Review

Molecular Cancer Therapeutics

The Use of Ribavirin as an Anticancer Therapeutic: Will It Go Viral?

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Abstract

The growing cost of medical care worldwide, particularly in oncology, has incentivized researchers and physicians to repurpose clinically used drugs to alleviate the financial burden of drug development and offer potential new therapeutics. Recent works have demonstrated anticancer properties of the FDA-approved drug ribavirin, a synthetic guanosine analogue and antiviral molecule used over the past four decades for the treatment of hepatitis C. The efficacy of ribavirin in cancer has been explored through several preclinical models and ongoing clinical trials in multiple cancers, including acute myeloid leukemia, oropharyngeal squamous cell carcinoma, and metastatic breast cancer. In this review, we summarize the role of ribavirin as an antiviral medication and focus our attention on its recent use as an antitumoral agent. We highlight current knowledge of the potential use and mechanisms of action of ribavirin in cancer. Because current therapeutics for patients with cancer still fail to cure, introducing new forms of treatment is essential. Converging evidence suggests that ribavirin represents a promising addition to a generation of newly repurposed safe and effective anticancer agents.

Introduction

Due to a renewed focus on pragmatism and cost effectiveness in the high-risk realm of drug development, there has been a strong push to uncover previously uncharacterized therapeutic properties among medications that have already been granted approval by the FDA. This drive is especially urgent in the fight against cancers that lack safe and effective therapeutic options. These trends, referred to as drug repurposing or repositioning, can save vast amounts of time and resources otherwise allocated to novel drug discovery, since rigorous drug safety and toxicity studies have already led to prior approval for use in humans (1). One such repurposing candidate is ribavirin. Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is an artificial guanosine nucleoside analogue with broad-spectrum antiviral properties that was first synthesized in 1972 (Table 1; ref. 2). Since its original discovery, ribavirin has been safely used in humans to treat multiple pathologies including respiratory syncytial virus (RSV), Lassa fever, and, most notably, hepatitis C virus (HCV) infections. More recently, ribavirin has shown promising antineoplastic activities and understanding the involved mechanisms could offer important insights into targeting conserved biological pathways that drive multiple cancers.

Ribavirin, the Anticancer Drug

Since its advent as an antiviral therapy over 40 years ago, a growing body of work has shown evidence for ribavirin as an anticancer agent. In particular, the elucidation of its antagonism toward the oncogenic eukaryotic translation initiation factor 4E (eIF4E) has led to promising preclinical and clinical results in monotherapy and combination therapy for several cancers. Moreover, several clinical trials have examined the safety and efficacy of ribavirin in malignancies and have validated its use as an antineoplastic agent, particularly in combination therapies (Table 2). In this section, the use of ribavirin in cancer is discussed and corresponding mechanisms of action are discussed subsequently.

Leukemia

Perhaps the greatest promise of ribavirin as an anticancer agent stems from its inhibition of eIF4E, specifically in leukemia [see also "Eukaryotic translation initiation factor 4E (eIF4E)"]. This oncogene has since been found to be elevated in approximately 30% of human cancers, including primary M4/M5 AML subtypes, blast crisis chronic myeloid leukemia (bcCML), acute lymphoid leukemia (ALL), and chronic-phase CML (3–5). Validated by preclinical studies examining the effects of ribavirin in M4/M5 AML cells, a phase I/II clinical trial examined the efficacy and safety of ribavirin monotherapy in patients with M4/M5 AML (6). Eleven patients with either relapsed or refractory disease received 6 or more 28-day cycles of daily oral ribavirin, with starting doses at 1,000 mg/day and eventual uptitration to 1,400, 2,000, and 2,800 mg/day doses. By day 30, one patient experienced complete remission, 2 patients had partial remissions, 2 others had blast responses, 4 continued to have stable disease, and 2 had progressive disease, while there were no therapy-related toxicities (6).

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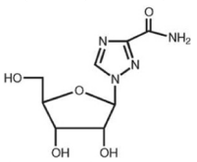
Table 1. Ribavirin's biometric information

Characteristics	Ribavirin
Structural name (other names)	1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (rebetol, ribamide, viramide, tribavirin)
Structure	
Formula	C_8-H_{12}-N_4-O_5
Molecular weight	244.207 g/mol
Density	2.09 g/cm^3
Solubility	Water, 142 mg/mL DMSO, 50 mg/mL at 25°C Ethanol, 1 mg/mL at 25°C
Melting point	175°C
Boiling point	639.78°C at 760 mm Hg
Color	White to yellow
CAS number	36791-04-5
PubChem substance ID	24278685

