). A total of 9 studies used males (8,10-12,16,18,22,27,29), 12 used females (9,14,15,19,20,23-26,28,30), and 4 studies did not specify the gender of the mice (13,17,21). Regarding the cancer models in each of the 25 studies, 3 were colorectal cancer (8,12,16), 5 were breast cancer (9,14,15,23,28), 2 were prostate cancer (10,18), 1 was ovarian cancer (11), 6 were lung cancer (13,19-21,24,27), 2 were liver cancer (17,22), 2 were melanoma (21,25), 1 was multiple myeloma (26), and 3 were sarcoma (29,30). Fucoidan sources included seaweed, vesicular brown algae, brown seaweed *Sargassum fusiforme*, American sea cucumber, sea melon, Icelandic sea cucumber, Arctic sea cucumber, glycoside moss, *undaria* spore leaf, and kelp. In 1 study, the intervention group was further specified as fucoidan FP08S2 (13), 3 were sea cucumber fucoidan sulfate (SC-FUC) (19,24,25), 1 was fucoidan from *Sargassum* SP (29), and 1 was low-molecular weight fucoidan (LMWF) (30). There were 6 studies with only 1 intervention group (8,10-12,17,20), 14 with 2 intervention groups (9,13-16,18,19,21,23-

26,28), and 5 with 3 intervention groups (22,27,29,30). A total of 16 studies (8,9,15,16,18-21,24-30) administered intraperitoneal fucoidan injection, 6 studies (10-12,14,22,23) used intragastric administration, 2 studies (17,30) involved oral administration, and 1 study (13) used caudal vein injection. There were 23 studies (8-17,19-25,27-30) with physiological saline in the control group and 2 studies (18,26) with phosphate buffered saline (PBS). The outcome indexes of each study varied. Tumor weight was an outcome index in 22 studies (9-15,17-27,29,30), tumor volume in 11 studies (8-11,13,15-18,26,28), and tumor number in 7 studies (12,19-21,24,25).

Results of bias risk assessment

The results of bias risk assessment are shown in *Table 2*. All 25 controlled trials were randomly grouped, but it was unclear which specific randomization methods were used and whether appropriate methods were adopted to achieve the unpredictability of random sequences. None of the 23 articles mentioned whether covert grouping was implemented, and the information provided was insufficient to determine whether breeders and researchers were blinded. The animals were randomly placed in 11 studies (8-12,14,15,17,18,26,30) and evaluated as "–". There was no experimental report on random selection of animals for result evaluation, and so the risk of bias was assessed as "+". None of the included studies blinded the evaluators of the results, however, this did not affect the determination of the indicators of the results (the outcome was an objective index), thus was evaluated as "–". The data of 17 studies (8-11,13,17-22,24-29) were reported completely.

Meta-analysis

A total of 22 studies (9-15,17-27,29,30) reported tumor weight. Meta-analysis showed that $I^2=98\%$ among the studies, and a REM was used for combined analysis. Compared with the control group, fucoidan inhibited tumor weight (MD =–0.94, 95% CI: –1.10 to –0.79, P<0.05), and the difference was statistically significant.

A total of 11 studies (8-11,13,15-18,26,28) reported tumor volume. Meta-analysis showed that $I^2=99\%$ among these studies, and REM was used for combined analysis. Compared with the control group, fucoidan inhibited tumor volume (MD =–0.78, 95% CI: –1.06 to –0.50, P<0.05), and the difference was statistically significant.

