



REVIEW ARTICLE

Ribonucleotide reductase M2 (RRM2): Regulation, function and targeting strategy in human cancer

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Abstract Ribonucleotide reductase M2 (RRM2) is a small subunit in ribonucleotide reductases, which participate in nucleotide metabolism and catalyze the conversion of nucleotides to deoxynucleotides, maintaining the dNTP pools for DNA biosynthesis, repair, and replication. RRM2 performs a critical role in the malignant biological behaviors of cancers. The structure, regulation, and function of RRM2 and its inhibitors were discussed. RRM2 gene can produce two transcripts encoding the same ORF. RRM2 expression is regulated at multiple levels during the processes from transcription to translation. Moreover, this gene is associated with resistance, regulated cell death, and tumor immunity. In order to develop and design inhibitors of RRM2,

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appropriate strategies can be adopted based on different mechanisms. Thus, a greater appreciation of the characteristics of RRM2 is a benefit for understanding tumorigenesis, resistance in cancer, and tumor microenvironment. Moreover, RRM2-targeted therapy will be more attention in future therapeutic approaches for enhancement of treatment effects and amelioration of the dismal prognosis.

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Introduction

Ribonucleotide reductases (RNR or RR) participate in nucleotide metabolism, by catalyzing the conversion of nucleotides to deoxynucleotides,¹ which is the rate-limiting step in the production of the precursors for DNA synthesis.² This is required for the maintenance of the relative ratios of dNTP pools, and DNA biosynthesis, repair, and replication.³ RR catalyzes the reaction that the hydroxyl at C-2' of ribonucleotide is replaced with a hydrogen using an organic radical. Four classes of RR are known according to the protein radical. Class I RR isolated from mammalian, some prokaryotes, and herpes simplex virus contains a tyrosyl radical at Tyr122.⁴ Class II enzyme identified in many prokaryotes has an adenosyl cobalamin as the radical.⁵ Class III enzyme has an S-adenosylmethionine.⁶ The RR from *Brevibacterium ammoniagenes* is believed to be included in Class IV.⁷ The mammalian RR contains two non-identical homodimeric subunits, a large protein M1 (RRM1) and a small protein M2 (RRM2) (Fig. 1). Throughout the cell cycle, the RRM1 level is excess and remains constant, while the level of RRM2 is fluctuating.¹⁰ Thus, RRM2 expression is exquisitely controlled in the cell cycle.

The coding region of RRM2 comprises 1,170 nucleotides, which codes a protein of 389 amino acids with a calculated molecular mass of 44,883 Da.¹¹ The amino-terminal portion of RRM2 from various species has a high degree of sequence divergence. For example, there is only 69.2% homology in the first 68 amino acids between the human and mouse RRM2 (Fig. 1C).¹² It indicates that the human RRM2 sequence from aa 1 to aa 68 is not critical for its enzymatic activity.¹¹ While the carboxy-terminal amino acid sequence from aa 69 to aa 389 has considerable homology, suggesting that this portion is essential for producing functions. RRM2 subunit consists of 13 helices and two β -sheet strands (Fig. 1D).¹³ These helices include the eight long helices named α A– α H and four shorter helices named α 1– α 4. After folding, the helices form into three layers, helices C, B, and D in layer one, helices F, E, and G in layer two, and helix H in layer three. The first layer contacts the other RRM2 subunit. RRM2 contains a non-heme iron center as well.¹⁴ RRM2 contains a dinuclear iron center enclosed by six hydrophilic residues from four helices and two essential tyrosine residues from C-helix.¹⁵ Hydrogen-bonding network connects the tyrosine and the iron cluster by the glutamine, which is a distinct way to regulate the enzymatic activities of RR. The tyrosine residue Tyr162 is essential for radical generation and stabilization.

RRM2 expression is regulated at multiple levels

In drug-resistant tumor cells, the RRM2 gene and its promoter region have significant amplification, leading to a high transcription level.¹⁶ Unlike RRM1, of which levels are constant during the cell cycle, RRM2 expression levels are fluctuating.¹⁰ The half-life of RRM2 ranges from 3 to 6 h.¹⁷ RRM2 levels are strictly regulated throughout the cell cycle. RRM2 is undetectable in the G₁ phase, is stable and accumulates constantly to reach its maximum during the S phase, and is degraded after entry into mitosis. Chabes et al found that both promoter-activating and repressive elements regulate S phase-specific transcription of RRM2 gene.¹⁸ Obviously, RRM2 expression is regulated at every stage all the time (Fig. 2).

Core elements in the promoter influence RRM2 gene transcription

Two RRM2 transcripts are expressed from the two separate promoters. For the first transcript, the fragment from –800 to +1 bp contributes the highest promoter activity, the fragment from –610 to +1 bp shows 80% of maximum activity, and the fragment from –125 to +1 bp has only more than 50% of maximum activity.⁹ For the second transcript, the fragments from –610 to –125 bp and –800 to –125 bp contribute the highest activities. Promoter fragment from –125 bp to +1 bp contains three CCAAT sequences and a non-canonical TATA-box. The CCAAT sequences instead of the atypical TATA-box are responsible for the substantial transcription and S-phase-specific expression of RRM2. Each CCAAT sequence loses, and the promoter activity is decreased by about 20%–40%. The transcriptional efficiency of atypical TATA-box only has 25% of the consensus TATA-box sequence.¹⁹ The TATA-box in mouse RRM2 promoter is dispensable for basal and S-phase-specific expression, but this element is required for full transcription.²⁰

Transcription factor II D (TFIID) is a multi-subunit complex consisting of the TATA-binding protein (TBP) and TBP-associated factors (TAFs) and binds the core promoter to assemble the pol II preinitiation complex.²¹ The TATA box is a core element for promoter function.²² Besides, other core promoter elements have been identified.²³ A region located at about 30 bp downstream of the conserved atypical TATA-box contains a highly conserved palindrome sequence, GTGCACC, interacting with TFIID subunits.²⁰

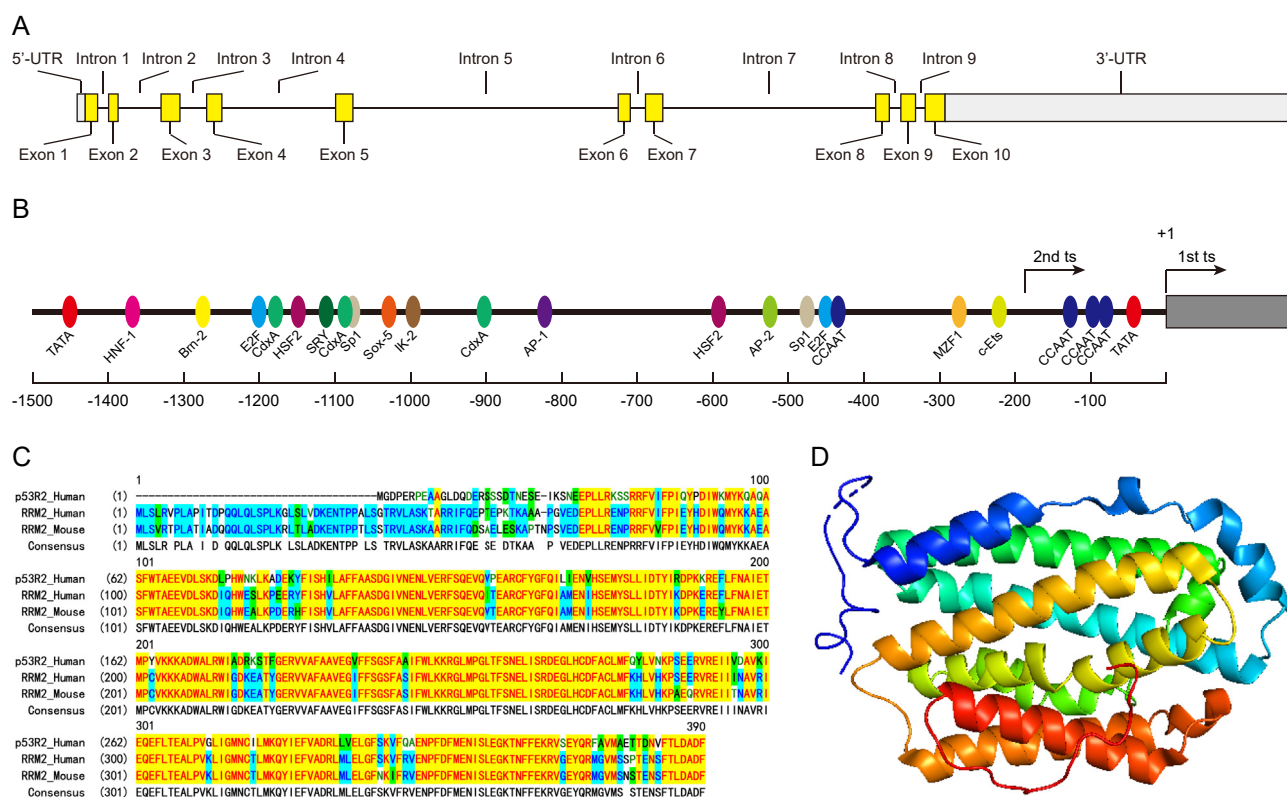


Figure 1 Structures of RRM2. (A) The RRM2 gene is transcribed to produce two transcripts, 1.65 kb, the first transcript, and 3.4 kb, the second transcript.⁸ The transcription initiation site (ts) of the first transcript is designated as +1 bp, and the second ts is located at -187 bp. The difference in the transcript is the length of 5'-UTR and 3'-UTR. A TTTAAA sequence instead of a TATAA sequence, a canonical TATA-box, is found in the proximal promoter, and only regulates the first transcript.⁹ (B) Potential DNA binding sites for transcription factors. ts, transcription initiation site. (C) Alignment of amino acid sequences of RRM2 from humans and mice and p53R2 from humans. (D) The tertiary structure of RRM2 protein (PDB code: 2UW2).

Methylation up-regulates RRM2 expression

In tumorigenesis, progression, and metastasis of breast cancer, aberrant gene expression results from many events including epigenetic modification such as DNA methylation.²⁴ More than 100 genes have an epigenetic modification in breast cancer.²⁵ Qi et al found that the up-regulated RRM2 is also hypomethylated.²⁶

In eukaryotes, DNA wraps around a histone octamer, which leads to forming the smallest subunit of chromatin, nucleosome.²⁷ Different modifications, such as methylation, on core histones, have been well studied and reported.²⁸ Histone methylation participates in multiple cellular processes and diseases through the regulation of gene expression.²⁹ It has been known that histone 3 lysine 4 trimethylation (H3K4me3), H3K79me3 and H3K36me3 active gene transcription, and H3K27me3 and H3K9me3 suppress transcription.³⁰ Xiao et al proposed that H3K36me3 is a promising target for epigenetic therapeutics in cancer.³¹ H3K36me3 provides a positive effect on the regulation of RRM2 transcription as well. SET-domain 2 protein (SETD2) is responsible for the methylation of H3K36 including its mono-, di-, and tri-methylation, and is believed to be a druggable target for precision cancer therapy as well.³² In the human osteosarcoma U2OS cells, RRM2 mRNA and protein levels are reduced by either SETD2

knockdown or CRISPR SETD2 knockout.³³ Reducing H3K36me3 also suppresses RRM2 levels. H3K36me3 activates gene expression through multiple mechanisms such as alteration of genomic H3K36me3 landscape, transcriptional elongation, and collaborative regulation with other histone marks.³¹ Based on the finding that TAFs are enriched at H3K36me3,³⁴ Pfister et al found that H3K36me3 recruits TAFs to facilitate transcription initiation.³³

RRM2 expression is regulated by multiple signaling pathways

Insulin-like growth factor 1 (IGF1) belongs to the insulin-like growth factor family, which binds specifically to the IGF1 receptor (IGF1R) and activates intracellular signaling pathways.³⁵ Subsequently, IGF1R activates the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) and the Ras/Raf/extracellular signal-regulated kinase (ERK) signaling pathways.³⁶

In MDA-MB-231 cells, Zhuang et al found that RRM2 inhibition decreases Akt phosphorylation level, suggesting that RRM2 can regulate PI3K/Akt pathway.³⁷ The RRM2/PI3K/Akt signal pathway also exists in liposarcoma cells.³⁸ Intriguingly, they also showed that, in RRM2-silenced MDA-