The safety and efficacy of ribavirin monotherapy prompted further investigation of its use in combination therapy for M4/M5 AML to increase the frequency and duration of clinical response. Anticancer agents commonly used in the treatment of AML including cytarabine (Ara-C), idarubicin, and azacitidine were each examined in combination with ribavirin on primary patient specimens. Interestingly, the addition of ribavirin to these chemotherapies permitted much lower concentrations of the more toxic Ara-C and idarubicin; Ara-C effects were observed at 1,000-fold lower concentrations than in standard 7 + 3 therapy (induction therapy including Ara-C along with either idarubicin or daunorubicin), and at 100-fold lower concentrations than in low-dose Ara-C regimens (4, 5). The safety profile of ribavirin's use in combination therapy was further validated in a follow-up phase I clinical trial, where the recommended phase II dose (RP2D) was found to be 28-day cycles of 1,400 mg oral b.i.d. continuous dosing for ribavirin and 10-mg subcutaneous injection b.i.d. for low-dose Ara-C (LDAC; ref. 7). The combination was well tolerated with no unexpected adverse effects. Hemolytic anemia, which is a known ribavirin toxicity in approximately 10% of patients (8), occurred in 4 (14%) patients. Anemia is likely due to accumulation of ribavirin in erythrocytes, the result of unidirectional transport (8). This did not change with dose reduction, but resolved when ribavirin was discontinued. Additionally, evidence of clinical response was observed. A complete response was observed in 2 patients, lasting 224 and 743 days each. Two additional patients had a partial response and blast response, respectively. Patients who demonstrated a clinical response had a median plasma ribavirin level of 33 μmol/L, while all 5 patients who responded had levels above 20 μmol/L (7). As a follow-up to this study, a phase II trial examining the efficacy of ribavirin and LDAC combination therapy has recently been completed, with release of results pending (NCT01056523), while a phase II trial examining further ribavirin drug combinations targeting mechanisms of resistance began May 2015 (NCT02073838; ref. 9).

Lymphoma

eIF4E is a factor that maintains the mRNA export and expression of driver oncogenes *MYC*, *BCL6*, and *BCL2*, which are pathogenic in diffuse large B-cell lymphoma (DLBCL). Culjkovic-Kraljacic and colleagues demonstrated preclinically that ribavirin inhibited eIF4E and abrogated eIF4E's activity in DLBCL mRNA export and expression, resulting in a significant reduction in tumor burden in both cell line– and patient-derived xenograft models (10). Furthermore, recent retrospective reports corroborate this potential role of ribavirin as an antilymphoma agent (11, 12). A recent review of clinical records reported outcomes of several patients with diagnosed lymphoma who underwent stem cell transplant who also happened to receive ribavirin for treatment of RSV infections within 6 months. Ten patients were identified and received ribavirin treatment for a median length of 10 days. Nine of 10 lymphoma patients were alive at the time of the report, with a median survival of 21.5 months and 8 patients demonstrating no evidence of disease. Although these data are limited, the observed outcomes were superior to expected outcomes per disease risk (12). These observations coupled with encouraging preclinical findings, formed the basis for a new phase I clinical trial investigating the effect of ribavirin in indolent follicular lymphoma and mantle cell lymphoma (NCT03585725).

Head and neck: squamous cell carcinoma and thyroid cancer

Combination therapies with ribavirin have been studied in several head and neck cancers, including human papillomavirus (HPV)–associated head and neck squamous cell carcinomas (HNSCC), and thyroid cancers. Preclinical xenograft experiments involving eIF4E-dependent HNSCC cell lines have provided proof-of-principle of anticancer effects of ribavirin on these tumors (13, 14). In 2017, a phase I study of induction chemotherapy with afatinib, ribavirin, and weekly carboplatin and paclitaxel for stage IVA/IVB HPV-associated HNSCC was reported (15). Current standard induction chemotherapy regimens consist of docetaxel, cisplatin, and 5-fluorouracil. However, these drugs can result in serious toxicities. The study included 10 patients diagnosed with previously untreated HPV-associated stage IVA or IVB HNSCC of the oropharynx. Investigators sought to assess the safety of this new regimen, which included the 2 biologically targeted agents, afatinib and ribavirin, along with deintensified doses of carboplatin and paclitaxel. The biological rationale being the capability of afatinib to target the enhanced activity of ErbB proteins associated with HPV infection, in combination with ribavirin targeting the oncogenic eIF4E. The study revealed no dose-limiting toxicities and no intensification of anemia thought to be related to ribavirin in any of the patients. Moreover, 6 of the 10 patients enrolled had unconfirmed objective partial responses to combined therapy with a 2-year progression-free survival rate of 75% (15). Although this study is limited in regard to therapeutic efficacy, the observed response rate with this new regimen was similar to those regimens using full-dose cytotoxic chemotherapy. This is a promising initial step for treatment strategies that combine lower-dose chemotherapies with rationally targeted agents, and it has spurred further studies to understand mechanisms of resistance to ribavirin (NCT02308241).