A total of 7 studies (12,19-21,24,25) reported the number
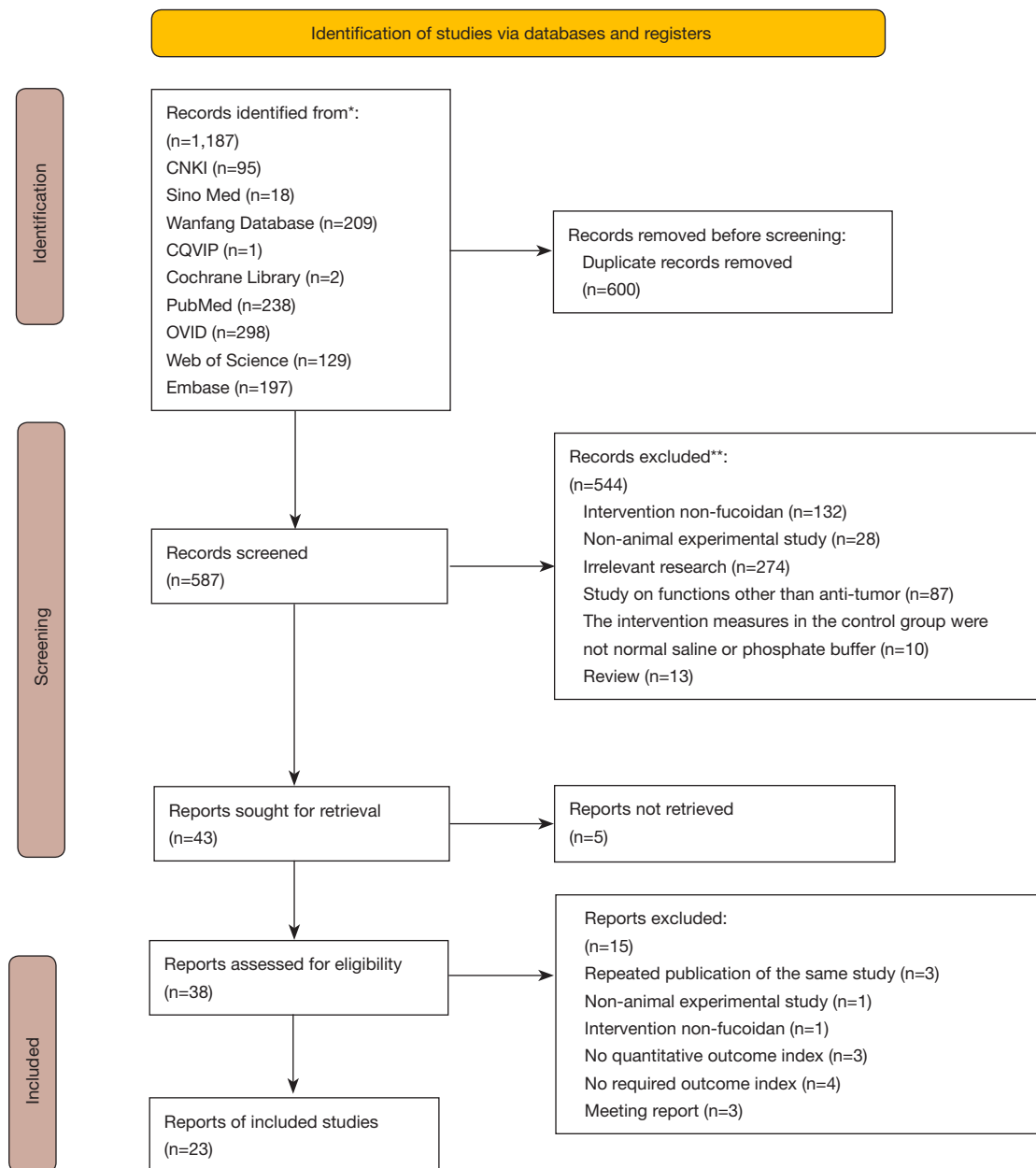
Figure 1 Literature screening flow chart.

Table 1 Basic characteristics of the included literature

Included study	Animal species	Animal gender	Animal weight	Animal age	Cancer type	Source of fucoidan	Dose	Medication time	Dosage (mg/Kg)	Administration method	Control group	Outcome indicator
Yun CW 2016 (8)	BALB/C nude mice	Male	?	?	Colorectal cancer	Seaweed	5 mg/kg	Every 2 days, a total of 9 times	22.5	ip.	NS	
Xue M 2012 (9)	BALB/c mice	Female	?	4 w	Breast cancer	?	5, 10 mg/kg	Every 2 days, a total of 10 times	25, 50	ip.	NS	
Rui X 2017 (10)	nude mice	Male	?	5 w	Prostate cancer	?	20 mg/kg	28 days	560	ig.	NS	
Liu S 2020 (11)	BALB/C nu/ nu mice	Male	?	6–8 w	Ovarian cancer	?	10 mg/kg	21 days	210	ig.	NS	
Xue M 2020 (12)	SD rats	Male	100–120 g	4 w	Colorectal cancer	Vesicular brown algae	400 mg/kg	Once a day for 22 weeks	61,600	ig.	NS	
Chen H 2016 (13)	BALB/CA nu/ nu mice	?	?	6–8 w	Lung cancer	Brown seaweed Sargassum fusiforme	0.5, 10 mg/kg	Once every 2 days for 26 days	6.5, 130	iv.	NS	
Xue ML 2017 (14)	SD rats	Female	150–180 g	6–8 w	Breast cancer	?	200, 400 mg/kg	Six times a week for 16 weeks	19,200, 38,400	ig.	NS	
Xue ML 2013 (15)	BALB/c mice	Female	?	4 w	Breast cancer	?	5, 10 mg/kg	Every 2 days, a total of 10 times	25, 50	ip.	NS	
Han YS 2015 (16)	BALB/C nude mice	Male	?	8 w	Colorectal cancer	?	5, 10 mg/kg	Once every 2 days for 30 days	75, 150	ip.	NS	
Duan Y 2020 (17)	nude mice	?	?	?	Liver cancer	?	25 mg/kg/d	Once a day for 3 weeks	525	po.	NS	
Choo GS 2016 (18)	BALB/c nude mice	Male	?	5 w	Prostate cancer	?	5, 10 mg/kg	5 times a week for 3 weeks	75, 150	ip.	PBS	
Zhang X 2011 (19)	C57BL/6J mice	Female	17–18 g	?	Lung cancer	American sea cucumber	10, 20 mg/kg	Once a day for 21 days	210, 420	ip.	NS	
Zhang X 2012a (20)	C57BL/6J mice	Female	17–18 g	?	Lung cancer	Sea melon, icelandic sea cucumber	100 mg/kg/d	Once a day for 21 days	2,100	ip.	NS	
Zhang X 2012b (21)	C57BL/6J mice	?	?	?	Lung cancer	Sea melon, icelandic sea cucumber, arctic sea cucumber	50, 100 mg/kg	Once a day for 21 days	1,050, 2,100	ip.	NS	

Table 1 (*continued*)

Table 1 (*continued*)