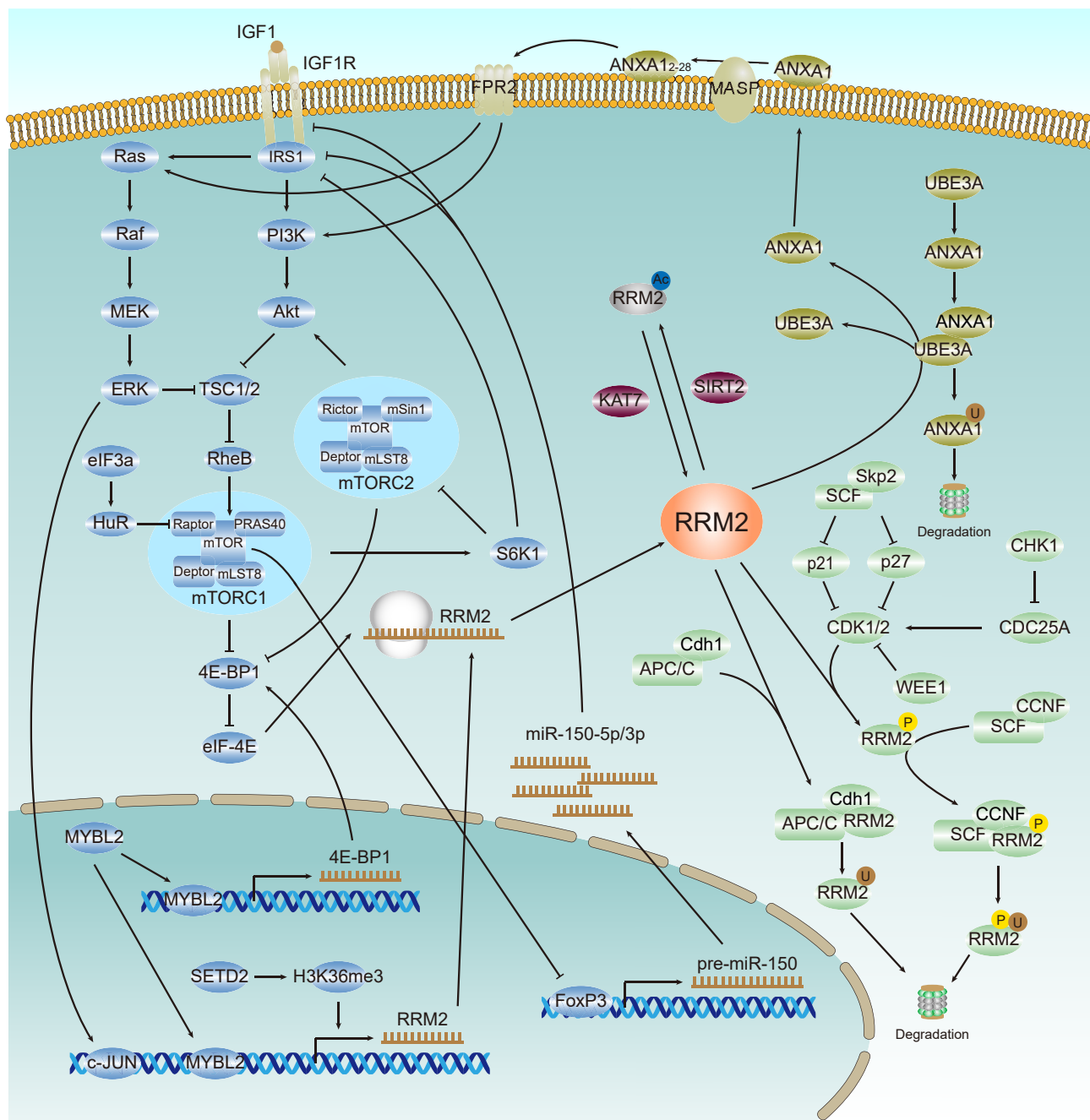


Figure 2 Mechanisms of regulation of RRM2. RRM2 expression is regulated at transcriptional, post-transcriptional, transcriptional, and post-transcriptional levels. Ac, acetylation; P, phosphorylation; U, ubiquitylation.

MB-231 cells, PI3K/Akt pathway agonist IGF1 restores the Akt phosphorylation level. It seems that PI3K/Akt pathway can be regulated both by IGF1/IGF1R and RRM2.

In the breast cancer cell line MCF-7, Li et al found that the phosphorylation levels of mitogen-activated protein kinase kinase (MEK), ERK, and p38 are decreased in the si-RRM2 cells, suggesting that the mitogen-activated protein kinase (MAPK) signaling pathway is inhibited by RRM2 down-regulation.³⁹ On the other hand, also in MCF-7 cells, Rieunier et al showed that MEK inhibitor trametinib inhibits RRM2 up-regulation induced by IGF1.⁴⁰ Moreover, c-JUN is a transcriptional effector of ERK,⁴¹ and c-JUN is a mediator of

IGF effects on RRM2,⁴⁰ suggesting that RRM2 expression is regulated by MEK/ERK/JUN.

IGF1R influences gene transcription by recruitment to promoters,⁴² but in MCF-7 cells, Rieunier et al did not detect IGF1R at the RRM2 promoter by chromatin immunoprecipitation sequencing (ChIP-seq).⁴⁰ Akt inhibitor AZD5363 inhibits RRM2 up-regulation induced by IGF1. Annexin A1 (ANXA1) is an anti-inflammatory protein and shows pro-invasive and pro-tumoral properties in cancers.^{43,44} In cervical carcinoma cells and renal cancer cells, ANXA1 interacts with ubiquitin-protein ligase E3A (UBE3A), thereby leading to degradation.⁴⁵ Furthermore, Xiong et al

found that in renal cancer cells RRM2 competes to seize ANXA1 from ANXA1-UBE3A which causes ANXA1 degradation, and stabilizes ANXA1.⁴⁵ ANXA1 will be externalized and is linked to the cell surface in a calcium-dependent manner.⁴⁶ After translocated to the outer cell surface, ANXA1 is cleaved between Ser28 and Lys29 by a membrane-associated serine protease, releasing a bioactive N-terminal acetyl Ala2-Ser28 (ANXA1₂₋₂₈) peptide. Then, the ANXA1₂₋₂₈ interacts with the formyl peptide receptor 2 (FPR2) belonging to the G protein-coupled receptor family,⁴⁷ leading to the activation of PI3K/Akt pathway in breast cancer⁴⁸ and glioma cells,⁴⁹ and also the activation of extracellular signal-regulated kinase (ERK) MAPK pathway through, probably, Ras/MEK⁵⁰ in melanoma cells⁴⁶ and breast cancer cells.⁵¹

Transcription factors positively or negatively regulate RRM2 expression

Besides the motif for FOS/JUN dimer AP-1, the RRM2 promoter also contains a motif binding with the transcription factor adenoviral early region 2 binding factor (E2F) (Fig. 3). The cyclin-dependent kinase (CDK)-retinoblastoma tumor suppressor (Rb)-E2F axis forms the core regulatory machinery to drive cell cycle progression. The regulation of

E2F transcription factors is the most critical event during the transition from G₁ to S.⁵² ERK can directly phosphorylate and activate cyclin D (CycD).⁵³ The activated CycD binds with CDK4/6 to form an active holoenzyme CDK4/6-CycD, which cooperates with or without CDK2-CycE in Rb phosphorylation.⁵⁴ The phosphorylated Rb release and activate E2F. The E2F family comprises eight distinct genes encoding transcriptional activators such as E2F1 and repressors such as E2F4.⁵² E2F1 induces RRM2 expression and E2F4 represses the activity of RRM2 promoter.^{18,40} E2F4 has a nuclear export signal and is predominantly cytoplasmic.⁵⁵ Associated with retinoblastoma protein family members, also called pocket proteins such as p107 and p130, E2F4 is localized in nuclear during G₀/G₁.⁵⁶ When the pocket protein is phosphorylated, the complex dissociates and E2F4 is free, resulting in the progressive increase of cytoplasmic E2F4.⁵⁶ E2F1 has a nuclear localization signal and is constitutively nuclear.⁵⁷ When being activated, E2F1 induces expression of cell cycle-related and S phase-promoting genes, and then the cells enter the S phase.⁵⁸

E2F1 has opposing functional roles in proliferation and apoptosis, called the “yin and yang” of E2F1.⁵⁹ The biological activity of E2F1 is regulated by arginine methylation.⁶⁰ E2F1 methylated by asymmetric dimethylating protein arginine methyltransferase (PRMT) 1 augments

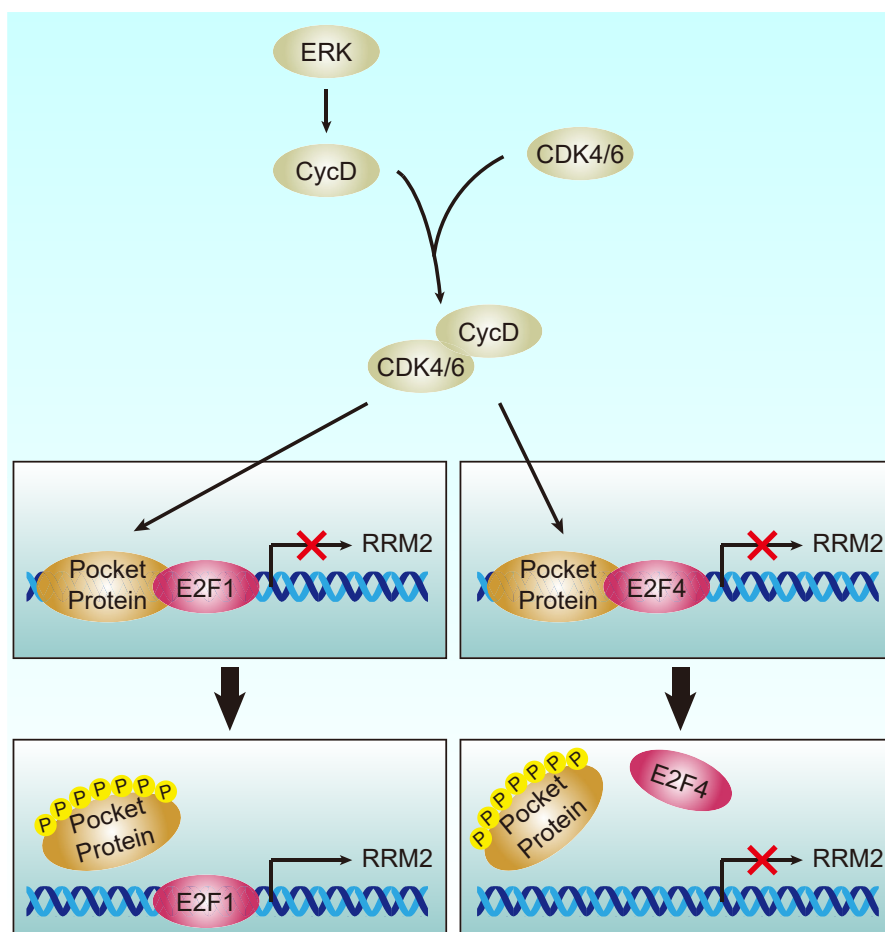


Figure 3 Regulation of RRM2 transcription by E2F1 and E2F4. Pocket proteins including Rb, p107, and p130 regulate the activation of E2Fs.

apoptosis, and symmetric dimethylating PRMT5-dependent methylation promotes E2F1-dependent proliferation.⁶¹ PRMT1 and PRMT5 competitively methylate E2F1, but cyclin A binding to E2F1 hinders PRMT1 methylation, thereby facilitating PRMT5 methylation. PRMT5 belongs to type II PRMT. Another type, type I PRMT, contains PRMT2, which directly binds and interacts with Rb through its S-adenosyl methionine binding motif, forming a ternary complex with E2F1, to negatively regulate E2F1 activity.⁶²

Breast cancer gene 1 (BRCA1) is a tumor suppressor. In breast cancer cells, BRCA1 suppresses epithelial-to-mesenchymal transition (EMT) through binding to the TWIST promoter and suppressing its activity,⁶³ while BRCA1 depletion promotes EMT activation through activation of transforming growth factor beta receptor 2 (TGFβR2) signaling pathway.⁶⁴ Interestingly, BRCA1 is found to be also a transcriptional co-activator of RRM2 in glioblastoma cells, but not in non-glioblastoma cells.⁶⁵

MYBL2 (B-Myb, MYB proto-oncogene like 2) belongs to the Myb transcription factor family and plays a critical role in the cell cycle and tumorigenesis.⁶⁶ MYBL2 is overexpressed and is believed to be a prognostic and predictive biomarker in several types of cancers including breast cancer.⁶⁷ Liu et al found that two MYBL2 binding motifs exist in the RRM2 promoter at −36 to −51 bp and −1857 to 1872 bp.⁶⁸ MYBL2 directly binds to the RRM2 promoter and promoted its transcription in colorectal cancer cells.

Non-coding RNAs (ncRNAs) engage in RRM2 gene expression

Long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) are different kinds of ncRNAs, and both participate in cancer initiation and development.^{69,70} lncRNAs can be a miRNA “sponge”. These sponges interact with miRNAs, resulting in the release of miRNA target genes. Using bioinformatical and experimental methods, some lncRNAs and miRNAs regulating the RRM2 gene have been identified, and the ones verified by experiments in breast cancer are listed in Table 1.

