ISSN 2307-8960 (online)

World Journal of *Clinical Cases*

World J Clin Cases 2022 November 6; 10(31): 11214-11664

Published by Baishideng Publishing Group Inc

World Journal of Clinical Cases

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NAME OF JOURNAL
World Journal of Clinical Cases

ISSN
ISSN 2307-8960 (online)

LAUNCH DATE
April 16, 2013

FREQUENCY
Thrice Monthly

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PUBLICATION DATE
November 6, 2022

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World J Clin Cases 2022 November 6; 10(31): 11299-11312

DOI: 10.12998/wjcc.v10.i31.11299

ISSN 2307-8960 (online)

ORIGINAL ARTICLE

Clinical and Translational Research

Screening of traditional Chinese medicine monomers as ribonucleotide reductase M2 inhibitors for tumor treatment

Ya-Ya Qin, Song Feng, Xiao-Dong Zhang, Bin Peng

Specialty type: Oncology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Gómez-Regalado F, Mexico; Kołat D, Poland; Rather AA, India

Received: June 23, 2022
Peer-review started: June 23, 2022
First decision: September 5, 2022
Revised: September 14, 2022
Accepted: September 29, 2022
Article in press: September 29, 2022
Published online: November 6, 2022

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Abstract

BACKGROUND

Ribonucleotide reductase (RR) is a key enzyme in tumor proliferation, especially its subunit-RRM2. Although there are multiple therapeutics for tumors, they all have certain limitations. Given their advantages, traditional Chinese medicine (TCM) monomers have become an important source of anti-tumor drugs. Therefore, screening and analysis of TCM monomers with RRM2 inhibition can provide a reference for further anti-tumor drug development.

AIM

To screen and analyze potential anti-tumor TCM monomers with a good binding capacity to RRM2.

METHODS

The Gene Expression Profiling Interactive Analysis database was used to analyze the level of RRM2 gene expression in normal and tumor tissues as well as RRM2's effect on the overall survival rate of tumor patients. TCM monomers that potentially act on RRM2 were screened *via* literature mining. Using AutoDock software, the screened monomers were docked with the RRM2 protein.

RESULTS

The expression of RRM2 mRNA in multiple tumor tissues was significantly higher than that in normal tissues, and it was negatively correlated with the overall survival rate of patients with the majority of tumor types. Through literature mining, we discovered that berberine, ursolic acid, gambogic acid, cinobufagin, quercetin, daphnetin, and osalmide have inhibitory effects on RRM2. The results of molecular docking identified that the above TCM monomers have a strong binding capacity with RRM2 protein, which mainly interacted through

hydrogen bonds and hydrophobic force. The main binding sites were Arg330, Tyr323, Ser263, and Met350.

CONCLUSION

RRM2 is an important tumor therapeutic target. The TCM monomers screened have a good binding capacity with the RRM2 protein.

Key Words: Tumor; Ribonucleotide reductase M2 inhibitor; Traditional Chinese medicine; Monomer; Molecular docking; Literature mining

Core Tip: Tumors seriously threaten human life and health. In our work, we found that ribonucleotide reductase M2 (RRM2) is highly expressed in most tumor tissues, and is related to poor prognosis. Seven traditional Chinese medicine monomers with good binding ability to RRM2 were identified, and their binding sites were summarized and analyzed. Those will provide ideas for the development of anti-tumor drugs with RRM2 inhibition in the future.

Citation: Qin YY, Feng S, Zhang XD, Peng B. Screening of traditional Chinese medicine monomers as ribonucleotide reductase M2 inhibitors for tumor treatment. *World J Clin Cases* 2022; 10(31): 11299-11312
URL: https://www.wjgnet.com/2307-8960/full/v10/i31/11299.htm
DOI: https://dx.doi.org/10.12998/wjcc.v10.i31.11299

INTRODUCTION

The tumor is a major contributor to endangering human health. In terms of disability-adjusted life years, it is only second to cardiovascular disease. The World Health Organization predicts that there will be a global increase in new tumor cases of more than 50%, from 18 million in 2018 to 27 million in 2040[1]. In addition to conventional surgical resection, drug adjuvant therapy still occupies a considerable part in the treatment of tumors. Although numerous chemotherapy medications have been developed, the majority of them have more or less side effects and some are rather pricey. Since natural chemicals are safer, cheaper, and more effective than synthetic ones, there has been an increasing interest in finding medications to prevent and cure tumors from natural compounds[2].

Ribonucleotide reductase (RR), the only multi-subunit enzyme existing in all biological cells that can catalyze the reduction of ribonucleotides to corresponding deoxyribonucleotides, is the rate-limiting enzyme of DNA synthesis. By regulating and balancing the content of different deoxyribonucleic acids (dNTPs) in the cell cycle, RR is mainly involved in DNA replication and repair, which is crucial for controlling cell proliferation and preserving genomic stability[3,4]. Human RR is composed of two large subunits M1 and two small subunits M2 (RRM2)[5]. Since RRM2 has the ability to regulate and catalyze substrates, the enzymatic activity of RR is primarily controlled by RRM2[6]. The tumor is a highly invasive disease, the tumor cell proliferation requires the participation of a large number of dNTPs[3]. Studies have found that most tumor cells express more RR than normal cells do. The overexpression of RRM2 is related to tumor malignancy, invasion, metastasis, drug resistance, and autophagy[7-9]. Inhibiting or reducing the expression of RRM2 may improve tumor patients' disease progression and prognosis, and lengthen their survival[10].

According to the target and mechanism of action, RRM2 inhibitors are roughly divided into gene expression regulators and protein inactivators. The gene expression regulators include R2 antisense inhibitors and siRNA inhibitors, whereas free radical scavengers, iron chelators, and iron mimics fall under the category of protein inactivators[11]. Due to a wide range of pharmacological properties, some traditional Chinese medicine (TCM) monomers have also been used as RRM2 inhibitors for research. Through literature mining, we found multiple TCM monomers that have inhibitory effects against RRM2 in tumors. However, there are few studies on their interaction sites. This paper aims to elucidate the relationship between RRM2 and malignant tumors and the prognosis of tumor patients, and then to screen out potential anti-tumor TCM monomers with good binding ability to RRM2. Through the analysis of their main binding sites, some thoughts for the development of new anti-tumor drugs with RRM2 inhibition are provided.

MATERIALS AND METHODS

Tumor patients' data acquisition

Through the Expression Profiling Interactive Analysis database (GEPIA) (http://gepia.cancer-pku.cn), we analyzed and obtained the mRNA level of the RRM2 gene in normal tissues and tumor tissues, as well as its effect on the overall survival rate of tumor patients. All tumor abbreviations were listed in Table 1.

Literature mining

PubMed database (http://www.ncbi.nlm.nih.gov/pubmed/) and the China National Knowledge Infrastructure database (CNKI, https://www.cnki.net) were used to retrieve and download the articles related to TCM monomers acting on RRM2 targets. Subsequently, the application of TCM monomers in tumors was summarized and analyzed one by one.

Molecular docking

According to the small molecule CAS number from the PubChem database, we downloaded the 3D structure of TCM monomers with small molecule SDF format, then imported them into chembio3d ultra 14.0 for energy minimization respectively. The three-dimensional structure of the RRM2 protein was obtained from the PDB (http://www.rcsb.org). AutoDock vina1.1.2 was used to complete the molecular docking between RRM2 protein and TCM monomers. The relevant parameters of RRM2 protein were set to center_ x = -4.715, center_ y = -3.6and 33, center_ Z = 15.668, the size of the grid box was set to 50 × 50 × 50 (the spacing of each grid point is 0.375 Å), and the other parameters were the default settings. Finally, analyzation of the interaction mode of the docking results was performed by Pymol 2.3.0 and ligplot V2.1.

RESULTS

RRM2 was identified as the tumor therapeutic target

In the GEPIA database, we found 31 types of tumor tissues with RRM2 differential expression and their paired normal samples. The findings revealed that, except for LAML, the mRNA expression of RRM2 in 30 types of tumor tissues was considerably higher than that in normal tissues (Figure 1). The investigation of the overall survival rate of 33 types of tumor patients revealed that the RRM2 gene expression of 23 types was negatively correlated with the overall survival rate (Figure 2), while it was positively correlated in 10 types (Figure 3). Among them, the reason for the few positive correlation results observed may be other issues exist that affect the overall survival rate.

Seven TCM monomers with inhibitory effect on RRM2 in tumors were screened

Through the literature search in the PubMed database and CNKI database, we found seven TCM monomers that may be used as RRM2 inhibitors in tumors (Table 2). They all will be described in subsequent sections separately.

Berberine: Berberine is a quaternary ammonium alkaloid extracted from medicinal plants such as Coptis chinensis, Berberis aristata, Hydrastis canadensis, and Coptis japonica[12]. Berberine and its derivatives have been identified to have pharmacological properties against multiple diseases, including digestive diseases, metabolic diseases, cardiovascular diseases, and neurological diseases[13]. Recent studies have discovered that berberine can also inhibit the invasion and metastasis of many kinds of tumors, such as oral squamous cell carcinoma, lung cancer, liver cancer, glioblastoma, breast cancer, and so on[12]. Through binding to P53, NF-κB, matrix metalloproteinase (MMP), Bcl-2, and receptors e.g. estrogen receptor, berberine could promote the cell cycle arrest and death of tumor cell lines, and induce the expression of pro-apoptotic factors[14-16]. In addition, some other information indicates that RRM2 may also be a potential target of berberine in the treatment of tumors. A bioinformatical analysis showed that RRM2 is the hub-gene for berberine to act on breast cancer[17]. After berberine treatment *in vitro*, the expression level of the RRM2 gene and protein in non-small cell lung cancer cell lines (A549, H1299, and H1975) was significantly reduced[18].