Included study	Animal species	Animal gender	Animal weight	Animal age	Cancer type	Source of fucoidan	Dose	Medication time	Dosage (mg/Kg)	Administration method	Control group	Outcome indicator
Zhang X 2012b (21)	C57BL/6J mice	?	?	?	Melanoma	Sea melon, icelandic sea cucumber, arctic sea cucumber	50, 100 mg/kg	Once a day for 26 days	1,300, 2,600	ip.	NS	
Zhang XD 2018 (22)	ICR mice	Male	?	?	Liver cancer	Glycoside moss	150, 350, 600 mg/kg	Once a day for 15 days, and once a day for 19 days after 3 days of cancer cell inoculation	5,100, 11,900, 20,400	ig.	NS	
Zhang T 2018 (23)	SD rats	Female	200–220 g	2 m	Breast cancer	?	200, 400 mg/kg	Once a day for 16 weeks	22,400, 45,200	ig.	NS	
Yang YH 2012 (24)	C57BL/6J mice	Female	17–18 g	?	Lung cancer	American meat ginseng	5, 20 mg/kg	Once a day for 21 days	105, 420	ip.	NS	
Wang JF 2012 (25)	C57BL/6J mice	Female	18–22 g	?	Melanoma	Icelandic sea cucumber	50, 100 mg/kg	Once a day for 26 days	1,300, 2,600	ip.	NS	
Liu F 2016 (26)	NOD/SCID mice	Female	15–20 g	4–6 w	Multiple myeloma	?	10, 50 mg/kg	Once every 2 days for 3 weeks	105, 525	ip.	PBS	
Li QM 2013 (27)	C57BL/6J mice	Male	18–22 g	6–8 w	Lung cancer	Undaria spore leaf	50, 100, 200 mg/kg	Once every 3 days, 10 times in total	166.7, 333.4, 666.7	ip.	NS	
Xue ML 2012 (28)	BALB/c mice	Female	14–18 g	4 w	Breast cancer	?	5, 10 mg/kg	Once every 2 days for 20 days	50, 100	ip.	NS	
Wang CZ 2010 (29)	Kunming mice	Male	18–22 g	?	Sarcoma	Kelp	25, 50, 100 mg/kg	Once a day for 10 days	250, 500, 1,000	ip.	NS	
Li ZJ 2001 (30)	Kunming mice	Female	22–24 g	?	Sarcoma	Kelp	125, 250, 500 mg/kg	Once a day for 3 days, and once a day for 10 days after 4 days of tumor inoculation	1,625, 3,250, 6,500	po.	NS	
Li ZJ 2001 (30)	Kunming mice	Female	22–24 g	?	Sarcoma	Kelp	25, 50, 100 mg/kg	Once every other day, 5 times in total	62.5, 125, 250	ip.	NS	

SD, Sprague-Dawley; ICR, Institute of Cancer Research; NOD/SCID, nonobese diabetic/severe combined immunodeficiency; g, gram; w, weeks; m, months; mg/kg, milligram/kilogram; mg/kg/d, milligram/kilogram/day; "?"indicates that no relevant information was provided in the article. *Dosage = Dose ÷ Time interval of medication × Medication days.* ip. intraperitoneal injection; ig. intragastric administration; iv. caudal vein injection; po. oral administration. NS, normal saline; PBS, phosphate buffered saline. represents tumor weight; represents tumor volume; represents tumor number.

Table 2 SYRCLE bias risk assessment results of included studies

Included study										
Yun CW 2016 (8)	?	?	?	—	?	+	—	—	—	?
Xue M 2012 (9)	?	?	?	—	?	+	—	—	—	?
Rui X 2017 (10)	?	?	?	—	?	+	—	—	—	?
Liu S 2020 (11)	?	?	?	—	?	+	—	—	?	?
Xue M 2020 (12)	?	?	?	—	?	+	—	+	?	?
Chen H 2016 (13)	?	?	?	?	?	+	—	—	—	?
Xue ML 2017 (14)	?	?	?	—	?	+	—	?	?	?
Xue ML 2013 (15)	?	?	?	—	?	+	—	?	—	?
Han YS 2015 (16)	?	?	?	?	?	+	—	?	—	?
Duan Y 2020 (17)	?	?	?	—	?	+	—	—	—	?
Choo GS 2016 (18)	?	?	?	—	?	+	—	—	+	?
Zhang X 2011 (19)	?	?	?	?	?	+	—	—	—	?
Zhang X 2012a (20)	?	?	?	?	?	+	—	—	—	?
Zhang X 2012b (21)	?	?	?	?	?	+	—	—	—	?
Zhang X 2012b (21)	?	?	?	?	?	+	—	—	—	?
Zhang XD 2018 (22)	?	?	?	?	?	+	—	—	—	?
Zhang T 2018 (23)	?	?	?	?	?	+	—	+	—	?
Yang YH 2012 (24)	?	?	?	?	?	+	—	—	—	?
Wang JF 2012 (25)	?	?	?	?	?	+	—	—	?	?
Liu F 2016 (26)	?	?	?	—	?	+	—	—	?	?
Li QM 2013 (27)	?	?	?	?	?	+	—	—	—	?
Xue ML 2012 (28)	?	?	?	?	?	+	—	—	?	?
Wang CZ 2010 (29)	?	?	?	?	?	+	—	—	—	?
Li ZJ 2001 (30)	?	?	?	—	?	+	—	+	—	?
Li ZJ 2001 (30)	?	?	?	—	?	+	—	+	—	?