The protein synthesizing machine regulates RRM2 gene translation

Protein synthesis is limited by mRNA translation. In eukaryotes, protein synthesis is regulated by eukaryotic translation initiation factors (eIFs) and translation repressors. The mTOR forms two structurally and functionally distinct complexes, mTOR complex 1 (mTORC1) and 2 (mTORC2), which participates in protein synthesis through mTORC1-mediated phosphorylation of 70-kDa ribosomal protein S6 kinase 1/2 (S6K1/2) and eIF4E-binding proteins 1/2/3 (4EBP1/2/3), and mTORC2-mediated phosphorylation of Akt (Akt1/2/3).^{74–76}

The eIFs act as complexes called eIF1, eIF2, eIF3, eIF4, eIF5, and eIF6. The eIF3 comprises 13 subunits,⁷⁷ of which eIF3a, the largest subunit, is believed to be a potential oncogene, and is also a potential drug target.⁷⁸ The eIF3a binds to the mRNA 5'-UTR of regulatory associated protein of mTOR (Raptor), a subunit of mTORC1, through

Table 1 lncRNAs and miRNAs regulating RRM2 gene in breast cancer cells.

lncRNA	miRNA	Target	Cell lines	Reference
DSCAM-AS1 ↑	miR-204-5p ↓	RRM2 ↑	HCC1937	71
SNHG16 ↑	miR-30a ↓	RRM2 ↑	MDA-MB-231	72
			MCF-7	
TTN-AS1 ↑	miR-524-5p ↓	RRM2 ↑	T47D	73
			BT549	
/	miR-4500 ↓	RRM2 ↑	MCF7	39
			BT474	
			MDA-MB-468	
			MDA-MB-231	

interacting with the RNA-binding protein human antigen R (HuR), and inhibits Raptor protein synthesis, resulting in negative regulation of mTORC1 activity.⁷⁹ The eIF4E-binding protein 1 (4E-BP1) belongs to a family of translation repressor proteins and a family of eIF4E-binding proteins. 4E-BP1 is regulated and phosphorylated by mTORC1/2.⁸⁰ The phosphorylated 4E-BP1 dissociates from eIF4E, thereby initiating cap-dependent translation. In rhabdomyosarcoma cells, mTORC1/eIF-4E cap-dependent protein translation participates in the regulation of RRM2 expression.⁸¹ Similar results are reported in sarcoma cell lines, in which 4E-BP1 is activated by inhibition of mTORC1/2, thereby inhibiting RRM2 translation.⁸² Moreover, 4E-BP1 transcription can be induced by MYBL2.⁸³ Thus, MYBL2 regulates RRM2 expression at transcriptional and translational levels. Simultaneously, Zhang et al found that RRM2 knockdown may suppress the activity of the Akt/mTOR/4E-BP1 pathway in retroperitoneal liposarcoma cells.⁸⁴ Perhaps, this mechanism is ANXA1 mediated.

Dong et al showed that eIF3a positively regulates RRM2 expression in HeLa and NIH3T3 cells.⁸⁵ He et al also found that depletion of S6K1, another target of mTORC1, leads to up-regulation of RRM2.⁸¹ Raptor binds to the tor signaling motif within S6K1 for promoting interaction with mTOR and mediating phosphorylation.⁸⁶ S6K1 has two downstream effectors, mTORC2 and insulin receptor substrate 1 (IRS1).^{86,87} S6K1 is implicated in the phosphorylation and negative regulation of Rictor (sirolimus-insensitive companion of mTOR) within mTORC2, which phosphorylates and activates Akt.⁷⁵ In addition to its role in the inhibition of mTORC2, S6K1 inhibits IRS1 through phosphorylation of serine of IRS1^{88,89}; while the activated IRS1 with tyrosine phosphorylation activates PI3K/Akt/mTOR pathway.⁹⁰ Forkhead box protein 3 (FoxP3), first found in regulatory T (Treg) cells, is a transcription factor involved in the development and function of Treg cells,⁹¹ and serves vital roles in cancers including breast cancer.⁹² The mTOR has the ability to block Foxp3 induction.⁹³ Zhang et al also showed that FoxP3 positively regulates miR-150-5p/3p by binding to the miR-150 promoter, and miR-150-5p/3p directly targets IRS1 and IGF1R.⁹⁰ This forms a feedback loop of FoxP3/miR-150/IGF1R-IRS1/PI3K/AKT/mTOR, like Akt/mTORC1/S6K1/mTORC2 and PI3K/Akt/mTORC1/S6K1/IRS1.

The post-translational modification will activate RRM2

Protein post-translational modifications (PTMs) are key steps in structural changes of proteins and participate in multiple biological processes.⁹⁴ Lysine acetylation is a versatile PTM and is involved in many important cellular processes.⁹⁵ Histone acetyltransferase KAT7 (MYST2 or HBO1), a member of the MYST KAT family, is essential for histone H3 lysine 14 acetylation and is involved in replication-associated processes.⁹⁶ Sirutin 2 (SIRT2), a member of the Sirtuin family, is a nicotinamide adenine dinucleotide (NAD⁺)-dependent deacetylase, and participates in various biological processes by deacetylation.⁹⁷ Chen et al found that acetylation/deacetylation of the RRM2 is a molecular switch of RR activity.⁹⁸ RRM2 is directly acetylated by KAT7. The acetylation of RRM2 at K95 disrupts RRM2 homodimerization, resulting in RR inactivation. While SIRT2 directly deacetylates RRM2, thereby leading to RR activation. They also found that SIRT2 regulation of RRM2 deacetylation is cell-cycle-dependent, and ionizing radiation or camptothecin significantly decreases RRM2 acetylation by promoting an interaction between SIRT2 and RRM2 instead of KAT7 and SIRT2 protein levels. Moreover, the RRM2 deacetylation is regulated by its phosphorylation induced by the serine/threonine-protein kinase ATR (ataxia telangiectasia and Rad-3 related protein), solving genome maintenance challenges and integrating the information stored in the DNA,⁹⁹ at the S150 site, the only one SQ motif in human RRM2.¹⁰⁰

Multiple pathways are responsible for RRM2 degradation

Two E3 ubiquitin protein ligases, the anaphase-promoting complex/cyclosome (APC/C) and the Skp1/cullin/F-box (SCF) complex, highly regulate the cell cycle and play an important role in tumorigenesis.¹⁰¹ RRM2 is regulated by the ubiquitination-proteasome system (UPS). The APC/C is an evolutionarily conserved multi-subunit E3 ubiquitin ligase. Cdh1 is an activator of APC/C.¹⁰² The APC/C^{Cdh1} can recognize a conserved KEN box located at the N-terminus of RRM2.¹⁰³ Then the RRM2 binds to APC/C^{Cdh1} and is polyubiquitinated.

The F-box protein is a component of SCF E3 ubiquitin ligase and is responsible for the recognition of target proteins for ubiquitylation and degradation.¹⁰⁴ Cyclin F (CCNF) is a member of the F-box protein family.¹⁰⁵ D'Angiolella et al identified that RRM2 is an interactor of the F-box protein CCNF, and found that RRM2 is degraded *via* SCF^{CCNF} after CDK-mediated phosphorylation of Thr33 during G₂, maintaining a balance of dNTP pools and stability of the genome.¹⁰⁶ They also showed that CCNF down-regulation in an ATR-dependent manner allows RRM2 accumulation in response to DNA damage, which is required for efficient DNA repair. Similar results are observed in breast cancer cell lines MCF-7 and MDA-MB-231. Chang et al showed that CCNF overexpression reduces the RRM2 protein level.¹⁰⁷

S-phase kinase-associated protein 2 (Skp2) is also a part of the SCF complex, an E3 ubiquitin ligase, and is a substrate-recruiting F-box protein.¹⁰⁸ During the G₁/S

transition, SCF^{Skp2} degrades the CDK1 inhibitors p21 and p27, thereby controlling cell cycle and cancer progression.^{109,110} Unexpectedly, Xiao et al found that Skp2 and RRM2 are synchronously overexpressed in the hydroxyurea (HU)-resistant cell line KB.¹¹¹ Furthermore, they demonstrated that Skp2 interacts with RRM2 directly, and the interaction enhances the RR activity.

WEE1 is a tyrosine kinase and is a master regulator of the G₂/M transition during the cell cycle.¹¹² CDK1 is a target of WEE1 and its phosphorylation at Tyr15 can be inhibited by WEE1.¹¹³ WEE1 inhibition can be applied in cancer therapy.¹¹⁴ Pfister et al showed that WEE1 inhibition activates CDK1/2, which increases RRM2 phosphorylation at Thr33, promoting RRM2 degradation.³³ The same results are confirmed in sarcoma cells by Gordon team, where inhibition of CHK1-WEE1 signaling promotes the CDK-mediated degradation of RRM2.^{82,115}

RRM2 has a dual function

RR has an important function in DNA synthesis, cell proliferation, and cancer development. RRM2, as the small subunit, has RR-related enzymatic functions and non-enzymatic functions.

Enzymatic function

DNA replication is a hallmark of cancer.¹¹⁶ Some chemotherapy drugs used in cancer treatment, such as Cisplatin,¹¹⁷ exert an anti-tumor function by interfering with DNA replication. Contrarily, sustained DNA synthesis and replication contribute to drug resistance. For example, RRM1 activation is an important hallmark of cancer cells acquiring drug resistance.¹¹⁸ Thus, RRM2 overexpression has been shown to be a potential factor causing drug resistance.

A HU-resistant human KB cell line was developed and exhibited a 15-fold resistance to HU, with a gene amplification of RRM2, increased levels of RRM2 mRNA and protein, and enhanced activity of RR.^{119,120} Inhibition of RRM2 can overcome the resistance and increase chemosensitivity in fibrosarcoma,¹²¹ pancreatic cancer,¹²² etc. Several studies also identified that MMR2 is a marker for resistance of HU,¹²³ tamoxifen,^{124–126} and Adriamycin.¹²⁷ ICBP90, also known as Uhrf1 (ubiquitin-like with PHD and ring finger domains 1) and Np95, is a Rb-associating transcription factor,¹²⁸ and has an essential role in the maintenance of DNA methylation by histone H3 ubiquitylation.¹²⁹ In human head and neck cancer cells, dNTP depletion caused by HU treatment induces ICBP90, thereby transactivating RRM2.¹²³ Gemcitabine can also increase RRM2 expression in a dose-dependent manner.¹³⁰ Nuclear factor Y (NF-Y) interacts with proximal CCAAT-boxed in the RRM2 promoter and is essential for its high-level expression of RRM2.¹³¹ Liu et al believed that this plays a pivotal role in human oropharyngeal epidermal carcinoma cell line KB with gemcitabine resistance (KB^{Gem}).¹³² Another transcription factor E2F1 can also be increased by gemcitabine and then induces RRM2 expression.¹³³ In KB^{Gem}, RRM2 overexpression increases the dCTP pool, and the available dCTP is able to compete with gemcitabine.^{16,134}

In breast cancer, several regulatory mechanisms of RRM2 overexpression have also been proposed, and involve Akt, NF- κ B, HIF1 α , and MAPK/c-Jun N-terminal kinase (JNK) pathways.^{124,125}

Non-enzymatic function

Crosstalk of RRM2 and regulated cell death

In 2012, Brent R. Stockwell team reports a new cell death modality, an iron-dependent form of regulated cell death, called ferroptosis, which is triggered by unrestricted lipid peroxidation and subsequent plasma membrane rupture.^{135,136} There are some characterizations, such as iron overload, glutathione (GSH) depletion, inactivation of glutathione peroxidase 4 (GPX4), and lipid peroxidation. GSH is a tripeptide synthesized by glutathione synthetase (GS) from glycine, glutamate, and cysteine.¹³⁷ RRM2 participates in nucleotide synthesis in a glutathione-dependent manner,¹³⁸ and is believed to be a ferroptosis-related gene.¹³⁹ Zhang et al showed that RRM2 is involved in ferroptosis in liver cancer.¹⁴⁰ RRM2 was also believed to be a ferroptosis-related metabolic gene in prostate cancer¹³⁹ and lung adenocarcinoma.¹⁴¹ It has been reported that RRM2 is an endogenous ferroptosis suppressor in liver cancer cells and lung adenocarcinoma, and RRM2 knockdown can induce ferroptosis.¹⁴¹ In liver cancer cells, the phosphorylation of RRM2 at T33 facilitates the expression of GS protein, which is critical for GSH synthesis. The dephosphorylation of RRM2 enhances the interaction of RRM2 and GS interaction and, at the same time, triggers proteasome degradation, thereby leading to their degradation.