Ursolic acid: Ursolic acid, a natural pentacyclic triterpene compound, is widely found in fruits and vegetables. It has been demonstrated to have multiple biological functions, including anti-inflammatory, antioxidant, anti-apoptotic, and anti-allergic activities[19]. At present, ursolic acid has also been reported to have anti-tumor pharmacological properties, acting as an active therapeutic agent for several malignancies such as breast cancer, colon cancer, pancreatic cancer, and liver cancer[20]. By regulating a variety of enzymes (ATPase, GST, COX-2), transcription factors (AP-1, NF-κB, STAT-3), growth factors (EGF, PDGF, HGF), receptors (EGFR, ER-a, HER-2, EAR), as well as inflammatory factors (MAP-K, PKA, PTK, IL-6, IL-1, IL-8, MIP), it could inhibit tumor proliferation, metastasis, and angiogenesis[21,22]. Recently, a network pharmacology analysis detected that RRM2 maybe also the

Table 1 Tumor abbreviations

Abbreviation	Full name	Abbreviation	Full name	Abbreviation	Full name
ACC	Adrenocortical carcinoma	BLCA	Bladder Urothelial Carcinoma	BRCA	Breast invasive carcinoma
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma	CHOL	Cholangio carcinoma	COAD	Colon adenocarcinoma
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma	ESCA	Esophageal carcinoma	GBM	Glioblastoma multiforme
HNSC	Head and Neck squamous cell carcinoma	KICH	Kidney Chromophobe	KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma	LAML	Acute Myeloid Leukemia	LGG	Brain Lower Grade Glioma
LIHC	Liver hepatocellular carcinoma	LUAD	Lung adenocarcinoma	LUSC	Lung squamous cell carcinoma
MESO	Mesothelioma	OV	Ovarian serous cystadeno-carcinoma	PAAD	Pancreatic adenocar-cinoma
PCPG	Pheochromocytoma and Paraganglioma	PRAD	Prostate adenocarcinoma	READ	Rectum adenocarcinoma
SARC	Sarcoma	SKCM	Skin Cutaneous Melanoma	STAD	Stomach adenocar-cinoma
UCEC	Uterine Corpus Endometrial Carcinoma	UCS	Uterine Carcinosarcoma	UVM	Uveal Melanoma
TGCT	Testicular Germ Cell Tumors	THCA	Thyroid carcinoma	THYM	Thymoma

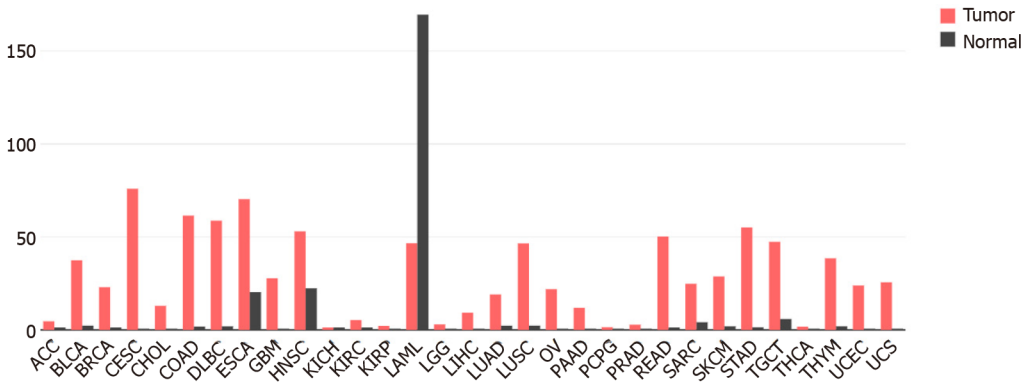
Table 2 The traditional Chinese medicine monomers confirmed or predicted having ribonucleotide reductase M2 inhibition in tumors

Name	*In vivo* study	*In vitro* study	Bioinformatics analysis
Berberine	-	Non-small cell lung cancer	Breast cancer
Ursolic acid	-	-	Hepatoma, Colon cancer
Gambogic acid	Pancreatic cancer	Pancreatic cancer	-
Cinobufagin	Endometrial carcinoma	Endometrial carcinoma	-
Quercetin	-	-	Hepatoma, Colon cancer
Daphnetin	-	-	-
Osalmide	Esophageal Cancer, Multiple myeloma, Hepato-cellular carcinoma, Diffuse large B-cell lymphoma	Esophageal Cancer, Multiple myeloma, Hepato-cellular carcinoma, Diffuse large B-cell lymphoma	Multiple myeloma, Hepato-cellular carcinoma

potential target of ursolic acid in tumors[23], but still needs to be further confirmed in clinical and experimental studies.

Gambogic acid: Gambogic acid, a kind of caged xanthone extracted from dry resin secreted by Garcinia hanburyi tree, has the functions of promoting blood circulation, anti-tumor, detoxification, and hemostasis[24]. According to numerous studies, multiple carcinomas, including breast cancer, lung cancer, liver cancer, colon cancer, and pancreatic cancer, were inhibited by gambogic acid[25]. Through the combination of several major targets such as VEGF, Bcl-2, MDM2, MMP-9, MMP-2, EGFR, and P53, gambogic acid promotes tumor cell apoptosis, autophagy, and arrests cell cycle, thereby inhibiting tumor invasion, metastasis, and angiogenesis[26]. An investigation in pancreatic cancer demonstrated that following treatment with gambogic acid *in vivo* and *in vitro*, the expression of RRM2 protein and mRNA was significantly decreased[6], suggesting that RRM2 may also be the target of gambogic acid in tumor treatment.

Cinobufagin: Bufadienolide cinobufagin, which is extracted from the Asiatic toad Bufo gargarizans, has analgesic, detoxifying, and detumescent properties[27]. Some investigations conducted recently have revealed that it has potent anti-tumor effects as well. In non-small cell lung cancer, cinobufagin could suppress proliferation, migration, and invasion of cancer cells by inhibiting the expression of G9a[27]. By interfering with the cell cycle, cinobufagin also inhibits the survival of cancer cells and promotes apoptosis[28]. Moreover, many other anti-tumor pathways are involved, such as the Notch signaling



DOI: 10.12998/wjcc.v10.i31.11299

Figure 1 The median ribonucleotide reductase M2 gene expression profile across all tumor samples and paired normal tissues (bar plot). ACC: Adrenocortical carcinoma; BLCA: Bladder Urothelial Carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangio carcinoma; COAD: Colon adenocarcinoma; DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; HNSC: Head and Neck squamous cell carcinoma; KICH: Kidney Chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute Myeloid Leukemia; LGG: Brain Lower Grade Glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; OV: Ovarian serous cystadenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and Paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin Cutaneous Melanoma; STAD: Stomach adenocarcinoma; TGCT: Testicular Germ Cell Tumors; THCA: Thyroid carcinoma; THYM: Thymoma; UCEC: Uterine Corpus Endometrial Carcinoma; UCS: Uterine Carcinosarcoma.

pathway[29], AURKA/mTOR/eIF4E axis[30], c-Myc pathway[31], and ROS/JNK/p38 signaling pathway[32]. After cinobufagin treatment, the expression of RRM2 in endometrial carcinoma (Ishikawa cell line) decreased significantly at gene and protein levels, inhibiting cell proliferation and reducing invasiveness[33]. *In vivo* studies likewise produced the same results[34]. Thus, cinobufagin is expected to be an RRM2 inhibitor with multiple anti-tumor effects.