Evaluation of bias risk was based on the following determinations: Whether the generation or application of allocation sequence was sufficient/correct; Whether the baseline of each group was the same or whether confounding factors had been adjusted; Whether allocation concealment was sufficient/correct; Whether the animals were randomly placed during the experiment; Whether animal breeders and researchers were blinded in the experiment to avoid knowing the intervention measures accepted by animals; Whether the animals were randomly selected for outcome assessment; Whether evaluators of results were blinded; Whether incomplete data were fully/correctly explained; Whether the research report had nothing to do with the selective result report; Whether there were other issues that would lead to high risk of bias; "−" means low risk; "?" indicates uncertainty; "+" means high risk.
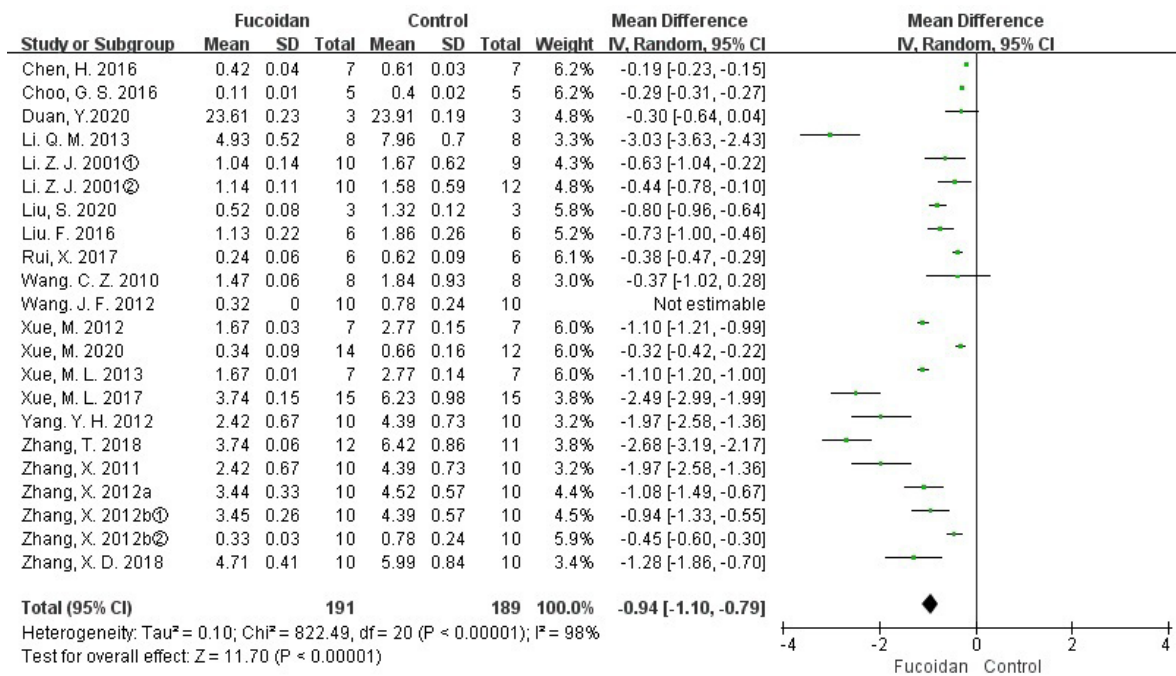
Figure 2 Meta-analysis results of tumor weight inhibition. SD, standard deviation; CI, confidence interval; IV, Inverse Variance; df, degree of freedom.

of tumors. As there were considerable differences in the outcome indicators of the various studies, the meta-analysis effect value was expressed by SMD and 95% CI. Meta-analysis showed that I^2=73% among the studies, and REM was used for combined analysis. Compared with the control group, fucoidan inhibited tumor number (SMD =−3.27, 95% CI: −4.30 to −2.23, P<0.05), and the difference was statistically significant.

The above results are shown in *Figures 2-4*, and *Table 3*.