Chen et al revealed a relationship between nucleotide pools and autophagy in cancer cells.¹⁴² RRM2 plays an important role in this process. Reduction of intracellular dNTP pools by knocking down RRM2 expression induced autophagy and conversely, an increase in intracellular dNTP pool concentrations by RRM2 overexpression attenuates autophagy induced by rapamycin.

RRM2 facilitates immune escape

Cancer immunotherapy is a novel strategy and a breakthrough approach for cancer treatment through the activation of the immune system.¹⁴³ Clinically, it has been proved that immunotherapy has achieved promising outcomes.¹⁴⁴ Based on experimental and bioinformatic studies, it has been confirmed that there is an association between RRM2 and tumor immunity, and RRM2 contributes to immune escape.¹⁴⁵

Immune checkpoint inhibition is an effective means of therapy for improving the prognosis of patients, such as programmed cell death 1/programmed cell death ligand 1 (PD-1/PD-L1) blockade.¹⁴⁶ PD-1, also known as CD279 and belonging to the CD28 family, is an immunoinhibitory receptor that is expressed on activated T cells, B cells, and macrophages.¹⁴⁷ PD-1 has a role in the inhibition of T-cell intracellular signaling, proliferation, and cytokine production.¹⁴⁸ PD-L1, PD-1 ligand, also known as CD274 and belonging to the B7 family, is expressed by lymphoid and nonlymphoid tissues, as well as various tumors.^{149,150} PD-L1 overexpression contributes to evading immune responses.¹⁵¹ Binding of PD-1 to PD-L1 and PD-L2 triggers a cascade of

intracellular signaling that results in T cell inhibition and exhaustion,¹⁵² thereby leading to tumor immune escape.¹⁵³ PD1/PD-L1 blockade therapy is considered a promising strategy in cancer treatment.¹⁵⁴ Based on RNA-seq data, Xiong et al found that RRM2 modulates the PD-1 pathway in renal cancer 786-O cells.⁴⁵ The GEPIA web tool also shows a significant association between RRM2 and PD-L1 levels in renal cancer, bladder cancer, breast cancer, and prostate cancer tissues. Then, experimentally, they confirmed that RRM2 silencing decreases and its overexpression increases the mRNA and protein levels of PD-L1 in 786-O and A498 cells. Hypoxia is a prominent feature of most tumors and leads to the stabilization of hypoxia inducible factor 1 subunit alpha (HIF-1 α) in cancer cells.¹⁵⁵ HIF-1 α inhibition promotes anti-tumor immunity in non-small cell lung cancer.¹⁵⁶ Patients with high HIF-1 α expression exhibit an immunosuppressive phenotype. Inhibition of HIF-1 α alleviates tumor immunosuppression through HIF-1 α /LOXL2 signaling pathway. Moreover, targeting HIF-1 α can also suppress PD-L1 expression in tumor cells.¹⁵⁷ In breast cancer, Shah et al showed that RRM2 overexpression specifically up-regulates HIF-1 α .¹²⁵ Furthermore, this may explain the reason that knockdown of RRM2 enhances the anti-tumor efficiency of PD-1 blockade in renal cancer.⁴⁵

Macrophages play significant roles in immunity and are generally categorized into the pro-inflammatory M1 and the anti-inflammatory M2 phenotypes.¹⁵⁸ In the tumor microenvironment (TME), M1 macrophages exhibit anti-tumor immunity, whereas M2 macrophages, also called "tumor-associated macrophages" (TAMs), have pro-tumor features (promoting tumor growth and metastasis).¹⁵⁹ Xiong et al found that RRM2 levels are positively correlated with the number of M2 macrophages in the TCGA-KIRC dataset.⁴⁵ Tang et al also showed that RRM2 promotes macrophage infiltration, and its inhibition suppresses macrophage infiltration in lung adenocarcinoma.¹⁴¹ RRM2 regulates macrophage polarization *in vitro* and *in vivo* as well. Inhibition of RRM2 effectively promotes M1 polarization and suppresses M2 polarization of macrophages.

Strategies for designing inhibitors targeting RRM2

RRM2 is the small subunit, two of which combine with two large subunits RRM1 to form a protein tetramer RR, catalyzing the reaction of nucleotides to deoxynucleotides. Stubbe and van der Donk proposed three approaches to targeting RR.¹ They involve nucleotide analogs, reduction of the essential tyrosyl radical, and inhibition of the interaction of RRM1 and RRM2. RR, as a holoenzyme, is directly involved in tumor growth and drug resistance. RRM2 expression is tightly regulated, but RRM1 overexpression does not affect RR activity.¹⁶⁰ As compared with RRM1, RRM2 is a promising and important target for the design of anti-cancer agents (Fig. S1). There are also several strategies for developing inhibitors targeting RRM2.

Destroying free radical and iron center

HU is synthesized for the first time in 1869 and is approved by the Food and Drug Administration (FDA) for the

treatment of solid tumors in 1967.¹⁶¹ HU inactivates RRM2 by destroying its tyrosyl-free radical and the iron center, accompanied by the release of iron from the protein.¹⁶²

Didox (3,4-Dihydroxybenzohydroxamic acid) is a derivative of HU. As an iron chelator and a free-radical scavenger for targeting RRM2,¹⁶³ didox is one of the most potent pharmaceutical inhibitors of RR^{163,164} and has been used as an anti-tumor agent for many years.^{165,166}

Resveratrol (3,5,4'-trihydroxy-trans-stilbene), a natural antioxidant,¹⁶⁷ has anti-tumor effects.¹⁶⁸ But its effects are controversial. Recently, Chen et al found that, in MDA-MB-231 cells, low concentrations of resveratrol have a pro-tumorigenic effect of promotion of proliferation and migration by activating the JAK3/STAT3 signaling pathway, while high concentrations of resveratrol exhibit anti-tumor performance of inhibition of cell growth and induction of autophagy and apoptosis through MAPK signaling pathway.¹⁶⁹ Resveratrol also has the ability to inhibition of RR by quenching the tyrosyl radical of RRM2.¹⁷⁰

Capturing iron from RRM2

Due to being iron-dependent, depleting iron can inhibit RRM2.¹²² Iron chelators such as DFX (desferrioxamine, deferoxamine mesylate), HBED (*N,N'*-bis(*o*-hydroxybenzyl) ethylenediamine-*N,N'*-diacetic acid) and BPYTA (2,2'-bipyridyl-6-carbothioamide) can be used as RRM2 inhibitors.^{171,172} Moreover, an agent inhibiting cellular iron incorporation can also decrease the activity of RRM2, such as transferrin-gallium.¹⁷³

Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone, 3-AP), one of the heterocyclic carboxaldehyde thiosemicarbazones (HCTs), is a synthesized RR inhibitor with antineoplastic activity.¹⁷⁴ Although several models are proposed to explain the mechanisms by which 3-AP inhibits RRM2 through chelating iron or producing oxygen reactive species (ROS), Aye et al presented another model, in which Fe(II)-(3-AP) directly reduces Y· of the [(Fe₂^{III}-Y·)(Fe₂^{III})] cofactor of RRM2.¹⁷⁵ Due to minor or even no anti-tumor activity and side effects, some clinical trials are stopped.^{176,177} Considering these problems, novel derivatives are discovered. Stefani et al designed a novel RBpT series, namely 2'-benzoylpyridine thiosemicarbazones bearing hydrophobic, electron-donating substituents at the *para* position of the phenyl group, especially *t*-BuBpT series.¹⁷⁸ Based on the formation of the Fe(II)(3-AP)₂ complex, Plamthottam et al synthesized three classes of 3-AP analogs including 12 compounds.¹⁷⁹ Group I compounds with methylation on the secondary amine show no RNR-specific activity. Group II compounds have the most similar structures with that of 3-AP, and have less potent (non-aromatic substituents on the pyridine ring) or slightly more potent (substituents to the primary amine part of the thiosemicarbazone moiety) than 3-AP. Group III compounds with isoquinolines instead of pyridine ring exhibit much stronger activity than 3-AP, in which IQ-2 is four-fold more potent than 3-AP.

Decreasing RRM2 mRNA level

Flavopiridol (also called alvocidib) is a synthetic analog of a natural flavone derivative that is isolated from *Dysoxylum*

binectariferum.¹⁸⁰ It is a CDK inhibitor, inhibits the growth of diverse human tumor cells, and treats acute myeloid leukemia and chronic lymphocytic leukemia.^{181,182} PP2, 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine, is a Src tyrosine kinase inhibitor.¹⁸³ It is found that both of them suppress RRM2 at the transcriptional level through regulation of E2F1.^{184,185}

Arginine is a non- or semi-essential amino acid for it can be synthesized from citrulline and aspartate using argininosuccinate synthase 1 (ASS1) and argininosuccinate lyase (ASL).¹⁸⁶ In most tumors, ASS1 transcription is suppressed and, thus, arginine deprivation is believed to be a novel antimetabolite strategy for treating arginine-dependent cancers.¹⁸⁷ Some enzymes such as arginase (ARG), arginine decarboxylase (ADC), and arginine deiminase (ADI) participate in arginine metabolism. Using these enzymes to target exogenous arginine is a promising therapy to treat cancers.¹⁸⁸ ADI-PEG 20 is a stabilized, pegylated arginine deiminase. It has been assessed against various cancers including breast cancer in clinical trials.¹⁸⁹ Prudner et al found that both acute and long-term treatment of ADI-PEG20 can significantly decrease RRM2 expression.¹⁹⁰ Daylami et al believed that this is PRMT2-mediated.¹³⁰

RRM2 mRNA can be effectively repressed by RNA interference. GTI-2040, a 20-mer antisense nucleotide against RRM2, decreases its mRNA and protein levels in various cancers including breast cancer.¹⁹¹ Interestingly, Xiao et al found that GTI-2040 can decrease both RRM2 and Skp2, suggesting that it interrupts their interaction by dissociation of the complex.¹¹¹ Moreover, in the less sensitive group, the RRM2 mRNA does not change. Duxbury et al used a novel engineered replication-deficient retrovirus to induce stable siRNA targeting RRM2.¹⁹² P-element induced wimpy testis (PIWI)-interacting RNA (piRNA) guide PIWI proteins to bind and cleave RNAs.¹⁹³ Das et al used a piRNA, piR-39980, to directly target 3'-UTR of RRM2 for repression.¹⁹⁴

5-Azacytidine (5-AZA) is a pyrimidine analog and is approved by FDA for the treatment of the myelodysplastic syndrome.¹⁹⁵ It also has anti-tumor effects on acute myeloid leukemia (AML).¹⁹⁶ Aimiwu et al found a novel molecular target of 5-AZA in AML.¹⁹⁷ It can attenuate RRM2 mRNA stability and induce its expression inhibition.

Promoting RRM2 protein degradation

GW8510 is also a CDK inhibitor and reverses chemoresistance through the inhibition of RRM2 in cancers such as lung squamous cell carcinoma¹³³ and breast cancer.¹²⁶ In human colon cancer HCT116 cells, Hsieh et al found that GW8510 targets the RRM2 protein and promotes its degradation, thereby resulting in inhibition.¹⁹⁸

Sorafenib (BAY 43-9006), an FDA-approved multikinase inhibitor, mainly targets the vascular endothelial growth factor receptor family and platelet-derived growth factor receptor family.¹⁹⁹ In human lung cancer cells, sorafenib reduces RRM2 gene expression.²⁰⁰ Yang et al showed that sorafenib inhibits RRM2 expression by suppressing its transcription and promoting its protein degradation. But the mechanism is unclear and needs further investigation.²⁰¹

Chen et al identified a synthetic analog of resveratrol, DHS (trans-4,4'-dihydroxystilbene), which can inhibit

pancreatic, ovarian, and colorectal cancer cells in mouse models of tumor xenografts.²⁰² Cellular and biochemical experiments confirmed that DHS binds directly to RRM2, and docking analysis showed that the binding involves residues Val146, Ser150, Gln151, Thr156, Arg159, Cys160, and Ile166. At the molecular level, DHS induces ubiquitination of RRM2 by cyclin F, and then the ubiquitinated RRM2 is degraded by the proteasome.