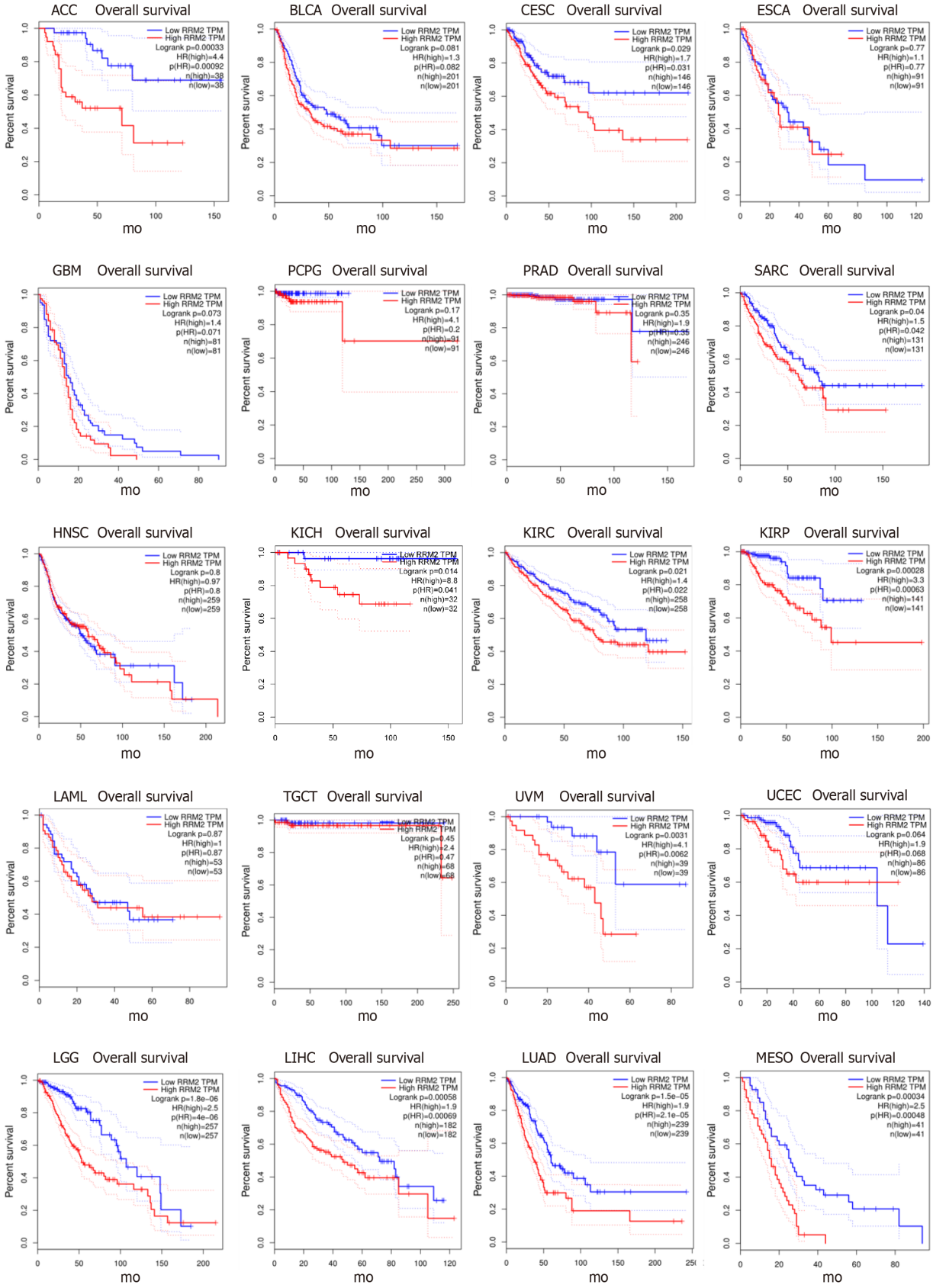
Quercetin: Quercetin, a flavonol compound widely existing in many plants, has been reported to have multiple pharmacological effects on preventing osteoporosis, cardiovascular disease, aging, and tumors [35]. In terms of anti-tumor properties, the main mechanisms are to regulate the viability, apoptosis, and autophagy of tumor cells through PI3K/Akt/mTOR, Wnt/β-Catenin, and MAPK/ERK1/2 pathways [36], and then exhibits inhibitory activities against a variety of tumors, such as colon cancer (Caco-2 cell line), lung cancer (NCI-H446, A549 cell line), and gastric cancer (MGC-803, SGC-7901 cell line)[37]. A comprehensive analysis based on differential genes and drug targets found that quercetin was closely related to RRM2[23]. After treatment with quercetin, the activity of Leishmania donovani was inhibited by targeting RR[38]. Therefore, we speculate that the anti-tumor effect of quercetin may be partially attributed to the inhibition of RRM2.

Daphnetin: Daphnetin is a coumarin derivative with rich pharmacological activity, extracted from Daphne odora. It is often used in the treatment and research of neurological diseases, malaria, parasites, and arthritis[39]. Currently, some studies suggest that daphnetin also has an inhibitory effect on tumor growth, with the mechanisms of action including downregulating Cyclin D1 expression in breast cancer (MCF-7 cell line), inducing G2/M and S phase arrest in hepatoma cells (SMMC-7721 cell line), suppressing the Akt/NF-κB signaling pathway in lung adenocarcinoma (A549 cell line), and inhibiting the AMPK/Akt/mTOR pathway in ovarian cancer (A2780 cell line)[40]. In addition, a study on malaria found that daphnetin could also inhibit the expression and activity of RR by binding to the iron-containing group (RRM2)[41]. However, as an indispensable key enzyme for tumor growth, whether daphnetin can inhibit RRM2 in human tumor cells needs further research to confirm.

Osalmid: In clinical practice, osalmid has been used to treat biliary tract inflammation, cholecystitis, and post cholecystectomy syndrome. By decreasing RRM2 activity and activating P53, it was found that osalmid also inhibits the progression of human hepatocellular carcinoma[42]. The expression of RRM2 in esophageal cancer was similarly inhibited by osalmid. In addition to promoting apoptosis, blocking cell cycle and DNA damage, and inhibiting the proliferation and migration of tumor cells, the radiosensitivity was enhanced[43]. Due to their powerful anti-tumor activities, osalmid and its derivatives have been used in numerous investigations as new RRM2 inhibitors[44-46].

The screened TCM monomers have a good binding capacity with RRM2 protein

Molecular docking presented interaction between the aforementioned seven TCM monomers and RRM2 protein, and the results showed that they all had a strong binding capacity. The specific results are


ACC Overall survival
Low RRM2 TPM
High RRM2 TPM
Logrank p=0.00033
HR(high)=4.4
p(HR)=0.00092
n(high)=38
n(low)=38
BLCA Overall survival
Logrank p=0.081
HR(high)=1.3
p(HR)=0.082
n(high)=201
n(low)=201
CESC Overall survival
Logrank p=0.029
HR(high)=1.7
p(HR)=0.031
n(high)=146
n(low)=146
ESCA Overall survival
Logrank p=0.77
HR(high)=1.1
p(HR)=0.77
n(high)=91
n(low)=91
GBM Overall survival
Logrank p=0.073
HR(high)=1.4
p(HR)=0.071
n(high)=81
n(low)=81
PCPG Overall survival
Logrank p=0.17
HR(high)=4.1
p(HR)=0.2
n(high)=91
n(low)=91
PRAD Overall survival
Logrank p=0.35
HR(high)=1.9
p(HR)=0.35
n(high)=246
n(low)=246
SARC Overall survival
Logrank p=0.04
HR(high)=1.5
p(HR)=0.042
n(high)=131
n(low)=131
HNSC Overall survival
Logrank p=0.8
HR(high)=0.97
p(HR)=0.8
n(high)=259
n(low)=259
KICH Overall survival
Logrank p=0.014
HR(high)=8.8
p(HR)=0.041
n(high)=32
n(low)=32
KIRC Overall survival
Logrank p=0.021
HR(high)=1.4
p(HR)=0.022
n(high)=258
n(low)=258
KIRP Overall survival
Logrank p=0.00028
HR(high)=3.3
p(HR)=0.00063
n(high)=141
n(low)=141
LAML Overall survival
Logrank p=0.87
HR(high)=1
p(HR)=0.87
n(high)=53
n(low)=53
TGCT Overall survival
Logrank p=0.45
HR(high)=2.4
p(HR)=0.47
n(high)=68
n(low)=68
UVM Overall survival
Logrank p=0.0031
HR(high)=4.1
p(HR)=0.0062
n(high)=39
n(low)=39
UCEC Overall survival
Logrank p=0.064
HR(high)=1.9
p(HR)=0.068
n(high)=86
n(low)=86
LGG Overall survival
Logrank p=1.8e-06
HR(high)=2.5
p(HR)=4e-06
n(high)=257
n(low)=257
LIHC Overall survival
Logrank p=0.00058
HR(high)=1.9
p(HR)=0.00069
n(high)=182
n(low)=182
LUAD Overall survival
Logrank p=1.5e-05
HR(high)=1.9
p(HR)=2.1e-05
n(high)=239
n(low)=239
MESO Overall survival
Logrank p=0.00034
HR(high)=2.5
p(HR)=0.00048
n(high)=41
n(low)=41
Percent survival
mo

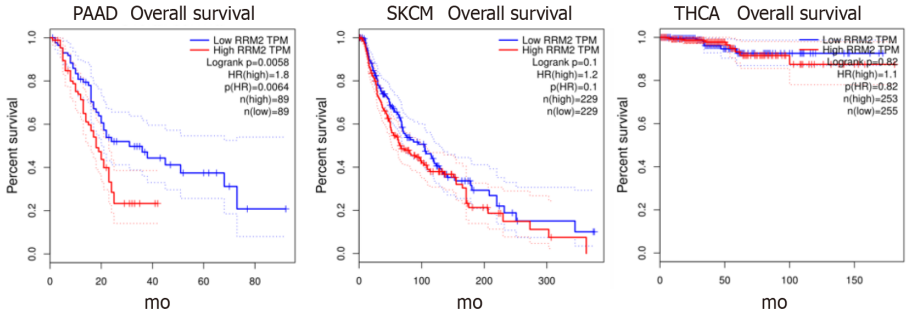


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Figure 2 The twenty-three types of tumors with a negative correlation between ribonucleotide reductase M2 gene expression and overall survival rate. ACC: Adrenocortical carcinoma; BLCA: Bladder Urothelial Carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; ESCA: Esophageal carcinoma; GBM: Glioblastoma multiforme; HNSC: Head and Neck squamous cell carcinoma; KICH: Kidney Chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LAML: Acute Myeloid Leukemia; LGG: Brain Lower Grade Glioma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and Paraganglioma; PRAD: Prostate adenocarcinoma; SARC: Sarcoma; SKCM: Skin Cutaneous Melanoma; UVM: Uveal Melanoma; TGCT: Testicular Germ Cell Tumors; THCA: Thyroid carcinoma; UCEC: Uterine Corpus Endometrial Carcinoma.