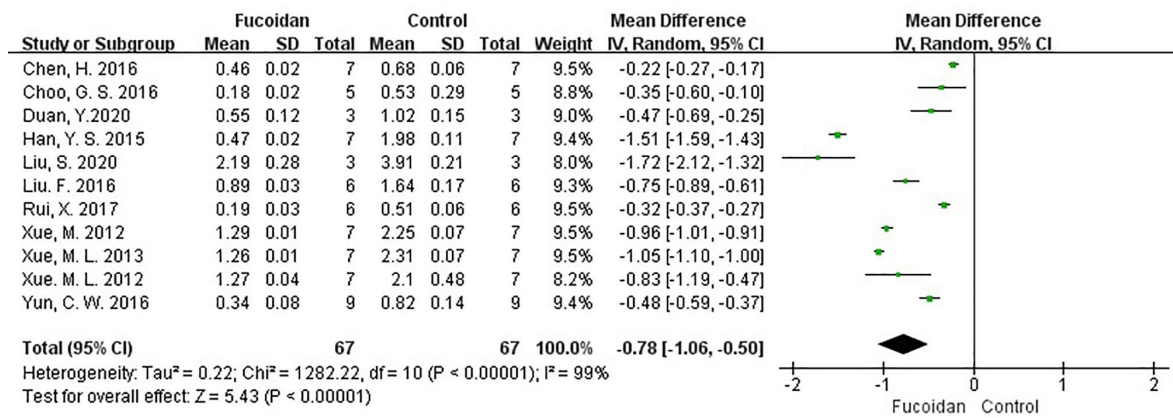### Analysis of source heterogeneity

Sensitivity analysis

Based on tumor weight, tumor volume, and tumor number, the outcome indicators of the included literature were combined and analyzed by FEM and REM, and the analysis results of the 2 models were consistent. A total of 22 studies (9-15,17-27,29,30) analyzed the tumor weight. After removing the studies 1 at a time, the I^2 of each combination scheme was still between 97% and 98%, and the heterogeneity had no obvious change. There were 11 studies (8-11,13,15-18,26,28) which analyzed tumor volume. After deleting each study 1 at a time, the heterogeneity remained unchanged, and I^2 was still 99%. A

total of 7 studies (12,19-21,24,25) analyzed the number of tumors, and after removing the study by Wang Jingfeng (25), I^2 decreased from 73% to 26%, indicating it was a source of heterogeneity (*Figure 5*).
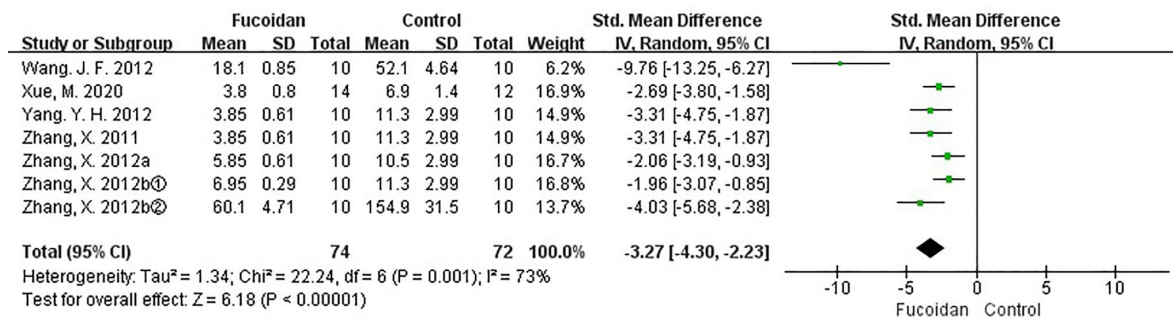
Subgroup analysis

In accordance with the Cochrane *Handbook* for *Systematic Reviews* of *Interventions* (7), because multiple intervention groups shared 1 control group, only the total number of subjects was divided in subgroup analysis, the original mean and standard deviation remaining unchanged. The results of the subgroup analysis are shown in *Table 4*.

Subgroup analysis of the effect of fucoidan intervention on tumor weight showed that the heterogeneity of studies in each dosage group was less than 50%, and dosage was one of the variables affecting the heterogeneity among studies. The tumor weight in the intervention group was lower than that in the control group, with different dosages, administration modes, and mouse types, and the difference was statistically significant. Except for prostate cancer and ovarian cancer, the tumor weights of the other cancer types in the intervention group were lower than those in the control group, and the difference was statistically significant.

Study or Subgroup	Fucoidan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Chen, H. 2016	0.46	0.02	7	0.68	0.06	7	9.5%	-0.22 [-0.27, -0.17]
Choo, G. S. 2016	0.18	0.02	5	0.53	0.29	5	8.8%	-0.35 [-0.60, -0.10]
Duan, Y.2020	0.55	0.12	3	1.02	0.15	3	9.0%	-0.47 [-0.69, -0.25]
Han, Y. S. 2015	0.47	0.02	7	1.98	0.11	7	9.4%	-1.51 [-1.59, -1.43]
Liu, S. 2020	2.19	0.28	3	3.91	0.21	3	8.0%	-1.72 [-2.12, -1.32]
Liu. F. 2016	0.89	0.03	6	1.64	0.17	6	9.3%	-0.75 [-0.89, -0.61]
Rui, X. 2017	0.19	0.03	6	0.51	0.06	6	9.5%	-0.32 [-0.37, -0.27]
Xue, M. 2012	1.29	0.01	7	2.25	0.07	7	9.5%	-0.96 [-1.01, -0.91]
Xue, M. L. 2013	1.26	0.01	7	2.31	0.07	7	9.5%	-1.05 [-1.10, -1.00]
Xue. M. L. 2012	1.27	0.04	7	2.1	0.48	7	8.2%	-0.83 [-1.19, -0.47]
Yun, C. W. 2016	0.34	0.08	9	0.82	0.14	9	9.4%	-0.48 [-0.59, -0.37]
Total (95% CI)			**67**			**67**	**100.0%**	**-0.78 [-1.06, -0.50]**

Heterogeneity: Tau² = 0.22; Chi² = 1282.22, df = 10 (P < 0.00001); I² = 99%
Test for overall effect: Z = 5.43 (P < 0.00001)

Figure 3 Meta-analysis results of tumor volume inhibition. SD, standard deviation; CI, confidence interval; IV, Inverse Variance; df, degree of freedom.