Developing small molecule inhibitors that target active sites of RRM2

Zhou et al identified five ligand binding pockets based on the human RRM2 structure and believed that Pocket 5 close to the RRM1-RRM2 interface is ideally suited for designing the inhibitor.²⁰³ Based on these structural features of the ligand binding pocket, the most effective inhibitor COH29, *N*-(4-(3,4-dihydroxyphenyl)-5-phenylthiazol-2-yl)-3,4-dihydroxybenzamide, is synthesized after structure-based optimization. Residues Gly233, Asp271, Tyr323, Phe326, Val327, Arg330, Glu334 and Met350 are involved in COH29 binding, and the 41 C-terminal amino acids of RRM2 are important for COH29 binding. COH29 can also disrupt RRM1-RRM2 interaction at higher doses, but enhance it at low doses. Mechanistically, COH29 directly affects enzyme activity and does not regulate protein expression. It inhibits most of the cancer cells including ovarian, leukemia, and prostate cancer cells.²⁰⁴ In KB epithelioid cells, COH29 has similar activity to gemcitabine, and is over 20-fold more active than HU; moreover, it overcomes resistance to both of them.²⁰³ COH29 also enhances the chemosensitivity to doxorubicin in breast cancer MDA-MB-231 cells.²⁰⁵

When Liu et al performed computer-assisted virtual screening against the RRM2 structure, they identified a potential RRM2 inhibitor osalmid from the Comprehensive Medicinal Chemistry (CMC) database, with 10-fold more activity than HU.²⁰⁶ They also found a novel derivative of osalmid, 4-cyclopropyl-2-fluoro-*N*-(4-hydroxyphenyl) benzamide (YZ51), which shows higher efficacy than osalmid. Docking studies show that residues Phe240, Phe244, Cys270, Asp271, Cys274, Tyr323, Arg330, Glu334, and Met350 are involved in the two compounds binding. Osalmid exhibits significant cytotoxicity in esophageal cancer²⁰⁷ and hepatocellular carcinoma cells.²⁰⁸ Wu et al studied the metabolic profile of osalmid.²⁰⁸ During the processes of hydroxylation, glucuronidation, sulfonation, acetylation, and degradation, osalmid will produce ten metabolites, in which metabolites M7, M8, and M10 have higher binding affinities with the RRM2 active site than osalmid.

Conclusion and prospects

Searching and identifying key genes from next-generation sequencing data of bulk normal and tumor samples is an effective strategy for tumor research. Undoubtedly, RRM2 is believed to be a promising target for diagnosis, treatment, and prognosis prediction, such as in breast cancer.^{209,210} RRM2, one of the subunits of RR which is essential for DNA replication and cancer cell proliferation, is tightly regulated. The molecular mechanisms of regulation are

extremely complicated. For example, RRM2 can be regulated by one pathway, and can also regulate this pathway, forming feedback signaling. During the process from transcription to translation, regulation happens in almost every step all the time.

Although chemotherapy treatment has a significant role in the treatment of cancers and prevention of their recurrence and spreading,²¹¹ the drug-resistance is the main problem. For example, gemcitabine is a famous RR inhibitor and is widely used in cancer therapies, but resistance limits its therapeutic efficacy.²¹² In the treatment of breast cancer cells, RRM2 is up-regulated and is involved in the resistance to GTI-2040, tamoxifen, adriamycin, and cisplatin. The acquired resistance is readily overcome by RRM2 inhibition. Besides chemoresistance, RRM2 participates in radiation resistance. After exposure to ionizing radiation or UV, the RRM2 level is increased.^{213,214} Chemotherapy or radical therapy combined with RRM2 inhibition significantly enhance the anti-tumor efficiency in cell models of fibrosarcoma cells¹²¹ and pancreatic ductal adenocarcinoma cells,²¹² and animal models of lung squamous cell carcinoma,¹³³ pancreatic cancer,²⁰² breast cancer,¹²⁶ and renal cell carcinoma.⁴⁵

However, unfortunately, many RRM2 inhibitors themselves will promote chemoresistance, and are effectless or have side effects. Thus, continued efforts to develop and design effective and less toxic RRM2 inhibitors are needed. A better understanding of the structure and mechanism of action of RRM2 may improve the design of novel RRM2 inhibitors for cancer therapy. Two main functions which are enzymatic and non-enzymatic provide two different kinds of inhibitors to be developed. Natural products can also be a source of anti-tumor agents. They have obvious advantages, such as safety, few side effects, and low toxicity, and exert chemopreventive activities against cancers including breast cancer.²¹⁵ For example, Zhou et al showed that curcumin can rescue part of adriamycin resistance in breast cancer, which may be associated with RRM2.¹²⁷ In addition to traditional therapies using small molecular compounds or natural products, gene therapy, such as RNA-based treatments using ncRNA including lncRNA and miRNA, creates a new avenue for treating diseases, such as cancers.²¹⁶

Overall, RRM2 has been uncovered to have a tremendous impact on the biological processes of cancer cells including breast cancer cells in proliferation, prognosis, and resistance. A deeper understanding of the regulatory mechanisms of RRM2 will advance our understanding of tumor development and resistance. Targeting RRM2 therapy is a promising approach in the treatment of breast cancer.

Author contributions

Zuo Zanwen: writing - original draft, writing - review & editing, visualization. Zhou Zerong: writing - original draft. Chang Yuzhou: writing - original draft. Liu Yan: writing - original draft, writing - review & editing. Shen Yuping: writing - original draft, writing - review & editing. Li Qizhang: conceptualization, writing - original draft, writing - review & editing, visualization, supervision, funding

acquisition. Zhang Lei: conceptualization, writing - review & editing. All authors read and approved the final version of the work to be published.

Conflict of interests

The authors report there are no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.gendis.2022.11.022>.

Abbreviations

4E-BP1	eIF4E-binding protein 1
ANXA1	annexin A1
Akt	protein kinase B
APC/C	anaphase-promoting complex/cyclosome
CCNF	cyclin F
CDC25A	cell division cycle 25A
CycD	cyclin D
Cdh1	epithelial-cadherin
CDK	cyclin-dependent kinase
CHK1	checkpoint kinase 1
eIF	eukaryotic translation initiation factor
ERK	extracellular signal-regulated kinase
FPR2	formyl peptide receptor 2
H3K36me3	histone 3 lysine 36 trimethylation
HuR	human antigen R
IGF1	insulin-like growth factor 1
IGF1R	insulin like growth factor 1 receptor
IRS1	insulin receptor substrate 1
KAT7	lysine acetyltransferase 7
MASP	membrane-associated serine protease
MEK	mitogen activated protein kinase kinase
mLST8	mammalian lethal with sec13 protein 8
mSin1	mammalian stress-activated mitogen-activated protein kinase-interacting protein 1
mTOR	mammalian target of rapamycin
mTORC	mammalian target of rapamycin complex
PI3K	phosphatidylinositol 3-kinase
PRAS40	proline-rich Akt substrate of 40 kDa
Raptor	regulatory associated protein of mammalian target of rapamycin
RheB	Ras homolog enriched in the brain

Rictor	rapamycin-insensitive companion of mammalian target of rapamycin
S6K1	70-kDa ribosomal protein S6 kinase
SETD2	methyltransferase SET-domain-containing 2
SCF	Skp1/cullin/F-box
SIRT2	sirtuin 2
Skp2	S-phase kinase-associated protein 2
TSC	tuberous sclerosis complex
UBE3A	ubiquitin protein ligase E3A

References

1. Stubbe J, van der Donk WA. Ribonucleotide reductases: radical enzymes with suicidal tendencies. *Chem Biol.* 1995; 2(12):793–801.
2. Reichard P. From RNA to DNA, why so many ribonucleotide reductases? *Science.* 1993;260(5115):1773–1777.
3. Thelander L, Reichard P. Reduction of ribonucleotides. *Annu Rev Biochem.* 1979;48:133–158.
4. Fontecave M, Nordlund P, Eklund H, et al. The redox centers of ribonucleotide reductase of *Escherichia coli*. *Adv Enzymol Relat Areas Mol Biol.* 1992;65:147–183.
5. Blakley RL, Barker HA. Cobamide stimulation of the reduction of ribotides to deoxyribotides in *Lactobacillus leichmannii*. *Biochem Biophys Res Commun.* 1964;16(5):391–397.
6. Eliasson R, Fontecave M, Jörnvall H, et al. The anaerobic ribonucleoside triphosphate reductase from *Escherichia coli* requires S-adenosylmethionine as a cofactor. *Proc Natl Acad Sci U S A.* 1990;87(9):3314–3318.
7. Willing A, Follmann H, Auling G. Ribonucleotide reductase of *Brevibacterium ammoniagenes* is a manganese enzyme. *Eur J Biochem.* 1988;170(3):603–611.
8. Zhou B, Yen Y. Characterization of the human ribonucleotide reductase M2 subunit gene; genomic structure and promoter analyses. *Cytogenet Cell Genet.* 2001;95(1–2):52–59.
9. Park JB, Levine M. Characterization of the promoter of the human ribonucleotide reductase R2 gene. *Biochem Biophys Res Commun.* 2000;267(2):651–657.
10. Chabes A, Thelander L. Controlled protein degradation regulates ribonucleotide reductase activity in proliferating mammalian cells during the normal cell cycle and in response to DNA damage and replication blocks. *J Biol Chem.* 2000; 275(23):17747–17753.
11. Pavloff N, Rivard D, Masson S, et al. Sequence analysis of the large and small subunits of human ribonucleotide reductase. *DNA Seq.* 1992;2(4):227–234.
12. Thelander M, Thelander L. Molecular cloning and expression of the functional gene encoding the M2 subunit of mouse ribonucleotide reductase: a new dominant marker gene. *EMBO J.* 1989;8(9):2475–2479.
13. Eklund H, Uhlin U, Färnegårdh M, et al. Structure and function of the radical enzyme ribonucleotide reductase. *Prog Biophys Mol Biol.* 2001;77(3):177–268.
14. Kolberg M, Strand KR, Graff P, et al. Structure, function, and mechanism of ribonucleotide reductases. *Biochim Biophys Acta.* 2004;1699(1–2):1–34.
15. Zhou B, Su L, Yuan YC, et al. Structural basis on the dityrosyl-diiron radical cluster and the functional differences of human ribonucleotide reductase small subunits hp53R2 and hRRM2. *Mol Cancer Ther.* 2010;9(6):1669–1679.
16. Zhou B, Mo X, Liu X, et al. Human ribonucleotide reductase M2 subunit gene amplification and transcriptional regulation in a homogeneous staining chromosome region responsible for the mechanism of drug resistance. *Cytogenet Cell Genet.* 2001; 95(1–2):34–42.