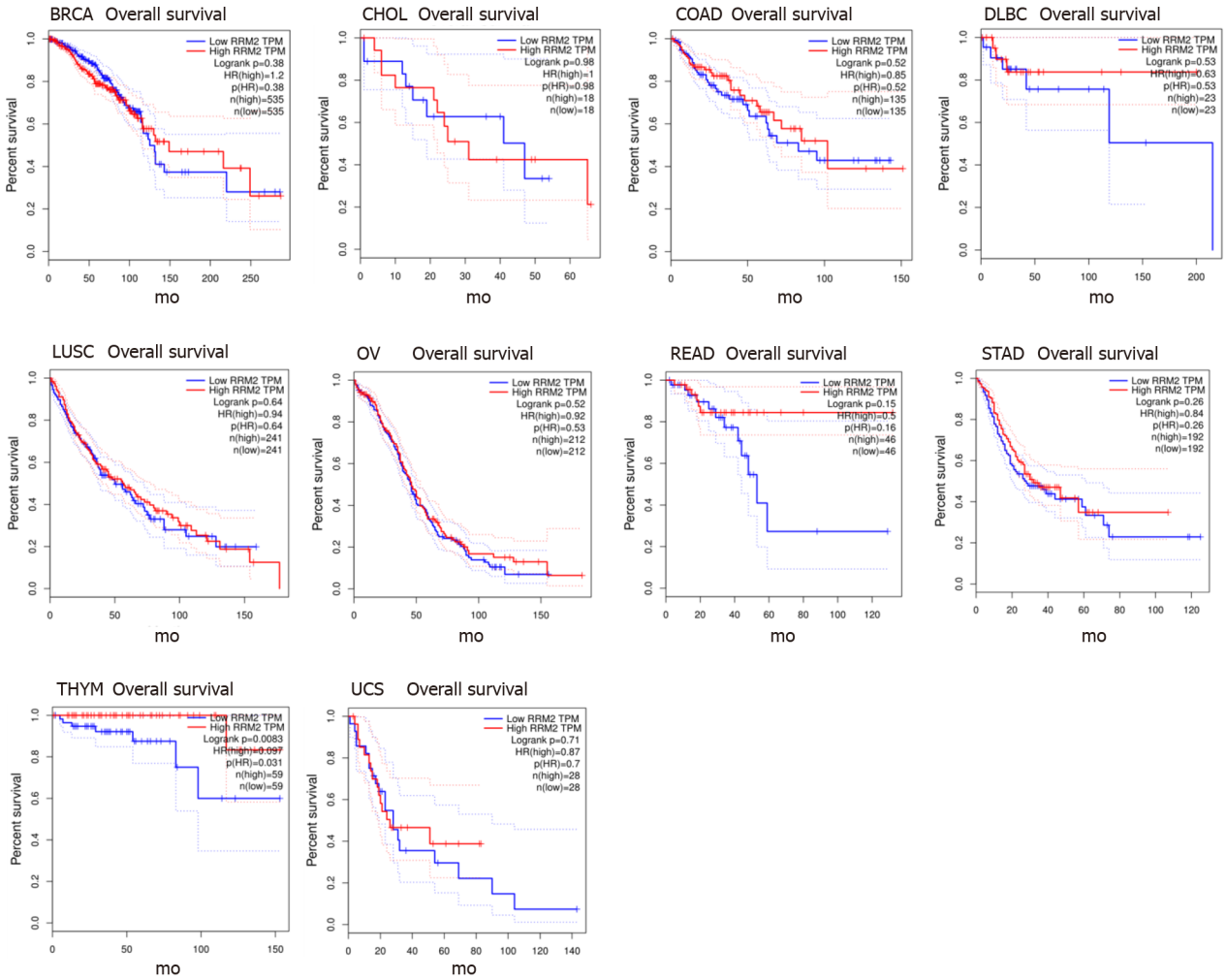

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Figure 3 The ten types of tumors with a positive correlation between ribonucleotide reductase M2 gene expression and overall survival rate. BRCA: Breast invasive carcinoma; CHOL: Cholangio carcinoma; COAD: Colon adenocarcinoma; DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; LUSC: Lung squamous cell carcinoma; OV: Ovarian serous cystadenocarcinoma; READ: Rectum adenocarcinoma; STAD: Stomach adenocarcinoma; THYM: Thymoma; UCS: Uterine Carcinosarcoma.

described in detail in the following sections (Table 3).

Berberine bonded to the RRM2 protein with a binding energy of -7.3 kcal/mol, mostly made up of one hydrogen bond and eight hydrophobic bonds (Figure 4A). The hydrogen bond was mainly localized at Ser263(A) of RRM2, with a length of 3.73 Å, and the hydrophobic sites were situated at Glu260(A), Arg264(A), Tyr323(A), Arg330(A), Gly233(A), Val327(A), Ser237(A) and Gly267(A) of RRM2.

Ursolic acid and RRM2 protein had binding energy of -8.6 kcal/mol (Figure 4B). There was just hydrophobic force between them. Arg264(A), Gly267(A), Cys270(A), Gly233(A), Arg330(A), Val327(A), Ser263(A), Phe244(A), Phe349(A), and Met350(A) of RRM2 were the primary hydrophobic action sites.

Gambogic acid and RRM2 protein bonded with a -8.6 kcal/mol binding energy (Figure 4C). Their interaction was achieved through the formation of hydrogen bonds and hydrophobic forces. The hydrogen bonds in RRM2 were at positions Arg330(A) and Tyr323(A), with lengths of 3.24 Å and 2.74 Å, respectively. The hydrophobic action sites in RRM2 were found at Gly267(A), Leu268(A), Ser100(A), Lys96(A), Arg264(A), Met350(A), and Ser263(A).

The binding energy between cinobufagin and RRM2 protein was -7.6 kcal/mol (Figure 4D). They interacted with each other through the formation of hydrogen bonds and hydrophobic force. The hydrogen bond lengths were 3.88 Å and 2.82 Å, respectively, which were located at Asp271(A) and Arg330(A) of RRM2. The hydrophobic effect was generated on Glu334(A), Leu331(A), Cys270(A), Gly267(A), Glu266(A), Ser263(A), Phe244(A), and Met350(A) of RRM2 and cinobufagin.

Quercetin and RRM2 protein had binding energy of -7.4 kcal/mol (Figure 5A). Quercetin mainly forms three hydrogen bonds and nine hydrophobic forces with RRM2. The hydrogen bond lengths were 3.16 Å, 3.05 Å, and 2.77 Å, respectively, which were mainly formed in Arg330(A) and Tyr323(A) of RRM2. The hydrophobic sites were found in the following positions in RRM2: Met350(A), Arg264(A), Glu260(A), Phe244(A), Ser263(A), Glu232(A), Gly233(A), Phe240(A) and Val327(A).

The binding energy between daphnetin and RRM2 protein was -6.7 kcal/mol (Figure 5B). Tyr323 (A) and Arg330 (A) of RRM2 made four hydrogen bonds with daphnetin, whereas Val327(A), Ser263(A), Phe240(A), Met350(A), Gly267(A), Gly233(A), and Gys270(A) of RRM2 formed seven hydrophobic forces with daphnetin. Whose hydrogen bonds had lengths of 2.87 Å, 3.19 Å, 2.80 Å, and 3.24 Å, respectively.

Osalmide and RRM2 protein had a -6.8 kcal/mol binding energy (Figure 5C). They only interacted hydrophobically, and their hydrophobic interaction sites were found in Phe244(A), Arg264(A), Tyr323(A), Phe240(A), Ser237(A), Met350(A), Gly233(A), and Ser263(A) of RRM2.