Study or Subgroup	Fucoidan Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Wang. J. F. 2012	18.1	0.85	10	52.1	4.64	10	6.2%	-9.76 [-13.25, -6.27]
Xue, M. 2020	3.8	0.8	14	6.9	1.4	12	16.9%	-2.69 [-3.80, -1.58]
Yang. Y. H. 2012	3.85	0.61	10	11.3	2.99	10	14.9%	-3.31 [-4.75, -1.87]
Zhang, X. 2011	3.85	0.61	10	11.3	2.99	10	14.9%	-3.31 [-4.75, -1.87]
Zhang, X. 2012a	5.85	0.61	10	10.5	2.99	10	16.7%	-2.06 [-3.19, -0.93]
Zhang, X. 2012b①	6.95	0.29	10	11.3	2.99	10	16.8%	-1.96 [-3.07, -0.85]
Zhang, X. 2012b②	60.1	4.71	10	154.9	31.5	10	13.7%	-4.03 [-5.68, -2.38]
Total (95% CI)			**74**			**72**	**100.0%**	**-3.27 [-4.30, -2.23]**

Heterogeneity: Tau² = 1.34; Chi² = 22.24, df = 6 (P = 0.001); I² = 73%
Test for overall effect: Z = 6.18 (P < 0.00001)

Figure 4 Meta-analysis results of tumor number inhibition. SD, standard deviation; CI, confidence interval; IV, Inverse Variance; df, degree of freedom; std, standard.
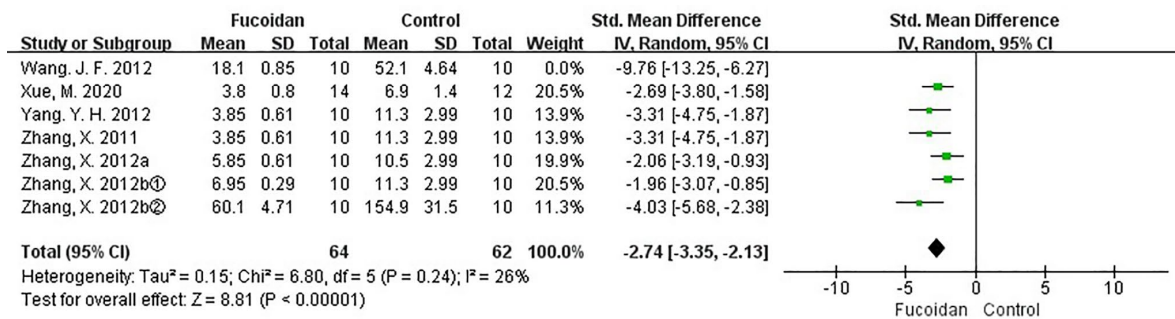
Table 3 Results of the meta-analysis of the tumor inhibition outcome index

Outcome indicator	Number of studies	Sample size T/C	MD/SMD	95% CI	I²/%	P value
Tumor weight (g)	22	191/189	MD =−0.94	−1.10, −0.79	98	<0.05
Tumor volume (cm³)	11	67/67	MD =−0.78	−1.06, −0.50	99	<0.05
Tumor number (pcs)	7	74/72	SMD =−3.27	−4.30, −2.23	73	<0.05

g, gram; cm³, cubic centimeter; pcs, pieces. T represents the intervention group, C represents the control group. MD, mean difference; SMD, standardized mean difference; CI, confidence interval.

Subgroup analysis of the effect of fucoidan intervention on tumor volume showed that the heterogeneity of dosage, administration mode, and cancer type was less than 50%, and these 3 variables affected the heterogeneity among studies. The tumor volume in the intervention group was smaller than that in the control group, with different dosages and mouse types, and the difference was statistically significant. Except for oral administration, there was no significant difference in tumor volume between treatment groups. The tumor volume of the administration methods other than oral in the intervention group was significantly lower than that in the control group. Except for lung

Study or Subgroup	Fucoidan Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Wang. J. F. 2012	18.1	0.85	10	52.1	4.64	10	0.0%	-9.76 [-13.25, -6.27]
Xue, M. 2020	3.8	0.8	14	6.9	1.4	12	20.5%	-2.69 [-3.80, -1.58]
Yang. Y. H. 2012	3.85	0.61	10	11.3	2.99	10	13.9%	-3.31 [-4.75, -1.87]
Zhang, X. 2011	3.85	0.61	10	11.3	2.99	10	13.9%	-3.31 [-4.75, -1.87]
Zhang, X. 2012a	5.85	0.61	10	10.5	2.99	10	19.9%	-2.06 [-3.19, -0.93]
Zhang, X. 2012b①	6.95	0.29	10	11.3	2.99	10	20.5%	-1.96 [-3.07, -0.85]
Zhang, X. 2012b②	60.1	4.71	10	154.9	31.5	10	11.3%	-4.03 [-5.68, -2.38]
Total (95% CI)			**64**			**62**	**100.0%**	**-2.74 [-3.35, -2.13]**

Heterogeneity: Tau² = 0.15; Chi² = 6.80, df = 5 (P = 0.24); I² = 26%
Test for overall effect: Z = 8.81 (P < 0.00001)

Figure 5 Sensitivity analysis results of tumor number inhibition. SD, standard deviation; CI, confidence interval; IV, Inverse Variance; df, degree of freedom; std, standard.