17. Eriksson S, Gräslund A, Skog S, et al. Cell cycle-dependent regulation of mammalian ribonucleotide reductase. The S phase-correlated increase in subunit M2 is regulated by *de novo* protein synthesis. *J Biol Chem.* 1984;259(19):11695–11700.
18. Chabes AL, Björklund S, Thelander LS. Phase-specific transcription of the mouse ribonucleotide reductase R2 gene requires both a proximal repressive E2F-binding site and an upstream promoter activating region. *J Biol Chem.* 2004;279(11):10796–10807.
19. Qian X, Strahs D, Schlick T. Dynamic simulations of 13 TATA variants refine kinetic hypotheses of sequence/activity relationships. *J Mol Biol.* 2001;308(4):681–703.
20. Kotova I, Chabes AL, Lobov S, et al. Sequences downstream of the transcription initiation site are important for proper initiation and regulation of mouse ribonucleotide reductase R2 gene transcription. *Eur J Biochem.* 2003;270(8):1791–1801.
21. Bhuiyan T, Timmers HTM. Promoter recognition: putting TFIID on the spot. *Trends Cell Biol.* 2019;29(9):752–763.
22. Wasyluk B, Derbyshire R, Guy A, et al. Specific *in vitro* transcription of conalbumin gene is drastically decreased by single-point mutation in T-A-T-a box homology sequence. *Proc Natl Acad Sci U S A.* 1980;77(12):7024–7028.
23. Vo Ngoc L, Wang YL, Kassavetis GA, et al. The punctilious RNA polymerase II core promoter. *Genes Dev.* 2017;31(13):1289–1301.
24. Pasculli B, Barbano R, Parrella P. Epigenetics of breast cancer: biology and clinical implication in the era of precision medicine. *Semin Cancer Biol.* 2018;51:22–35.
25. Jovanovic J, Rønneberg JA, Tost J, et al. The epigenetics of breast cancer. *Mol Oncol.* 2010;4(3):242–254.
26. Qi L, Zhou B, Chen J, et al. Significant prognostic values of differentially expressed-aberrantly methylated hub genes in breast cancer. *J Cancer.* 2019;10(26):6618–6634.
27. Luger K, Mäder AW, Richmond RK, et al. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature.* 1997;389(6648):251–260.
28. Strahl BD, Allis CD. The language of covalent histone modifications. *Nature.* 2000;403(6765):41–45.
29. Li J, Ahn JH, Wang GG. Understanding histone H3 lysine 36 methylation and its deregulation in disease. *Cell Mol Life Sci.* 2019;76(15):2899–2916.
30. Zhang T, Cooper S, Brockdorff N. The interplay of histone modifications - writers that read. *EMBO Rep.* 2015;16(11):1467–1481.
31. Xiao C, Fan T, Tian H, et al. H3K36 trimethylation-mediated biological functions in cancer. *Clin Epigenetics.* 2021;13:199.
32. Lam UTF, Chen ES. Molecular mechanisms in governing genomic stability and tumor suppression by the SETD2 H3K36 methyltransferase. *Int J Biochem Cell Biol.* 2022;144:106155.
33. Pfister SX, Markkanen E, Jiang Y, et al. Inhibiting WEE1 selectively kills histone H3K36me3-deficient cancers by dNTP starvation. *Cancer Cell.* 2015;28(5):557–568.
34. Vermeulen M, Eberl HC, Matarese F, et al. Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers. *Cell.* 2010;142(6):967–980.
35. Baxter RC. IGF binding proteins in cancer: mechanistic and clinical insights. *Nat Rev Cancer.* 2014;14(5):329–341.
36. Hakuno F, Takahashi SI. IGF₁ receptor signaling pathways. *J Mol Endocrinol.* 2018;61(1):T69–T86.
37. Zhuang S, Li L, Zang Y, et al. RRM2 elicits the metastatic potential of breast cancer cells by regulating cell invasion, migration and VEGF expression via the PI3K/AKT signaling. *Oncol Lett.* 2020;19(4):3349–3355.
38. Cui L, Yan L, Guan X, et al. Anti-tumor effect of apatinib and relevant mechanisms in liposarcoma. *Front Oncol.* 2021;11:739139.
39. Li S, Mai H, Zhu Y, et al. MicroRNA-4500 inhibits migration, invasion, and angiogenesis of breast cancer cells via RRM2-dependent MAPK signaling pathway. *Mol Ther Nucleic Acids.* 2020;21:278–289.
40. Rieunier G, Wu X, Harris LE, et al. Targeting IGF perturbs global replication through ribonucleotide reductase dysfunction. *Cancer Res.* 2021;81(8):2128–2141.
41. Lee H, Kim WJ, Kang HG, et al. Upregulation of LAMB1 via ERK/c-Jun axis promotes gastric cancer growth and motility. *Int J Mol Sci.* 2021;22(2):626.
42. Aleksic T, Gray N, Wu X, et al. Nuclear IGF1R interacts with regulatory regions of chromatin to promote RNA polymerase II recruitment and gene expression associated with advanced tumor stage. *Cancer Res.* 2018;78(13):3497–3509.
43. Fu Z, Zhang S, Wang B, et al. Annexin A1: a double-edged sword as novel cancer biomarker. *Clin Chim Acta.* 2020;504:36–42.
44. Araújo TG, Mota STS, Ferreira HSV, et al. Annexin A1 as a regulator of immune response in cancer. *Cells.* 2021;10(9):2245.
45. Xiong W, Zhang B, Yu H, et al. RRM2 regulates sensitivity to sunitinib and PD-1 blockade in renal cancer by stabilizing ANXA1 and activating the AKT pathway. *Adv Sci (Weinh).* 2021;8(18):e2100881.
46. Boudhraa Z, Merle C, Mazzocut D, et al. Characterization of pro-invasive mechanisms and N-terminal cleavage of ANXA1 in melanoma. *Arch Dermatol Res.* 2014;306(10):903–914.
47. Li Y, Ye D. Molecular biology for formyl peptide receptors in human diseases. *J Mol Med.* 2013;91(7):781–789.
48. Khau T, Langenbach SY, Schuliga M, et al. Annexin-1 signals mitogen-stimulated breast tumor cell proliferation by activation of the formyl peptide receptors (FPRs) 1 and 2. *FASEB J.* 2011;25(2):483–496.
49. Wei L, Li L, Liu L, et al. Knockdown of annexin-A1 inhibits growth, migration and invasion of glioma cells by suppressing the PI3K/Akt signaling pathway. *ASN Neuro.* 2021;13:17590914211001218.
50. Barbosa CMV, Fock RA, Hastreiter AA, et al. Extracellular annexin-A1 promotes myeloid/granulocytic differentiation of hematopoietic stem/progenitor cells via the Ca²⁺/MAPK signalling transduction pathway. *Cell Death Discov.* 2019;5:135.
51. Bist P, Phua QH, Shu S, et al. Annexin-A1 controls an ERK-RhoA-NFκB activation loop in breast cancer cells. *Biochem Biophys Res Commun.* 2015;461(1):47–53.
52. Kent LN, Leone G. The broken cycle: E2F dysfunction in cancer. *Nat Rev Cancer.* 2019;19(6):326–338.
53. Lavoie JN, L'Allemain G, Brunet A, et al. Cyclin D1 expression is regulated positively by the p42/p44^{MAPK} and negatively by the p38/HOG^{MAPK} pathway. *J Biol Chem.* 1996;271(34):20608–20616.
54. Rubin SM, Sage J, Skotheim JM. Integrating old and new paradigms of G1/S control. *Mol Cell.* 2020;80(2):183–192.
55. Lindeman GJ, Gaubatz S, Livingston DM, et al. The subcellular localization of E2F-4 is cell-cycle dependent. *Proc Natl Acad Sci U S A.* 1997;94(10):5095–5100.
56. Magae J, Wu CL, Illenye S, et al. Nuclear localization of DP and E2F transcription factors by heterodimeric partners and retinoblastoma protein family members. *J Cell Sci.* 1996;109(Pt 7):1717–1726.
57. Verona R, Moberg K, Estes S, et al. E2F activity is regulated by cell cycle-dependent changes in subcellular localization. *Mol Cell Biol.* 1997;17(12):7268–7282.
58. Wang R, Li X, Sun C, et al. The ATPase Pontin is a key cell cycle regulator by amplifying E2F1 transcription response in glioma. *Cell Death Dis.* 2021;12(2):141.
59. La Thangue NB. The Yin and Yang of E2F-1: balancing life and death. *Nat Cell Biol.* 2003;5(7):587–589.
60. Cho EC, Zheng S, Munro S, et al. Arginine methylation controls growth regulation by E2F-1. *EMBO J.* 2012;31(7):1785–1797.

61. Zheng S, Moehlenbrink J, Lu YC, et al. Arginine methylation-dependent reader-writer interplay governs growth control by E2F-1. *Mol Cell*. 2013;52(1):37–51.
62. Cura V, Cavarelli J. Structure, activity and function of the PRMT2 protein arginine methyltransferase. *Life (Basel)*. 2021;11(11):1263.
63. Bai F, Chan HL, Scott A, et al. BRCA1 suppresses epithelial-to-mesenchymal transition and stem cell dedifferentiation during mammary and tumor development. *Cancer Res*. 2014;74(21):6161–6172.
64. Bai F, Wang C, Liu X, et al. Loss of function of BRCA1 promotes EMT in mammary tumors through activation of TGF β R2 signaling pathway. *Cell Death Dis*. 2022;13(3):195.
65. Rasmussen RD, Gajjar MK, Tuckova L, et al. BRCA1-regulated RRM2 expression protects glioblastoma cells from endogenous replication stress and promotes tumorigenicity. *Nat Commun*. 2016;7:13398.
66. Musa J, Aynaud MM, Mirabeau O, et al. MYBL2 (B-Myb): a central regulator of cell proliferation, cell survival and differentiation involved in tumorigenesis. *Cell Death Dis*. 2017;8(6):e2895.
67. Bayley R, Ward C, Garcia P. MYBL2 amplification in breast cancer: molecular mechanisms and therapeutic potential. *Biochim Biophys Acta Rev Cancer*. 2020;1874(2):188407.
68. Liu Q, Guo L, Qi H, et al. A MYBL2 complex for RRM2 transactivation and the synthetic effect of MYBL2 knockdown with WEE1 inhibition against colorectal cancer. *Cell Death Dis*. 2021;12(7):683.
69. Xing Z, Lin A, Li C, et al. lncRNA directs cooperative epigenetic regulation downstream of chemokine signals. *Cell*. 2014;159(5):1110–1125.
70. Kandettu A, Radhakrishnan R, Chakrabarty S, et al. The emerging role of miRNA clusters in breast cancer progression. *Biochim Biophys Acta Rev Cancer*. 2020;1874(2):188413.
71. Liang WH, Li N, Yuan ZQ, et al. DSCAM-AS1 promotes tumor growth of breast cancer by reducing miR-204-5p and up-regulating RRM2. *Mol Carcinog*. 2019;58(4):461–473.
72. Du SM. The SNHG16/miR-30a axis promotes breast cancer cell proliferation and invasion by regulating RRM2. *Neoplasma*. 2020;67(3):567–575.
73. Feng H, Wang Q, Xiao W, et al. lncRNA TTN-AS1 regulates miR-524-5p and RRM2 to promote breast cancer progression. *Onco Targets Ther*. 2020;13:4799–4811.
74. Tait-Mulder J, Hodge K, Sumpton D, et al. The conversion of formate into purines stimulates mTORC1 leading to CAD-dependent activation of pyrimidine synthesis. *Cancer Metab*. 2020;8:20.
75. Fu X, Li P, Zhang L, et al. Activation of Rictor/mTORC2 signaling acts as a pivotal strategy to protect against sensorineural hearing loss. *Proc Natl Acad Sci U S A*. 2022;119(10):e2107357119.
76. Shams R, Ito Y, Miyatake H. Mapping of mTOR drug targets: featured platforms for anti-cancer drug discovery. *Pharmacol Ther*. 2022;232:108012.
77. Yin Y, Long J, Sun Y, et al. The function and clinical significance of eIF $_3$ in cancer. *Gene*. 2018;673:130–133.
78. Chen Q, Yang B, Nass N, et al. Impact of eukaryotic translation initiation factors on breast cancer: still much to investigate. *Cancers*. 2020;12(7):1984.
79. Ma S, Dong Z, Huang Y, et al. eIF $_3$ a regulation of mTOR signaling and translational control via HuR in cellular response to DNA damage. *Oncogene*. 2022;41(17):2431–2443.
80. Qin X, Jiang B, Zhang Y. 4E-BP1, a multifactor regulated multifunctional protein. *Cell Cycle*. 2016;15(6):781–786.
81. He Z, Hu X, Liu W, et al. P53 suppresses ribonucleotide reductase via inhibiting mTORC1. *Oncotarget*. 2017;8(25):41422–41431.
82. Goss KL, Koppenhafer SL, Waters T, et al. The translational repressor 4E-BP1 regulates RRM2 levels and functions as a tumor suppressor in Ewing sarcoma tumors. *Oncogene*. 2021;40(3):564–577.
83. Hauffe L, Picard D, Musa J, et al. Eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1) expression in glioblastoma is driven by ETS1- and MYBL2-dependent transcriptional activation. *Cell Death Discov*. 2022;8(1):91.
84. Zhang S, Yan L, Cui C, et al. Downregulation of RRM2 attenuates retroperitoneal liposarcoma progression via the Akt/mTOR/4EBP1 pathway: clinical, biological, and therapeutic significance. *Onco Targets Ther*. 2020;13:6523–6537.
85. Dong Z, Liu LH, Han B, et al. Role of eIF $_3$ p170 in controlling synthesis of ribonucleotide reductase M2 and cell growth. *Oncogene*. 2004;23(21):3790–3801.
86. Sridharan S, Basu A. Distinct roles of mTOR targets S6K1 and S6K2 in breast cancer. *Int J Mol Sci*. 2020;21(4):1199.
87. Julien LA, Carriere A, Moreau J, et al. mTORC1-activated S6K1 phosphorylates rictor on threonine 1135 and regulates mTORC2 signaling. *Mol Cell Biol*. 2010;30(4):908–921.
88. Greene MW, Sakaue H, Wang L, et al. Modulation of insulin-stimulated degradation of human insulin receptor substrate-1 by serine 312 phosphorylation. *J Biol Chem*. 2003;278(10):8199–8211.
89. Denhez B, Rousseau M, Spino C, et al. Saturated fatty acids induce insulin resistance in podocytes through inhibition of IRS1 via activation of both IKK β and mTORC1. *Sci Rep*. 2020;10:21628.
90. Zhang Q, Zhou X, Wan M, et al. FoxP3-miR-150-5p/3p suppresses ovarian tumorigenesis via an IGF1R/IRS1 pathway feedback loop. *Cell Death Dis*. 2021;12(3):275.
91. Williams LM, Rudensky AY. Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. *Nat Immunol*. 2007;8(3):277–284.
92. Hashemi V, Maleki LA, Esmaily M, et al. Regulatory T cells in breast cancer as a potent anti-cancer therapeutic target. *Int Immunopharmacol*. 2020;78:106087.
93. Haxhinasto S, Mathis D, Benoist C. The AKT-mTOR axis regulates *de novo* differentiation of CD4 $^{+}$ Foxp3 $^{+}$ cells. *J Exp Med*. 2008;205(3):565–574.
94. Li W, Li F, Zhang X, et al. Insights into the post-translational modification and its emerging role in shaping the tumor microenvironment. *Signal Transduct Target Ther*. 2021;6:422.
95. Shvedunova M, Akhtar A. Modulation of cellular processes by histone and non-histone protein acetylation. *Nat Rev Mol Cell Biol*. 2022;23(5):329–349.
96. Newman DM, Voss AK, Thomas T, et al. Essential role for the histone acetyltransferase KAT7 in T cell development, fitness, and survival. *J Leukoc Biol*. 2017;101(4):887–892.
97. Zhang Y, Long X, Ruan X, et al. SIRT2-mediated deacetylation and deubiquitination of C/EBP β prevents ethanol-induced liver injury. *Cell Discov*. 2021;7:93.
98. Chen G, Luo Y, Warncke K, et al. Acetylation regulates ribonucleotide reductase activity and cancer cell growth. *Nat Commun*. 2019;10:3213.
99. Saldívar JC, Cortez D, Cimprich KA. The essential kinase ATR: ensuring faithful duplication of a challenging genome. *Nat Rev Mol Cell Biol*. 2017;18(10):622–636.
100. Kim ST, Lim DS, Canman CE, et al. Substrate specificities and identification of putative substrates of ATM kinase family members. *J Biol Chem*. 1999;274(53):37538–37543.
101. Dang F, Nie L, Wei W. Ubiquitin signaling in cell cycle control and tumorigenesis. *Cell Death Differ*. 2021;28(2):427–438.
102. da Fonseca PCA, Kong EH, Zhang Z, et al. Structures of APC/C(Cdh1) with substrates identify Cdh1 and Apc10 as the D-box co-receptor. *Nature*. 2011;470(7333):274–278.