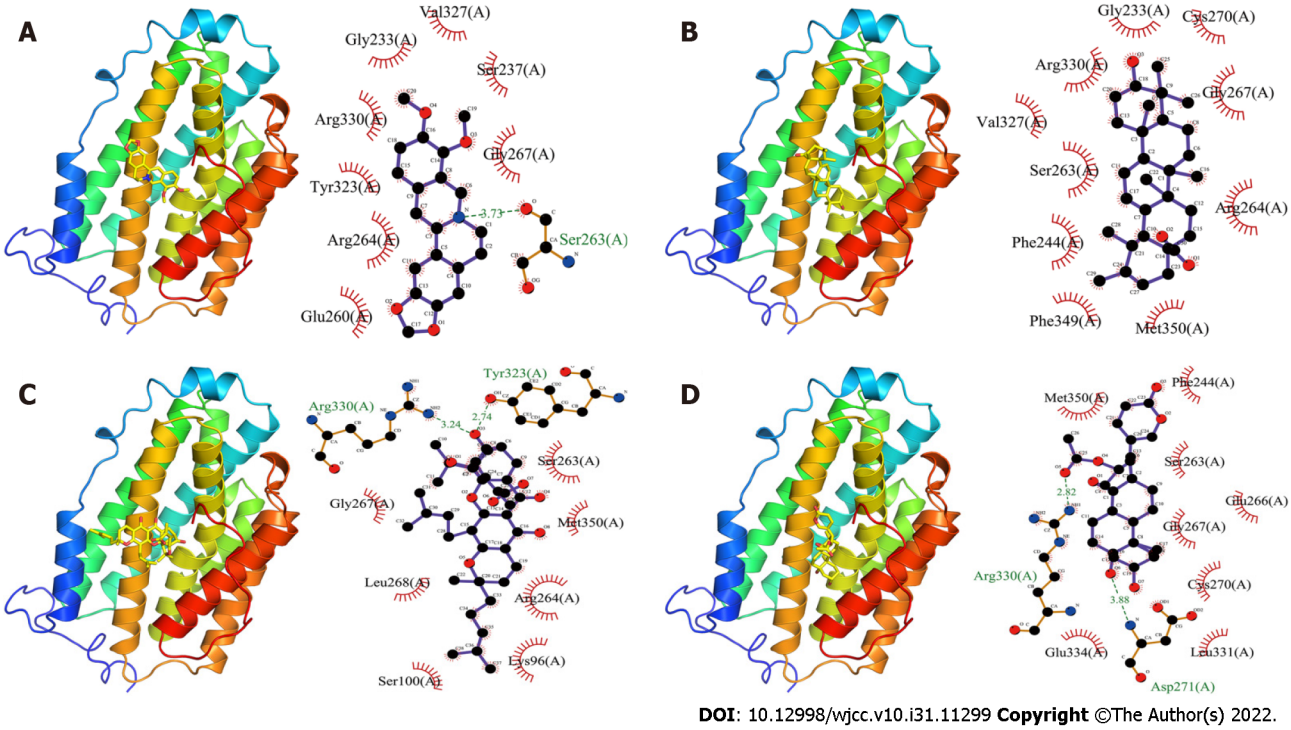
DISCUSSION

Nowadays, the acknowledged tumor treatment strategies include surgical resection, chemotherapy, and radiotherapy, as well as biotherapy, immunotherapy, and targeted therapy developed in recent decades. Due to some limitations and defects, monotherapy does not seem to be able to fully achieve the ideal effect[47]. Therefore, combination therapy and adjuvant therapy are often required. As a natural medicine, some active ingredients of TCM have been proven to have excellent anti-tumor activity. TCM can not only inhibit the proliferation of tumor cells through multiple targets, improve the cancer microenvironment, and strengthen the function of anti-tumor immunity, but also enhance the efficacy of chemotherapy, radiotherapy, targeted therapy, and immunotherapy, and reduce the damage caused by these therapies, to prolong the survival time of tumor patients and improve the quality of life to a certain extent[48]. Because of their advantages of broad spectrum, high efficacy, low toxicity, and strong specificity, TCMs and extracts are widely used as adjuvant therapy for tumors in clinics[49]. Paclitaxel, vinblastine, and hydroxycamptothecin are three examples of commonly used clinical chemotherapeutic medicines[50-52]. Compared with traditional synthetic medications, the anti-tumor mechanisms of TCMs are more complex and extensive. They involve multiple signaling pathways and biological targets related to cancer. Despite the long history of TCM study, part of the mechanism of action and molecular targets are not completely clear[53]. TCM monomers, as the active compound of TCM, including their functions still need to be further explored and studied.

Deoxyribonucleotide triphosphate (dNTP), the building block for DNA synthesis, is in high demand in tumors. As the key enzyme of DNA synthesis, RR not only participates in DNA synthesis and repair *via* producing dNTP but is also involved in cell cycle regulation[5,54]. RRM2 is an important subunit of RR, which also play a regulatory role in multiple biological processes, including the survival, proliferation, apoptosis, and chemoresistance of various cancer cells[7]. According to GEPIA database analysis, we found that RRM2 is highly expressed in more than 30 types of tumor tissues, and negatively correlated with the overall survival rate of patients with the majority of tumor types. A study in prostate cancer has found that RRM2 is a driver of aggressive subtypes, and elevated RRM2 contributes to tumor cell immune escape[55]. The overexpression of RRM2 in breast cancer cells activated NF-κB and MMP-9 to alter the tumor microenvironment, thereby enhancing the migratory abilities of tumor cells[56]. Increased RRM2 expression is also associated with tamoxifen resistance, inhibition of RRM2 not only reduced migration and invasion characteristics of cancer cells *in vitro* but also reversed tamoxifen resistance of breast cancer cells, which may be mediated by NF-κB, HIF-1α, and

Table 3 The binding energy and binding sites of the selected traditional Chinese medicine monomers with ribonucleotide reductase M2

Names	Binding energy	Hydrogen bond site	Hydrophobic bond site
Berberine	-7.3 kcal/mol	Ser263 (A)	Glu260(A), Arg264(A), Tyr323(A), Arg330(A), Gly233(A), Val327(A), Ser237(A), and Gly267(A)
Ursolic acid	-8.6 kcal/mol	-	Arg264(A), Gly267(A), Cys270(A), Gly233(A), Arg330(A), Val327(A), Ser263(A), Phe244(A), Phe349(A), and Met350(A)
Gambogic acid	-8.6 kcal/mol	Arg330(A) and Tyr323(A)	Gly267(A), Leu268(A), Ser100(A), Lys96(A), Arg264(A), Met350(A), and Ser263(A)
Cinobufagin	-7.6 kcal/mol	Asp271 (A) and Arg330 (A)	Glu334 (A), Leu331 (A), Cys270 (A), Gly267 (A), Glu266 (A), Ser263 (A), Phe244 (A), and Met350 (A)
Quercetin	-7.4 kcal/mol	Arg330 (A) and Tyr323 (A)	Met350 (A), Arg264 (A), Glu260 (A), Phe244 (A), Ser263 (A), Glu232 (A), Gly233 (A), Phe240 (A) and Val327 (A)
Daphnetin	-6.7 kcal/mol	Tyr323 (A) and Arg330 (A)	Val327 (A), Ser263 (A), Phe240 (A), Met350 (A), Gly267 (A), Gly233 (A), and Gys270 (A)
Osalmide	-6.8 kcal/mol	-	Phe244 (A), Arg264 (A), Tyr323 (A), Phe240 (A), Ser237 (A), Met350 (A), Gly233 (A), and Ser263 (A)

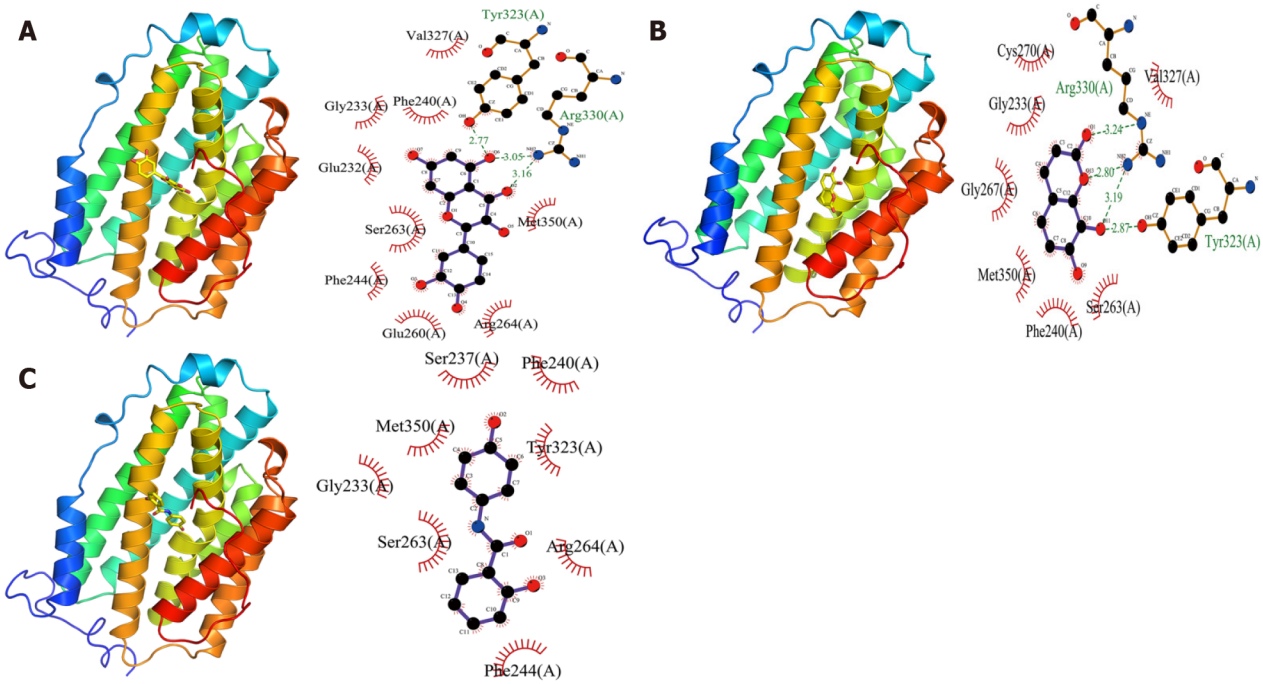


DOI: 10.12998/wjcc.v10.i31.11299

Figure 4 Berberine/ursolic acid/gambogic acid/cinobufagin and RRM2 protein have binding energy. A: The molecular docking diagram of berberine with ribonucleotide reductase M2 (RRM2) protein; B: The molecular docking diagram of ursolic acid with RRM2 protein; C: The molecular docking diagram of gambogic acid with RRM2 protein; D: The molecular docking diagram of cinobufagin with RRM2 protein.