Table 4 Results of the meta-analysis of the tumor inhibition outcome index

Outcome indicator	Variable	Subgroup	Number of studies	SMD (95% CI)	I^2/%	P value
Tumor weight (g)	Dosage of fucoidan	Low	18	−2.59 (−3.56, −1.62)	75	<0.05
		Medium	2	−2.88 (−4.01, −1.74)	0	<0.05
		High	24	−2.24 (−2.67, −1.81)	47	<0.05
	Administration mode	ip.	13	−2.96 (−3.97, −1.95)	81	<0.05
		iv.	1	−5.03 (−7.47, −2.60)	–	<0.0001
		ig.	6	−3.16 (−4.11, −2.21)	55	<0.05
		po.	2	−1.32 (−2.23, −0.42)	0	<0.05
	Cancer type	Lung cancer	6	−2.84 (−3.62, −2.06)	43	<0.05
		Liver cancer	2	−1.68 (−2.63, −0.74)	0	<0.05
		Breast cancer	4	−5.94 (−8.46, −3.43)	79	<0.05
		Melanoma	2	−2.52 (−3.76, −1.28)	–	<0.05
		Sarcoma	3	−0.95 (−1.51, −0.39)	0	<0.05
		Colorectal cancer	1	−2.44 (−3.50, −1.39)	–	<0.00001
		Multiple myeloma	1	−2.80 (−4.57, −1.02)	–	0.002
		Prostate cancer	2	−9.68 (−21.29, 1.93)	83	>0.05
		Ovarian cancer	1	−6.28 (−12.54, −0.01)	–	0.05
	Mouse type	Nude mice	5	−4.87 (−7.84, −1.90)	75	<0.05
		Non-nude mice	17	−2.64 (−3.32, −1.96)	76	<0.05

Table 4 (*continued*)

Table 4 (*continued*)

Outcome indicator	Variable	Subgroup	Number of studies	SMD (95% CI)	I²/%	P value
Tumor volume (cm³)	Dosage of fucoidan	Low	15	–4.86 (–6.46, –3.26)	76	<0.05
		High	3	–4.74 (–6.88, –2.59)	20	<0.05
	Administration mode	ip.	7	–7.01 (–10.00, –4.02)	88	<0.05
		iv.	1	–4.61 (–6.87, –2.34)	–	<0.0001
		ig.	2	–6.06 (–8.87, –3.25)	0	<0.05
		po.	1	–2.77 (–5.88, 0.35)	–	0.08
	Cancer type	Lung cancer	1	–4.61 (–6.87, –2.34)	–	<0.0001
		Liver cancer	1	–2.77 (–5.88, 0.35)	–	0.08
		Breast cancer	3	–12.81 (–26.02, 0.39)	93	>0.05
		Colorectal cancer	2	–10.39 (–23.94, 3.16)	91	>0.05
		Multiple myeloma	1	–5.67 (–8.66, –2.68)	–	0.0002
		Prostate cancer	2	–3.65 (–8.23, 0.92)	85	>0.05
		Ovarian cancer	1	–5.56 (–11.16, 0.04)	–	0.05
	Mouse type	Nude mice	7	–4.63 (–6.73, –2.54)	75	<0.05
		Non-nude mice	4	–9.88 (–16.06, –3.71)	76	<0.05
Tumor number (pcs)	Dosage of fucoidan	Low	2	–2.72 (–3.82, –1.62)	0	<0.05
		Medium	2	–4.70 (–6.28, –3.12)	0	<0.05
		High	8	–3.80 (–5.10, –2.50)	76	<0.05
	Administration mode	ip.	6	–3.47 (–4.76, –2.19)	77	<0.05
		ig.	1	–2.69 (–3.80, –1.58)	–	<0.00001
	Cancer type	Lung cancer	4	–2.53 (–3.25, –1.82)	23	<0.05
		Melanoma	2	–6.68 (–12.28, –1.08)	88	<0.05
		Colorectal cancer	1	–2.69 (–3.80, –1.58)	–	<0.00001

Dosages less than 420 mg/kg were considered low, 420 mg/kg medium, and greater than 420 mg/kg high. g, gram; cm³, cubic centimeter; pcs, pieces. ip. intraperitoneal injection; ig. intragastric administration; iv. caudal vein injection; po. oral administration. SMD, standardized mean difference; CI, confidence interval.

cancer and multiple myeloma, the tumor volume in the intervention group was lower than that in the control group, and the difference was statistically significant.

Subgroup analysis of the effect of fucoidan intervention on the number of tumors showed that the heterogeneity of different drug delivery methods and cancer types in the study group was less than 50%, and these 2 variables affected the heterogeneity between studies. Different dosages, administration modes, and cancer types in the intervention group were lower than those in the control

group, and the difference was statistically significant.