103. Chabes AL, Pfeleger CM, Kirschner MW, et al. Mouse ribonucleotide reductase R2 protein: a new target for anaphase-promoting complex-Cdh1-mediated proteolysis. *Proc Natl Acad Sci U S A*. 2003;100(7):3925–3929.
104. Kim YJ, Zhao Y, Myung JK, et al. Suppression of breast cancer progression by FBXL16 via oxygen-independent regulation of HIF1 α stability. *Cell Rep*. 2021;37(8):109996.
105. Bai C, Richman R, Elledge SJ. Human cyclin F. *EMBO J*. 1994;13(24):6087–6098.
106. D'Angiolella V, Donato V, Forrester FM, et al. Cyclin F-mediated degradation of ribonucleotide reductase M2 controls genome integrity and DNA repair. *Cell*. 2012;149(5):1023–1034.
107. Chang SC, Hung CS, Zhang BX, et al. A novel signature of CCNF-associated E3 ligases collaborate and counter each other in breast cancer. *Cancers*. 2021;13(12):2873.
108. Cardozo T, Pagano M. The SCF ubiquitin ligase: insights into a molecular machine. *Nat Rev Mol Cell Biol*. 2004;5(9):739–751.
109. Carrano AC, Eytan E, Hershko A, et al. SKP₂ is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nat Cell Biol*. 1999;1(4):193–199.
110. Bornstein G, Bloom J, Sitry-Shevah D, et al. Role of the SCF^{SKP2} ubiquitin ligase in the degradation of p21^{Cip1} in S phase. *J Biol Chem*. 2003;278(28):25752–25757.
111. Xiao GG, Zhou BS, Somlo G, et al. Identification of F-box/LLR-repeated protein 17 as potential useful biomarker for breast cancer therapy. *Cancer Genomics Proteomics*. 2008;5(3–4):151–160.
112. Nurse P, Thuriaux P. Regulatory genes controlling mitosis in the fission yeast *Schizosaccharomyces pombe*. *Genetics*. 1980;96(3):627–637.
113. McGowan CH, Russell P. Human Wee1 kinase inhibits cell division by phosphorylating p34cdc2 exclusively on Tyr15. *EMBO J*. 1993;12(1):75–85.
114. Elbæk CR, Petrosius V, Sørensen CS. WEE1 kinase limits CDK activities to safeguard DNA replication and mitotic entry. *Mutat Res*. 2020;819–820:111694.
115. Koppenhafer SL, Goss KL, Terry WW, et al. Inhibition of the ATR-CHK₁ pathway in ewing sarcoma cells causes DNA damage and apoptosis via the CDK2-mediated degradation of RRM2. *Mol Cancer Res*. 2020;18(1):91–104.
116. Gaillard H, Garcia-Muse T, Aguilera A. Replication stress and cancer. *Nat Rev Cancer*. 2015;15(5):276–289.
117. Wang L, Zhao X, Fu J, et al. The role of tumour metabolism in cisplatin resistance. *Front Mol Biosci*. 2021;8:691795.
118. Kato T, Ono H, Fujii M, et al. Cytoplasmic RRM1 activation as an acute response to gemcitabine treatment is involved in drug resistance of pancreatic cancer cells. *PLoS One*. 2021;16(6):e0252917.
119. Yen Y, Grill SP, Dutschman GE, et al. Characterization of a hydroxyurea-resistant human KB cell line with supersensitivity to 6-thioguanine. *Cancer Res*. 1994;54(14):3686–3691.
120. Zhou BS, Hsu NY, Pan BC, et al. Overexpression of ribonucleotide reductase in transfected human KB cells increases their resistance to hydroxyurea: M2 but not M1 is sufficient to increase resistance to hydroxyurea in transfected cells. *Cancer Res*. 1995;55(6):1328–1333.
121. Das B, Jain N, Mallick B. piR-39980 mediates doxorubicin resistance in fibrosarcoma by regulating drug accumulation and DNA repair. *Commun Biol*. 2021;4:1312.
122. Zhao Y, Zheng Y, Zhu Y, et al. M1 macrophage-derived exosomes loaded with gemcitabine and deferasirox against chemoresistant pancreatic cancer. *Pharmaceutics*. 2021;13(9):1493.
123. Un F, Qi C, Prosser M, et al. Modulating ICBP90 to suppress human ribonucleotide reductase M2 induction restores sensitivity to hydroxyurea cytotoxicity. *Anticancer Res*. 2006;26(4B):2761–2767.
124. Shah KN, Mehta KR, Peterson D, et al. AKT-induced tamoxifen resistance is overturned by RRM2 inhibition. *Mol Cancer Res*. 2014;12(3):394–407.
125. Shah KN, Wilson EA, Malla R, et al. Targeting ribonucleotide reductase M2 and NF- κ B activation with didox to circumvent tamoxifen resistance in breast cancer. *Mol Cancer Ther*. 2015;14(11):2411–2421.
126. Li ZN, Shu Y, Chen CG, et al. Acquired tamoxifen resistance is surmounted by GW8510 through ribonucleotide reductase M2 downregulation-mediated autophagy induction. *Biochem Biophys Res Commun*. 2020;528(3):554–560.
127. Zhou S, Li J, Xu H, et al. Liposomal curcumin alters chemosensitivity of breast cancer cells to Adriamycin via regulating microRNA expression. *Gene*. 2017;622:1–12.
128. Muto M, Kanari Y, Kubo E, et al. Targeted disruption of Np95 gene renders murine embryonic stem cells hypersensitive to DNA damaging agents and DNA replication blocks. *J Biol Chem*. 2002;277(37):34549–34555.
129. Nishiyama A, Yamaguchi L, Sharif J, et al. Uhrf1-dependent H3K23 ubiquitylation couples maintenance DNA methylation and replication. *Nature*. 2013;502(7470):249–253.
130. Daylami R, Muilenburg DJ, Virudachalam S, et al. Pegylated arginine deiminase synergistically increases the cytotoxicity of gemcitabine in human pancreatic cancer. *J Exp Clin Cancer Res*. 2014;33:102.
131. Kotova I, Chabes AL, Segerman B, et al. A mouse *in vitro* transcription system reconstituted from highly purified RNA polymerase II, TFIIF and recombinant TBP, TFIIB, TFIIE and TFIIH. *Eur J Biochem*. 2001;268(16):4527–4536.
132. Liu X, Zhou B, Xue L, et al. Nuclear factor Y regulation and promoter transactivation of human ribonucleotide reductase subunit M2 gene in a Gemcitabine resistant KB clone. *Biochem Pharmacol*. 2004;67(8):1499–1511.
133. Chen P, Wu JN, Shu Y, et al. Gemcitabine resistance mediated by ribonucleotide reductase M2 in lung squamous cell carcinoma is reversed by GW8510 through autophagy induction. *Clin Sci (Lond)*. 2018;132(13):1417–1433.
134. Goan YG, Zhou B, Hu E, et al. Overexpression of ribonucleotide reductase as a mechanism of resistance to 2, 2-difluorodeoxycytidine in the human KB cancer cell line. *Cancer Res*. 1999;59(17):4204–4207.
135. Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell*. 2012;149(5):1060–1072.
136. Stockwell BR, Friedmann Angeli JP, Bayir H, et al. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. *Cell*. 2017;171(2):273–285.
137. Jiang Y, Tao R, Shen Z, et al. Enzymatic production of glutathione by bifunctional γ -glutamylcysteine synthetase/glutathione synthetase coupled with *in vitro* acetate kinase-based ATP generation. *Appl Biochem Biotechnol*. 2016;180(7):1446–1455.
138. Tarangelo A, Rodencal J, Kim JT, et al. Nucleotide biosynthesis links glutathione metabolism to ferroptosis sensitivity. *Life Sci Alliance*. 2022;5(4):e202101157.
139. Ke ZB, You Q, Sun JB, et al. A novel ferroptosis-based molecular signature associated with biochemical recurrence-free survival and tumor immune microenvironment of prostate cancer. *Front Cell Dev Biol*. 2021;9:774625.
140. Zhang X, Du L, Qiao Y, et al. Ferroptosis is governed by differential regulation of transcription in liver cancer. *Redox Biol*. 2019;24:101211.
141. Tang B, Xu W, Wang Y, et al. Identification of critical ferroptosis regulators in lung adenocarcinoma that RRM2 facilitates tumor immune infiltration by inhibiting ferroptotic death. *Clin Immunol*. 2021;232:108872.
142. Chen W, Zhang L, Zhang K, et al. Reciprocal regulation of autophagy and dNTP pools in human cancer cells. *Autophagy*. 2014;10(7):1272–1284.