MAPK/JNK pathways[57]. GW8510 acts as an RRM2 inhibitor, improving acquired tamoxifen resistance in breast cancer cells by autophagy induction, a similar effect was seen in lung squamous cell carcinoma cells[58,59]. Besides, knockdown of RRM2 enhanced the drug sensitivity of chronic myeloid leukemia to imatinib treatment by activating the Bcl-2/caspase apoptosis pathway and inhibiting the Akt cell signaling pathway[60]. These results indicate that RRM2 is an independent predictor of poor prognosis in a variety of tumors and could be a good target for tumor therapy.

RRM2 has two important drug binding targets: tyrosine free radical and divalent iron radical, most of the currently developed RRM2 inhibitors act on these two targets[61]. Hydroxyurea is a common anti-tumor chemotherapy drug as well as an RRM2 inhibitor, which can inhibit RRM2 activity by scavenging tyrosine free radicals, and then inhibit DNA synthesis[62]. Gallium, an iron analog, has chemical characteristics similar to iron. Though interacting with iron-binding protein, gallium interferes with cellular iron uptake and damages iron homeostasis in cells, resulting in the inhibition of RRM2 function[63]. Triapine also inhibits RRM2 activity by forming iron chelates with iron groups[64]. However, some



DOI: 10.12998/wjcc.v10.i31.11299

Figure 5 Quercetin/daphnetin/osalmide and ribonucleotide reductase M2 protein have binding energy. A: The molecular docking diagram of quercetin with ribonucleotide reductase M2 (RRM2) protein; B: The molecular docking diagram of daphnetin with RRM2 protein; C: The molecular docking diagram of osalmide with RRM2 protein.

RRM2 inhibitors may lead to different degrees of side effects such as blood and lymphatic system metabolic disorders, liver and kidney dysfunction, gastrointestinal reactions, and reproductive toxicity [65,66]. Therefore, it is urgent to develop or find new RRM2 inhibitors that are safer, more effective, and more specific.

Through literature mining, we retrieved seven TCM monomers with an inhibitory effect on RRM2 in tumors. They all have good binding capacities with RRM2, according to molecular docking analysis, with binding energies ranging from -8.6 to -6.8 kcal/mol. The hydrogen bonds and/or hydrophobic forces are the main contributors to these binding energies, their major active sites are Arg330, Tyr323, Ser263, and Met350 of RRM2. Among them, Arg330 is the site where the most hydrogen bonds are formed between TCM monomer and RRM2, followed by Tyr323. The locations with the highest frequency of hydrophobic action are Ser263 and Met350, the next two are Gly267 and Arg264. These findings imply that Arg330, Tyr323, Ser263, and Met350 may be important binding sites of RRM2 inhibitors with RRM2, which will provide some thoughts for the development of new anti-tumor drugs with RRM2 inhibition based on these sites.

CONCLUSION

RRM2 is a crucial tumor therapeutic target. It is highly expressed in almost all tumors and negatively correlated with the overall survival rate of patients with the majority of tumor types. The seven screened TCM monomers have a good binding capacity to RRM2, and their binding sites are mainly concentrated in Arg330, Tyr323, Ser263, and Met350 of RRM2. This will provide theoretical support and a point for the development of anti-tumor medications with RRM2 inhibition based on these binding sites. Meanwhile, natural drugs with abundant structures are an important source for the development of anti-tumor drugs, it is anticipated that more effective RRM2 inhibitors will be developed through in-depth research.

ARTICLE HIGHLIGHTS

Research background

The tumor is a major contributor to endangering human health, traditional Chinese medicine (TCM) monomer is an important source of anti-tumor drugs. Ribonucleotide reductase (RR) is a key enzyme in tumor proliferation, especially its subunit-RRM2. Screening and analysis of TCM monomers with RRM2

inhibition can provide a reference for further anti-tumor drug development.

Research motivation
To screen and analyze potential anti-tumor TCM monomers with a good binding capacity to RRM2, and provide some thoughts for the development of anti-tumor drugs with RRM2 inhibition in the future.

Research objectives
To clarify the relationship between RRM2 and malignant tumors. To clarify the relationship between RRM2 and the prognosis of tumor patients. To screen and analyze potential anti-tumor TCM monomers with a good binding capacity to RRM2, and provide some thoughts for the development of anti-tumor drugs with RRM2 inhibition in the future.

Research methods
The GEPIA database was used to analyze the level of RRM2 gene expression in normal and tumor tissues as well as RRM2's effect on the overall survival rate of tumor patients. TCM monomers that potentially act on RRM2 were screened *via* literature mining. Using AutoDock software, the screened monomers were docked with the RRM2 protein.

Research results
The expression of RRM2 mRNA in multiple tumor tissues was significantly higher than that in normal tissues, and RRM2 was negatively correlated with the overall survival rate of patients with the majority of tumor types. Berberine, ursolic acid, gambogic acid, cinobufagin, quercetin, daphnetin, and osalmide have inhibitory effects on RRM2. The screened TCM monomers had a strong binding capacity with RRM2 protein.

Research conclusions
RRM2 is an important tumor therapeutic target. The screened TCM monomers have a good binding ability with the RRM2.

Research perspectives
Their main binding sites could provide new thoughts for the development of anti-tumor drugs with RRM2 inhibition.

FOOTNOTES

Author contributions: Qin YY wrote the manuscript, designed the study, and acquired data; Feng S acquired, analyzed, and interpreted the data; Zhang XD supervised, reviewed, and edited the manuscript; Peng B designed and coordinated the study, collected funds; all authors approved the final draft.

Supported by Nanchong City School's Science and Technology Strategic Cooperation, China, No. 20SXQT0304; Research and Development Project Plan of Affiliated Hospital of North Sichuan Medical College, China, No. 2020ZD003.

Institutional review board statement: Not applicable.

Informed consent statement: Not applicable.

Conflict-of-interest statement: All authors declare that they have no conflicts of interest.

Data sharing statement: No additional data are available.

Country/Territory of origin: China

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S-Editor: Liu JH
L-Editor: A

P-Editor: Liu JH

REFERENCES

1 **Wild CP WE**, Stewart BW. World Cancer Report: Cancer Research for Cancer Prevention: world health organization; 2021

2 **Sharma P**, McClees SF, Afaq F. Pomegranate for Prevention and Treatment of Cancer: An Update. *Molecules* 2017; **22** [PMID: 28125044 DOI: 10.3390/molecules22010177]

3 **Nordlund P**, Reichard P. Ribonucleotide reductases. *Annu Rev Biochem* 2006; **75**: 681-706 [PMID: 16756507 DOI: 10.1146/annurev.biochem.75.103004.142443]

4 **Subramaniam R**, Lamb NA, Hwang Y, Johengen L, Surtees JA. Extracting and Measuring dNTP Pools in Saccharomyces cerevisiae. *Methods Mol Biol* 2019; **1999**: 103-127 [PMID: 31127572 DOI: 10.1007/978-1-4939-9500-4_6]

5 **Li J**, Pang J, Liu Y, Zhang J, Zhang C, Shen G, Song L. Suppression of RRM2 inhibits cell proliferation, causes cell cycle arrest and promotes the apoptosis of human neuroblastoma cells and in human neuroblastoma RRM2 is suppressed following chemotherapy. *Oncol Rep* 2018; **40**: 355-360 [PMID: 29749541 DOI: 10.3892/or.2018.6420]

6 **Xia G**, Wang H, Song Z, Meng Q, Huang X. Gambogic acid sensitizes gemcitabine efficacy in pancreatic cancer by reducing the expression of ribonucleotide reductase subunit-M2 (RRM2). *J Exp Clin Cancer Res* 2017; **36**: 107 [PMID: 28797284 DOI: 10.1186/s13046-017-0579-0]

7 **Zhan Y**, Jiang L, Jin X, Ying S, Wu Z, Wang L, Yu W, Tong J, Zhang L, Lou Y, Qiu Y. Inhibiting RRM2 to enhance the anticancer activity of chemotherapy. *Biomed Pharmacother* 2021; **133**: 110996 [PMID: 33227712 DOI: 10.1016/j.biopha.2020.110996]

8 **Knighton LE**, Delgado LE, Truman AW. Novel insights into molecular chaperone regulation of ribonucleotide reductase. *Curr Genet* 2019; **65**: 477-482 [PMID: 30519713 DOI: 10.1007/s00294-018-0916-7]

9 **Chen WX**, Yang LG, Xu LY, Cheng L, Qian Q, Sun L, Zhu YL. Bioinformatics analysis revealing prognostic significance of *RRM2* gene in breast cancer. *Biosci Rep* 2019; **39** [PMID: 30898978 DOI: 10.1042/BSR20182062]

10 **Aye Y**, Li M, Long MJ, Weiss RS. Ribonucleotide reductase and cancer: biological mechanisms and targeted therapies. *Oncogene* 2015; **34**: 2011-2021 [PMID: 24909171 DOI: 10.1038/onc.2014.155]

11 **Mannargudi MB**, Deb S. Clinical pharmacology and clinical trials of ribonucleotide reductase inhibitors: is it a viable cancer therapy? *J Cancer Res Clin Oncol* 2017; **143**: 1499-1529 [PMID: 28624910 DOI: 10.1007/s00432-017-2457-8]