Results of bias analysis

An inverted funnel diagram was used to judge the publication bias of the included studies. Since the number of studies with tumor quantitative indicators was fewer than 10, it was deemed too few a number, and the test efficiency too low to conduct publication bias evaluation. Only publication bias for tumor weight and tumor volume was
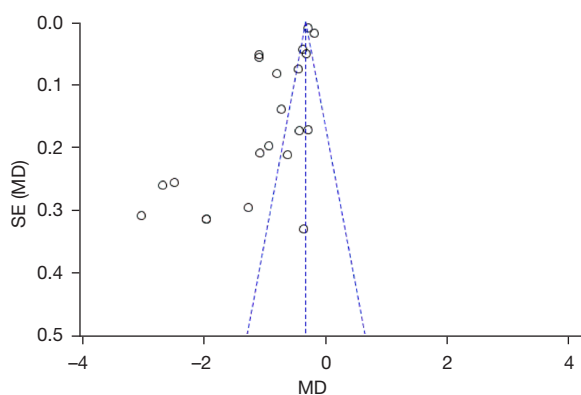
Figure 6 Funnel diagram of studies of tumor weight inhibition by fucoidan. SE, standard error; MD, mean difference.
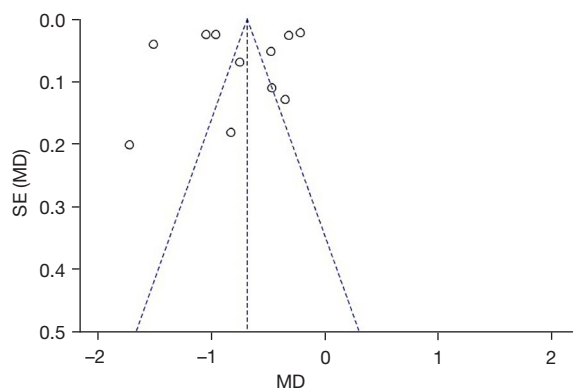
Figure 7 Funnel diagram of studies of tumor volume inhibition by fucoidan. SE, standard error; MD, mean difference.

evaluated (see *Figures 6*,*7*).

As shown in *Figure 6*, the funnel chart of the literature included in the tumor weight analysis is asymmetric and skewed, and most of the selected studies are outside the funnel chart's 95% CI, indicating that those studies contained publication bias.

As displayed in *Figure 7*, the funnel chart structure of the literature included in tumor volume analysis is basically symmetrical; however, most of the selected studies were outside the funnel chart's 95% CI, indicating that those studies contained publication bias.

Discussion

This study involved a meta-analysis of animal studies exploring the effect of fucoidan on antitumor function

in vivo in successfully established animal disease models. Based on the systematic evaluation of results, fucoidan can significantly inhibit tumor weight, tumor volume, and tumor number. In terms of the effect on tumor weight, the best effect was achieved with low dosage and intragastric administration in breast cancer models. In controlling tumor volume, the best effect was observed with low dosage and intraperitoneal injection in multiple myeloma models. In controlling the number of tumors, the best effect was achieved in melanoma models with high dosage and intraperitoneal injection. Different effects were shown across different tumor models, dosage, and administration methods, and the mechanism requires further study.

Meta-analysis showed that there was considerable heterogeneity among the studies. Subgroup analysis revealed that different dosages, modes of administration, and cancer types may have been the main reasons for heterogeneity between studies. As meta-analysis is based on data from the existing literature, conclusion reliability and literature bias are greatly influenced by the quality of the included studies (36). In this paper, although keywords and MeSH were used to search Chinese and international databases, the sources, components, concentrations, doses, administration methods, and intervention time among the 23 studies included in our analysis were inconsistent. Additionally, experimental animal subjects and models differed, and there was variation in index measurement methods and numerical units, leading to the heterogeneity and bias of the meta-analysis in this paper. Furthermore, the results included in the literature were all positive results, and there was no published literature with negative results. The results of bias analysis in this paper showed that all the included studies contained some publication bias. However, the results of sensitivity analysis showed good stability, which may reflect, to a certain extent, the reliability of the results showing the antitumor activity of fucoidan. In order to improve the repeatability of the research results, it is necessary to unify the dosage, components, and administration methods of fucoidan in future animal experiment research.

In this study, the SYRCLE animal experiment risk assessment tool was used to evaluate the quality of the included literature. In animal experiments, the randomization of animals has an important impact on the experimental results. If the animals are not randomly allocated, researchers may predict the behavior of each group of animals, resulting in implementation bias (36). In this study, only 11 studies (8-12,14,15,17,18,26,30) utilized the randomized placement of animals. In addition,

if the results were measured without randomization and the samples were only measured and evaluated in a certain period of time, there may be a risk of measurement bias (36). The literature included in this study did not report whether the outcome indicators were randomly evaluated. In animal experiments, the application of blind methods in the intervention, implementation, and result measurement stages differs from their execution in RCTs. Although there is no need to blind animals, most researchers involved in the process of intervention implementation are also animal breeders. If animal breeders are not blinded, subjective bias may arise in their expected experimental results (36). None of the studies included in this review described whether the breeders and researchers were blinded. More attention should be given to the implementation and full reporting of measures, including the balance of baseline characteristics, random result measurement, randomized animal placement, and blinding methods. This is especially important for promoting the establishment and implementation of animal experiment registries to enhance the transparency of the entire animal research process, promote the improvement of its quality, and ultimately improve the usefulness of basic research and provide a reliable theoretical basis for the transformation of basic research to clinical research.

In summary, this study investigated the effectiveness of the antitumor activity of fucoidan based on published animal experimental studies. The existing evidence showed that fucoidan inhibited the growth and spontaneous metastasis of tumors in numerous animal models, the effect of intraperitoneal injection at a low dose was best for most tumors, and the mechanism of fucoidan's antitumor activity warrants further research. However, as the design quality of the included studies was not high, heterogeneity and bias may have affected the accuracy of the results.

Acknowledgments

Footnote

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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