143. Kennedy LB, Salama AKS. A review of cancer immunotherapy toxicity. *CA Cancer J Clin.* 2020;70(2):86–104.
144. Nam J, Son S, Park KS, et al. Cancer nanomedicine for combination cancer immunotherapy. *Nat Rev Mater.* 2019;4(6):398–414.
145. Mazzu YZ, Armenia J, Nandakumar S, et al. Ribonucleotide reductase small subunit M2 is a master driver of aggressive prostate cancer. *Mol Oncol.* 2020;14(8):1881–1897.
146. Roviello G, Iannone LF, Bersanelli M, et al. The gut microbiome and efficacy of cancer immunotherapy. *Pharmacol Ther.* 2022;231:107973.
147. Sharpe AH, Wherry EJ, Ahmed R, et al. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat Immunol.* 2007;8(3):239–245.
148. Iwai Y, Hamanishi J, Chamoto K, et al. Cancer immunotherapies targeting the PD-1 signaling pathway. *J Biomed Sci.* 2017;24:26.
149. Okazaki T, Honjo T. The PD-1-PD-L pathway in immunological tolerance. *Trends Immunol.* 2006;27(4):195–201.
150. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med.* 2002;8(8):793–800.
151. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations. *Sci Transl Med.* 2016;8(328):328rv4.
152. Fife BT, Pauken KE, Eagar TN, et al. Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal. *Nat Immunol.* 2009;10(11):1185–1192.
153. Zhang J, Fang W, Qin T, et al. Co-expression of PD-1 and PD-L1 predicts poor outcome in nasopharyngeal carcinoma. *Med Oncol.* 2015;32(3):86.
154. Ren D, Hua Y, Yu B, et al. Predictive biomarkers and mechanisms underlying resistance to PD1/PD-L1 blockade cancer immunotherapy. *Mol Cancer.* 2020;19:19.
155. Palazon A, Goldrath AW, Nizet V, et al. HIF transcription factors, inflammation, and immunity. *Immunity.* 2014;41(4):518–528.
156. Luo F, Lu FT, Cao JX, et al. HIF-1 α inhibition promotes the efficacy of immune checkpoint blockade in the treatment of non-small cell lung cancer. *Cancer Lett.* 2022;531:39–56.
157. Bailey CM, Liu Y, Liu M, et al. Targeting HIF-1 α abrogates PD-L1-mediated immune evasion in tumor microenvironment but promotes tolerance in normal tissues. *J Clin Invest.* 2022;132(9):e150846.
158. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity.* 2016;44(3):450–462.
159. Mantovani A, Marchesi F, Malesci A, et al. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol.* 2017;14(7):399–416.
160. Kuo ML, Hwang HS, Sosnay PR, et al. Overexpression of the R2 subunit of ribonucleotide reductase in human nasopharyngeal cancer cells reduces radiosensitivity. *Cancer J.* 2003;9(4):277–285.
161. Griesshammer M, Wille K, Sadjadian P, et al. A review of hydroxyurea-related cutaneous adverse events. *Expert Opin Drug Saf.* 2021;20(12):1515–1521.
162. Nyholm S, Thelander L, Gräslund A. Reduction and loss of the iron center in the reaction of the small subunit of mouse ribonucleotide reductase with hydroxyurea. *Biochemistry.* 1993;32(43):11569–11574.
163. Asperti M, Cantamessa L, Ghidinelli S, et al. The antitumor didox acts as an iron *Chelator* in hepatocellular carcinoma cells. *Pharmaceuticals (Basel).* 2019;12(3):129.
164. Elfrod HL, Wampler GL, van't Riet B. New ribonucleotide reductase inhibitors with antineoplastic activity. *Cancer Res.* 1979;39(3):844–851.
165. Horvath Z, Bauer W, Hoechtl T, et al. Combination chemotherapy of BCNU and Didox acts synergistically in 9L glioma cells. *Nucleosides Nucleotides Nucleic Acids.* 2004;23(8–9):1531–1535.
166. Khaleel SA, Al-Abd AM, Ali AA, et al. Didox and resveratrol sensitize colorectal cancer cells to doxorubicin via activating apoptosis and ameliorating P-glycoprotein activity. *Sci Rep.* 2016;6:36855.
167. Shang Y, Li X, Li Z, et al. Theoretical study on the radical scavenging activity and mechanism of four kinds of Gnetin molecule. *Food Chem.* 2022;378:131975.
168. Sun Q, Ye Z, Qin Y, et al. Oncogenic function of TRIM2 in pancreatic cancer by activating ROS-related NRF2/ITGB7/FAK axis. *Oncogene.* 2020;39(42):6572–6588.
169. Cheng T, Wang C, Lu Q, et al. Metformin inhibits the tumor-promoting effect of low-dose resveratrol, and enhances the anti-tumor activity of high-dose resveratrol by increasing its reducibility in triple negative breast cancer. *Free Radic Biol Med.* 2022;180:108–120.
170. Fontecave M, Lepoivre M, Elleingand E, et al. Resveratrol, a remarkable inhibitor of ribonucleotide reductase. *FEBS Lett.* 1998;421(3):277–279.
171. Lundberg JH, Chitambar CR. Interaction of gallium nitrate with fludarabine and iron chelators: effects on the proliferation of human leukemic HL60 cells. *Cancer Res.* 1990;50(20):6466–6470.
172. Nocentini G, Federici F, Armellini R, et al. Isolation of two cellular lines resistant to ribonucleotide reductase inhibitors to investigate the inhibitory activity of 2, 2'-bipyridyl-6-carbothioamide. *Anticancer Drugs.* 1990;1(2):171–177.
173. Chitambar CR, Mattheus WG, Antholine WE, et al. Inhibition of leukemic HL60 cell growth by transferrin-Gallium: effects on ribonucleotide reductase and demonstration of drug synergy with hydroxyurea. *Blood.* 1988;72(6):1930–1936.
174. Li J, Zheng LM, King I, et al. Syntheses and antitumor activities of potent inhibitors of ribonucleotide reductase: 3-amino-4-methylpyridine-2-carboxaldehyde-thiosemicarbazone (3-AMP), 3-amino-pyridine-2-carboxaldehyde-thiosemicarbazone (3-AP) and its water-soluble prodrugs. *Curr Med Chem.* 2001;8(2):121–133.
175. Aye Y, Long MJC, Stubbe J. Mechanistic studies of semicarbazone triapine targeting human ribonucleotide reductase *in vitro* and in mammalian cells: tyrosyl radical quenching not involving reactive oxygen species. *J Biol Chem.* 2012;287(42):35768–35778.
176. Nutting CM, van Herpen CML, Miah AB, et al. Phase II study of 3-AP Triapine in patients with recurrent or metastatic head and neck squamous cell carcinoma. *Ann Oncol.* 2009;20(7):1275–1279.
177. Traynor AM, Lee JW, Bayer GK, et al. A phase II trial of Triapine® (NSC# 663249) and gemcitabine as second line treatment of advanced non-small cell lung cancer: eastern Cooperative Oncology Group Study 1503. *Invest New Drugs.* 2010;28(1):91–97.
178. Stefani C, Jansson PJ, Gutierrez E, et al. Alkyl substituted 2'-benzoylpyridine thiosemicarbazone chelators with potent and selective anti-neoplastic activity: novel ligands that limit methemoglobin formation. *J Med Chem.* 2013;56(1):357–370.
179. Plamthottam S, Sun D, Van Valkenburgh J, et al. Activity and electrochemical properties: iron complexes of the anticancer drug triapine and its analogs. *J Biol Inorg Chem.* 2019;24(5):621–632.
180. Senderowicz AM, Sausville EA. Preclinical and clinical development of cyclin-dependent kinase modulators. *J Natl Cancer Inst.* 2000;92(5):376–387.
181. Zeidner JF, Karp JE. Clinical activity of alvocidib (flavopiridol) in acute myeloid leukemia. *Leuk Res.* 2015;39(12):1312–1318.

182. Wiernik PH. Alvocidib (flavopiridol) for the treatment of chronic lymphocytic leukemia. *Expert Opin Investig Drugs*. 2016;25(6):729–734.
183. Wilson MB, Schreiner SJ, Choi HJ, et al. Selective pyrrolo-pyrimidine inhibitors reveal a necessary role for Src family kinases in Bcr-Abl signal transduction and oncogenesis. *Oncogene*. 2002;21(53):8075–8088.
184. Jung CP, Motwani MV, Schwartz GK. Flavopiridol increases sensitization to gemcitabine in human gastrointestinal cancer cell lines and correlates with down-regulation of ribonucleotide reductase M2 subunit. *Clin Cancer Res*. 2001;7(8):2527–2536.
185. Duxbury MS, Ito H, Zinner MJ, et al. Inhibition of SRC tyrosine kinase impairs inherent and acquired gemcitabine resistance in human pancreatic adenocarcinoma cells. *Clin Cancer Res*. 2004;10(7):2307–2318.
186. Chen CL, Hsu SC, Ann DK, et al. Arginine signaling and cancer metabolism. *Cancers*. 2021;13(14):3541.
187. Matos A, Carvalho M, Bicho M, et al. Arginine and arginases modulate metabolism, tumor microenvironment and prostate cancer progression. *Nutrients*. 2021;13(12):4503.
188. Riess C, Shokraie F, Classen CF, et al. Arginine-depleting enzymes - an increasingly recognized treatment strategy for therapy-refractory malignancies. *Cell Physiol Biochem*. 2018;51(2):854–870.
189. Yao S, Janku F, Koenig K, et al. Phase 1 trial of ADI-PEG 20 and liposomal doxorubicin in patients with metastatic solid tumors. *Cancer Med*. 2022;11(2):340–347.
190. Prudner BC, Rathore R, Robinson AM, et al. Arginine starvation and docetaxel induce c-Myc-driven hENT1 surface expression to overcome gemcitabine resistance in ASS1-negative tumors. *Clin Cancer Res*. 2019;25(16):5122–5134.
191. 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(8):1499–1529.
192. Duxbury MS, Ito H, Benoit E, et al. Retrovirally mediated RNA interference targeting the M2 subunit of ribonucleotide reductase: a novel therapeutic strategy in pancreatic cancer. *Surgery*. 2004;136(2):261–269.
193. Marzec M. Size does matter: piRNA and miRNA targeting. *Trends Biochem Sci*. 2022;47(4):287–288.
194. Das B, Roy J, Jain N, et al. Tumor suppressive activity of PIWI-interacting RNA in human fibrosarcoma mediated through repression of RRM2. *Mol Carcinog*. 2019;58(3):344–357.
195. Comont T, Heiblig M, Rivière E, et al. Azacitidine for patients with Vacuoles, E1 Enzyme, X-linked, Autoinflammatory, Somatic syndrome (VEXAS) and myelodysplastic syndrome: data from the French VEXAS registry. *Br J Haematol*. 2022;196(4):969–974.
196. Li Z, Zhang J, Zhou M, et al. Epigenetic therapy with chidamide alone or combined with 5-azacitidine exerts antitumor effects on acute myeloid leukaemia cells *in vitro*. *Oncol Rep*. 2022;47(4):66.
197. Aimiwu J, Wang H, Chen P, et al. RNA-dependent inhibition of ribonucleotide reductase is a major pathway for 5-azacytidine activity in acute myeloid leukemia. *Blood*. 2012;119(22):5229–5238.
198. Hsieh YY, Chou CJ, Lo HL, et al. Repositioning of a cyclin-dependent kinase inhibitor GW8510 as a ribonucleotide reductase M2 inhibitor to treat human colorectal cancer. *Cell Death Discov*. 2016;2:16027.
199. Wilhelm SM, Carter C, Tang L, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res*. 2004;64(19):7099–7109.
200. Pasqualetti G, Ricciardi S, Mey V, et al. Synergistic cytotoxicity, inhibition of signal transduction pathways and pharmacogenetics of sorafenib and gemcitabine in human NSCLC cell lines. *Lung Cancer*. 2011;74(2):197–205.
201. Yang PM, Lin LS, Liu TP. Sorafenib inhibits ribonucleotide reductase regulatory subunit M2 (RRM2) in hepatocellular carcinoma cells. *Biomolecules*. 2020;10(1):117.
202. Chen CW, Li Y, Hu S, et al. DHS (trans-4, 4'-dihydroxystilbene) suppresses DNA replication and tumor growth by inhibiting RRM2 (ribonucleotide reductase regulatory subunit M2). *Oncogene*. 2019;38(13):2364–2379.
203. Zhou B, Su L, Hu S, et al. A small-molecule blocking ribonucleotide reductase holoenzyme formation inhibits cancer cell growth and overcomes drug resistance. *Cancer Res*. 2013;73(21):6484–6493.
204. Mazzu YZ, Armenia J, Chakraborty G, et al. A novel mechanism driving poor-prognosis prostate cancer: overexpression of the DNA repair gene, ribonucleotide reductase small subunit M2 (RRM2). *Clin Cancer Res*. 2019;25(14):4480–4492.
205. Zhang H, Liu X, Warden CD, et al. Prognostic and therapeutic significance of ribonucleotide reductase small subunit M2 in estrogen-negative breast cancers. *BMC Cancer*. 2014;14:664.
206. Liu X, Xu Z, Hou C, et al. Inhibition of hepatitis B virus replication by targeting ribonucleotide reductase M2 protein. *Biochem Pharmacol*. 2016;103:118–128.
207. Tang Q, Wu L, Xu M, et al. Osalmid, a novel identified RRM2 inhibitor, enhances radiosensitivity of esophageal cancer. *Int J Radiat Oncol*. 2020;108(5):1368–1379.
208. Wu Z, Zhan Y, Wang L, et al. Identification of osalmid metabolic profile and active metabolites with anti-tumor activity in human hepatocellular carcinoma cells. *Biomed Pharmacother*. 2020;130:110556.
209. Wang Y, Liang F, Zhou Y, et al. Sharp downregulation of hub genes associated with the pathogenesis of breast cancer from ductal carcinoma *in situ* to invasive ductal carcinoma. *Front Oncol*. 2021;11:634569.
210. Kretschmer C, Sterner-Kock A, Siedentopf F, et al. Identification of early molecular markers for breast cancer. *Mol Cancer*. 2011;10:15.
211. Lainetti PF, Leis-Filho AF, Laufer-Amorim R, et al. Mechanisms of resistance to chemotherapy in breast cancer and possible targets in drug delivery systems. *Pharmaceutics*. 2020;12(12):1193.
212. Rüstem DG, Atay S, Aydin HH, et al. Synergistic interactions between GW8510 and gemcitabine in an *in vitro* model of pancreatic cancer. *Anticancer Agents Med Chem*. 2021;21(16):2204–2215.
213. Kuo ML, Kinsella TJ. Expression of ribonucleotide reductase after ionizing radiation in human cervical carcinoma cells. *Cancer Res*. 1998;58(10):2245–2252.
214. Zhou B, Liu X, Mo X, et al. The human ribonucleotide reductase subunit hRRM2 complements p53R2 in response to UV-induced DNA repair in cells with mutant p53. *Cancer Res*. 2003;63(20):6583–6594.
215. Ko EY, Moon A. Natural products for chemoprevention of breast cancer. *J Cancer Prev*. 2015;20(4):223–231.
216. Winkle M, El-Daly SM, Fabbri M, et al. Noncoding RNA therapeutics - challenges and potential solutions. *Nat Rev Drug Discov*. 2021;20(8):629–651.