12 **Ortiz LM**, Lombardi P, Tillhon M, Scovassi AI. Berberine, an epiphany against cancer. *Molecules* 2014; **19**: 12349-12367 [PMID: 25153862 DOI: 10.3390/molecules190812349]

13 **Song D**, Hao J, Fan D. Biological properties and clinical applications of berberine. *Front Med* 2020; **14**: 564-582 [PMID: 32335802 DOI: 10.1007/s11684-019-0724-6]

14 **Liu Q**, Jiang H, Liu Z, Wang Y, Zhao M, Hao C, Feng S, Guo H, Xu B, Yang Q, Gong Y, Shao C. Berberine radiosensitizes human esophageal cancer cells by downregulating homologous recombination repair protein RAD51. *PLoS One* 2011; **6**: e23427 [PMID: 21858113 DOI: 10.1371/journal.pone.0023427]

15 **Tillhon M**, Guamán Ortiz LM, Lombardi P, Scovassi AI. Berberine: new perspectives for old remedies. *Biochem Pharmacol* 2012; **84**: 1260-1267 [PMID: 22842630 DOI: 10.1016/j.bcp.2012.07.018]

16 **Li J**, Gu L, Zhang H, Liu T, Tian D, Zhou M, Zhou S. Berberine represses DAXX gene transcription and induces cancer cell apoptosis. *Lab Invest* 2013; **93**: 354-364 [PMID: 23295648 DOI: 10.1038/labinvest.2012.172]

17 **Hao M**, Liu W, Ding C, Peng X, Zhang Y, Chen H, Dong L, Liu X, Zhao Y, Chen X, Khatoon S, Zheng Y. Identification of hub genes and small molecule therapeutic drugs related to breast cancer with comprehensive bioinformatics analysis. *PeerJ* 2020; **8**: e9946 [PMID: 33083112 DOI: 10.7717/peerj.9946]

18 **Ni L**, Li Z, Ren H, Kong L, Chen X, Xiong M, Zhang X, Ning B, Li J. Berberine inhibits non-small cell lung cancer cell growth through repressing DNA repair and replication rather than through apoptosis. *Clin Exp Pharmacol Physiol* 2022; **49**: 134-144 [PMID: 34448246 DOI: 10.1111/1440-1681.13582]

19 **Jinhua W**. Ursolic acid: Pharmacokinetics process in vitro and in vivo, a mini review. *Arch Pharm (Weinheim)* 2019; **352**: e1800222 [PMID: 30663087 DOI: 10.1002/ardp.201800222]

20 **Seo DY**, Lee SR, Heo JW, No MH, Rhee BD, Ko KS, Kwak HB, Han J. Ursolic acid in health and disease. *Korean J Physiol Pharmacol* 2018; **22**: 235-248 [PMID: 29719446 DOI: 10.4196/kjpp.2018.22.3.235]

21 **Alam M**, Ali S, Ahmed S, Elasbali AM, Adnan M, Islam A, Hassan MI, Yadav DK. Therapeutic Potential of Ursolic Acid in Cancer and Diabetic Neuropathy Diseases. *Int J Mol Sci* 2021; **22** [PMID: 34830043 DOI: 10.3390/ijms222212162]

22 **Khwaza V**, Oyedeji OO, Aderibigbe BA. Ursolic Acid-Based Derivatives as Potential Anti-Cancer Agents: An Update. *Int J Mol Sci* 2020; **21** [PMID: 32824664 DOI: 10.3390/ijms21165920]

23 **Zhu HZ**, Sang TQ, JI Y, Li WT, Wu MH. [Study on Target of Treating Tumors Based on Active Constituents of Baihuasheshecao (Hedyotis diffusa) in XIaoai Jiedu Decoction]. *Zhonghua Zhong Yi Yao Xue Kan* 2020; **38**: 132-135+276

24 **Liu Q**, Shan P, Li H. Gambogic acid prevents angiotensin IIinduced abdominal aortic aneurysm through inflammatory and oxidative stress dependent targeting the PI3K/Akt/mTOR and NFκB signaling pathways. *Mol Med Rep* 2019; **19**: 1396-1402 [PMID: 30535428 DOI: 10.3892/mmr.2018.9720]

25 **Hatami E**, Jaggi M, Chauhan SC, Yallapu MM. Gambogic acid: A shining natural compound to nanomedicine for cancer therapeutics. *Biochim Biophys Acta Rev Cancer* 2020; **1874**: 188381 [PMID: 32492470 DOI: 10.1016/j.bbcan.2020.188381]

26 **Liu Y**, Chen Y, Lin L, Li H. Gambogic Acid as a Candidate for Cancer Therapy: A Review. *Int J Nanomedicine* 2020; **15**: 10385-10399 [PMID: 33376327 DOI: 10.2147/IJN.S277645]

27 **Zhang L**, Liang B, Xu H, Gong Y, Hu W, Jin Z, Wu X, Chen X, Li M, Shi L, Shi Y, Wang Y, Yang L. Cinobufagin induces FOXO1-regulated apoptosis, proliferation, migration, and invasion by inhibiting G9a in non-small-cell lung cancer

A549 cells. *J Ethnopharmacol* 2022; **291**: 115095 [PMID: 35176466 DOI: 10.1016/j.jep.2022.115095]
28 **Niu J**, Wang J, Zhang Q, Zou Z, Ding Y. Cinobufagin-induced DNA damage response activates G_2/M checkpoint and apoptosis to cause selective cytotoxicity in cancer cells. *Cancer Cell Int* 2021; **21**: 446 [PMID: 34425836 DOI: 10.1186/s12935-021-02150-0]
29 **Cao Y**, Yu L, Dai G, Zhang S, Zhang Z, Gao T, Guo W. Cinobufagin induces apoptosis of osteosarcoma cells through inactivation of Notch signaling. *Eur J Pharmacol* 2017; **794**: 77-84 [PMID: 27845066 DOI: 10.1016/j.ejphar.2016.11.016]
30 **Jin X**, Wang J, Zou S, Xu R, Cao J, Zhang Y, Guo J, Wen X, Deng S, Zheng Y, Zhu Y, Wang F, Xu Z. Cinobufagin Triggers Defects in Spindle Formation and Cap-Dependent Translation in Liver Cancer Cells by Inhibiting the AURKA-mTOR-eIF4E Axis. *Am J Chin Med* 2020; **48**: 651-678 [PMID: 32349518 DOI: 10.1142/S0192415X20500330]
31 **Hirasaki Y**, Okabe A, Fukuyo M, Rahmutulla B, Mano Y, Seki M, Hoshii T, Namiki T, Kaneda A. Cinobufagin inhibits proliferation of acute myeloid leukaemia cells by repressing c-Myc pathway-associated genes. *Chem Biol Interact* 2022; **360**: 109936 [PMID: 35447139 DOI: 10.1016/j.cbi.2022.109936]
32 **Ma K**, Zhang C, Huang MY, Li WY, Hu GQ. Cinobufagin induces autophagy-mediated cell death in human osteosarcoma U2OS cells through the ROS/JNK/p38 signaling pathway. *Oncol Rep* 2016; **36**: 90-98 [PMID: 27176794 DOI: 10.3892/or.2016.4782]
33 **Wang LL**, Zhou HJ, Hu YL, Xun QY. [Effect and significance of cinobufagin on RRM2 expression in the Ishikawa cells]. *Xian Dai Fu Chan Ke Jin Zhan* 2009; **18**: 269-272 (in Chinese)
34 **Feng K**, Zhou HJ, Hu YL. [Effect of cinobufotalin on growth of xenograft of endometrial carcinoma cell line ishikawa in nude mouse and its impact on RRM2 expression]. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 2010; **30**: 1183-1185 [PMID: 21275172]
35 **Boots AW**, Haenen GR, Bast A. Health effects of quercetin: from antioxidant to nutraceutical. *Eur J Pharmacol* 2008; **585**: 325-337 [PMID: 18417116 DOI: 10.1016/j.ejphar.2008.03.008]
36 **Reyes-Farias M**, Carrasco-Pozo C. The Anti-Cancer Effect of Quercetin: Molecular Implications in Cancer Metabolism. *Int J Mol Sci* 2019; **20** [PMID: 31261749 DOI: 10.3390/ijms20133177]
37 **Massi A**, Bortolini O, Ragno D, Bernardi T, Sacchetti G, Tacchini M, De Risi C. Research Progress in the Modification of Quercetin Leading to Anticancer Agents. *Molecules* 2017; **22** [PMID: 28758919 DOI: 10.3390/molecules22081270]
38 **Sen G**, Mukhopadhyay S, Ray M, Biswas T. Quercetin interferes with iron metabolism in Leishmania donovani and targets ribonucleotide reductase to exert leishmanicidal activity. *J Antimicrob Chemother* 2008; **61**: 1066-1075 [PMID: 18285311 DOI: 10.1093/jac/dkn053]
39 **Singh L**, Singh AP, Bhatti R. Mechanistic interplay of various mediators involved in mediating the neuroprotective effect of daphnetin. *Pharmacol Rep* 2021; **73**: 1220-1229 [PMID: 33860917 DOI: 10.1007/s43440-021-00261-z]
40 **Fan X**, Xie M, Zhao F, Li J, Fan C, Zheng H, Wei Z, Ci X, Zhang S. Daphnetin triggers ROS-induced cell death and induces cytoprotective autophagy by modulating the AMPK/Akt/mTOR pathway in ovarian cancer. *Phytomedicine* 2021; **82**: 153465 [PMID: 33486268 DOI: 10.1016/j.phymed.2021.153465]
41 **Huang F**, Tang LH, Chen B, Ni YC, Wang QM. [In vitro effect of daphnetin on cytochrome C oxidase and ribonucleotide reductase of plasmodium falciparum]. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* 2006; **24**: 179-182 [PMID: 17094616]
42 **Wu Z**, Zhan Y, Wang L, Tong J, Zhang L, Lin M, Jin X, Jiang L, Lou Y, Qiu Y. Identification of osalmid metabolic profile and active metabolites with anti-tumor activity in human hepatocellular carcinoma cells. *Biomed Pharmacother* 2020; **130**: 110556 [PMID: 32763815 DOI: 10.1016/j.biopha.2020.110556]
43 **Tang Q**, Wu L, Xu M, Yan D, Shao J, Yan S. Osalmid, a Novel Identified RRM2 Inhibitor, Enhances Radiosensitivity of Esophageal Cancer. *Int J Radiat Oncol Biol Phys* 2020; **108**: 1368-1379 [PMID: 32763454 DOI: 10.1016/j.ijrobp.2020.07.2322]
44 **Lu K**, Li B, Zhang H, Xu Z, Song D, Gao L, Sun H, Li L, Wang Y, Feng Q, Chen G, Hu L, Wei R, Xie Y, Yu D, Wu X, Zhu W, Shi J. A novel silicone derivative of natural osalmid (DCZ0858) induces apoptosis and cell cycle arrest in diffuse large B-cell lymphoma *via* the JAK2/STAT3 pathway. *Signal Transduct Target Ther* 2020; **5**: 31 [PMID: 32296013 DOI: 10.1038/s41392-020-0123-0]
45 **Zhou J**, Zhang M, Zhang Y, Shi X, Liu L, Yao R. Identification of Potential Prognostic Biomarker for Predicting Survival in Multiple Myeloma Using Bioinformatics Analysis and Experiments. *Front Genet* 2021; **12**: 722132 [PMID: 34567073 DOI: 10.3389/fgene.2021.722132]
46 **Hu X**, Zhou J, Zhang Y, Zeng Y, Jie G, Wang S, Yang A, Zhang M. Identifying potential prognosis markers in hepatocellular carcinoma *via* integrated bioinformatics analysis and biological experiments. *Front Genet* 2022; **13**: 942454 [PMID: 35928445 DOI: 10.3389/fgene.2022.942454]
47 **Xiang Y**, Guo Z, Zhu P, Chen J, Huang Y. Traditional Chinese medicine as a cancer treatment: Modern perspectives of ancient but advanced science. *Cancer Med* 2019; **8**: 1958-1975 [PMID: 30945475 DOI: 10.1002/cam4.2108]
48 **Wang S**, Long S, Deng Z, Wu W. Positive Role of Chinese Herbal Medicine in Cancer Immune Regulation. *Am J Chin Med* 2020; **48**: 1577-1592 [PMID: 33202152 DOI: 10.1142/S0192415X20500780]
49 **Liu Y**, Yang S, Wang K, Lu J, Bao X, Wang R, Qiu Y, Wang T, Yu H. Cellular senescence and cancer: Focusing on traditional Chinese medicine and natural products. *Cell Prolif* 2020; **53**: e12894 [PMID: 32881115 DOI: 10.1111/cpr.12894]
50 **Zhu L**, Chen L. Progress in research on paclitaxel and tumor immunotherapy. *Cell Mol Biol Lett* 2019; **24**: 40 [PMID: 31223315 DOI: 10.1186/s11658-019-0164-y]
51 **Meynard L**, Galtier J, Favre S, Debus L, Lascaux A, Dilhuydy MS, Gros FX, Sauvezie M, Milpied N, Bouabdallah K, Dimicoli S. Vinblastine for elderly and frail patients with Hodgkin lymphoma. *Leuk Lymphoma* 2020; **61**: 3239-3242 [PMID: 32716269 DOI: 10.1080/10428194.2020.1797009]
52 **Wang T**, Ding Y, Yang Y, Wang Z, Gao W, Li D, Wei J, Sun Y. Synergistic antitumour effects of triptolide plus 10-hydroxycamptothecin onbladder cancer. *Biomed Pharmacother* 2019; **115**: 108899 [PMID: 31063955 DOI: 10.1016/j.biopha.2019.108899]
53 **Yan Z**, Lai Z, Lin J. Anticancer Properties of Traditional Chinese Medicine. *Comb Chem High Throughput Screen* 2017;

20: 423-429 [PMID: 28093974 DOI: 10.2174/1386207320666170116141818]

54 **Greene BL**, Kang G, Cui C, Bennati M, Nocera DG, Drennan CL, Stubbe J. Ribonucleotide Reductases: Structure, Chemistry, and Metabolism Suggest New Therapeutic Targets. *Annu Rev Biochem* 2020; **89**: 45-75 [PMID: 32569524 DOI: 10.1146/annurev-biochem-013118-111843]

55 **Mazzu YZ**, Armenia J, Nandakumar S, Chakraborty G, Yoshikawa Y, Jehane LE, Lee GM, Atiq M, Khan N, Schultz N, Kantoff PW. Ribonucleotide reductase small subunit M2 is a master driver of aggressive prostate cancer. *Mol Oncol* 2020; **14**: 1881-1897 [PMID: 32385899 DOI: 10.1002/1878-0261.12706]

56 **Duxbury MS**, Whang EE. RRM2 induces NF-kappaB-dependent MMP-9 activation and enhances cellular invasiveness. *Biochem Biophys Res Commun* 2007; **354**: 190-196 [PMID: 17222798 DOI: 10.1016/j.bbrc.2006.12.177]

57 **Shah KN**, Wilson EA, Malla R, Elford HL, Faridi JS. Targeting Ribonucleotide Reductase M2 and NF-κB Activation with Didox to Circumvent Tamoxifen Resistance in Breast Cancer. *Mol Cancer Ther* 2015; **14**: 2411-2421 [PMID: 26333382 DOI: 10.1158/1535-7163.MCT-14-0689]

58 **Li ZN**, Shu Y, Chen CG, Li XQ, Li MY, Zhao XH, Wang S, Li J. Acquired tamoxifen resistance is surmounted by GW8510 through ribonucleotide reductase M2 downregulation-mediated autophagy induction. *Biochem Biophys Res Commun* 2020; **528**: 554-560 [PMID: 32505349 DOI: 10.1016/j.bbrc.2020.05.149]

59 **Chen YR**, Tsou B, Hu S, Ma H, Liu X, Yen Y, Ann DK. Autophagy induction causes a synthetic lethal sensitization to ribonucleotide reductase inhibition in breast cancer cells. *Oncotarget* 2016; **7**: 1984-1999 [PMID: 26675256 DOI: 10.18632/oncotarget.6539]

60 **Liu C**, Li Y, Hu R, Han W, Gao S. Knockdown of ribonucleotide reductase regulatory subunit M2 increases the drug sensitivity of chronic myeloid leukemia to imatinibbased therapy. *Oncol Rep* 2019; **42**: 571-580 [PMID: 31233186 DOI: 10.3892/or.2019.7194]

61 **Fairman JW**, Wijerathna SR, Ahmad MF, Xu H, Nakano R, Jha S, Prendergast J, Welin RM, Flodin S, Roos A, Nordlund P, Li Z, Walz T, Dealwis CG. Structural basis for allosteric regulation of human ribonucleotide reductase by nucleotide-induced oligomerization. *Nat Struct Mol Biol* 2011; **18**: 316-322 [PMID: 21336276 DOI: 10.1038/nsmb.2007]

62 **Donehower RC**. An overview of the clinical experience with hydroxyurea. *Semin Oncol* 1992; **19**: 11-19 [PMID: 1641651]

63 **Chitambar CR**. Gallium Complexes as Anticancer Drugs. *Met Ions Life Sci* 2018; **18** [PMID: 29394029 DOI: 10.1515/9783110470734-016]

64 **Mortazavi A**, Ling Y, Martin LK, Wei L, Phelps MA, Liu Z, Harper EJ, Ivy SP, Wu X, Zhou BS, Liu X, Deam D, Monk JP, Hicks WJ, Yen Y, Otterson GA, Grever MR, Bekaii-Saab T. A phase I study of prolonged infusion of triapine in combination with fixed dose rate gemcitabine in patients with advanced solid tumors. *Invest New Drugs* 2013; **31**: 685-695 [PMID: 22847785 DOI: 10.1007/s10637-012-9863-1]

65 **Fan X**, Zhu Y, Wang N, Zhang B, Zhang C, Wang Y. Therapeutic Dose of Hydroxyurea-Induced Synaptic Abnormalities on the Mouse Spermatocyte. *Front Physiol* 2021; **12**: 666339 [PMID: 34305635 DOI: 10.3389/fphys.2021.666339]

66 **Botzenhardt S**, Li N, Chan EW, Sing CW, Wong IC, Neubert A. Safety profiles of iron chelators in young patients with haemoglobinopathies. *Eur J Haematol* 2017; **98**: 198-217 [PMID: 27893170 DOI: 10.1111/ejh.12833]

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