Metastatic breast cancer

The oncogene eIF4E is overexpressed in at least 50% of breast cancers and is associated with increased tumor formation, metastatic disease, and tumor invasion (16–21). Previous studies examining eIF4E knockdown models of breast cancer have shown reduction of cell migration and invasion, along with suppression of the proliferative and clonogenic potential *in vitro* (18). Follow-up studies inhibiting eIF4E phosphorylation also

Table 2. Past, present, and future clinical trials using ribavirin in cancer

Clinical trials	ID number	Status as of December 2018	Condition	Treatment	Phase	References
Low-dose Peg-interferon plus ribavirin (IFN/RBV) for prevention of hepatocellular carcinoma (HCC) recurrence in patients who had surgery to remove primary HCC	NCT00375661	Completed	Hepatocellular carcinoma	Interferon-alfa-2b, ribavirin	4	NA
A study of ribavirin to treat M4 and M5 acute myelocytic leukemia	NCT00559091	Completed	Acute myeloid leukemia	Ribavirin	2	(6)
Effect of biological therapy on biomarkers in patients with untreated hepatitis C, metastatic melanoma, or Crohn disease	NCT00897312	Terminated	Melanoma	Infliximab, pegylated-interferon alfa, ticilimumab, ribavirin	NA	NA
Use of ribavirin and low-dose ara-C to treat acute myeloid leukemia	NCT01056523	Completed (has results)	Acute myeloid leukemia	Ribavirin, cytarabine arabinoside	1–2	(6, 7)
Treatment with ribavirin for patients with metastatic breast cancer	NCT01056757	Terminated	Breast cancer	Ribavirin	1–2	NA
Peg-interferon plus ribavirin for hepatitis C patients concomitant with hepatocellular carcinoma	NCT00834860	Unknown	Hepatocellular carcinoma	Peg-interferon alpha-2a, ribavirin	4	(26)
Phase I/II study of ribavirin given as monotherapy in solid tumor cancer patients	NCT01309490	Unknown	Malignant solid tumor	Ribavirin	1–2	NA
Ribavirin and Hedgehog inhibitor with or without decitabine in AML	NCT02073838	Recruiting	Acute myeloid leukemia	Ribavirin, Hedegehog inhibitor, decitabine	2	NA
Study of decitabine in combination with sequential rapamycin or ribavirin in high-risk AML patients (AML)	NCT02109744	Recruiting	Acute myeloid leukemia	Decitabine, rapamycin, ribavirin	1–2	NA
Oral hepatitis C treatment for indolent lymphoma (OPTImaL) study (Optimal)	NCT02717949	Terminated (results submitted)	Lymphoma	Sofosbuvir, ledipasvir, ribavirin	4	NA
Impact of interferon free regimens in patients with chronic HCV and successfully treated HCC (FRI-STC)	NCT02771405	Recruiting	Hepatocellular carcinoma	Sofosbuvir, ribavirin, simeprevir, daclatasvir, ledipasvir	3	(29)
Sofosbuvir + ledipasvir ± ribavirin and sofosbuvir + ribavirin for pts with indolent B-cell lymphoma associated with HCV infection	NCT02836925	Recruiting	B-Cell lymphoma	Sofosbuvir, ledipasvir, ribavirin	2	NA
Pharmacodynamic effects of ribavirin in patients with tonsil and/or base of tongue squamous cell carcinoma	NCT01268579	Active, not recruiting	Head and neck cancer	Ribavirin	NA	NA
Induction chemotherapy with afatinib, ribavirin, and weekly carboplatin/paclitaxel for stage IVA/IVB HPV-associated oropharynx squamous cell cancer (OPSCC)	NCT01721525	Completed	Head and neck cancer	Afatinib, ribavirin, carboplatin/paclitaxel	1	(15)
Peg-interferon plus ribavirin for hepatitis C patients concomitant with malignancy other than hepatocellular carcinoma	NCT00630084	Completed	Neoplasms	Peg-interferon alpha-2a, ribavirin	4	NA
A pilot investigator-initiated study of ribavirin in indolent follicular lymphoma and mantle cell lymphoma	NCT03585725	Recruiting	Follicular/mantle cell lymphoma	Ribavirin	1	NA
A clinical study to evaluate the efficacy and safety of docetaxel with ribavirin in patients with progressive castration-resistant prostate cancer who have previously received docetaxel alone	UMIN 000012521	Completed	Castration-resistant prostate cancer	Ribavirin, docetaxel	1–2	(43)
A phase 1/2a trial of docetaxel plus ribavirin for reprogramming efficacy in patients with progressive metastatic castration-resistant prostate cancer who previously received docetaxel alone: DRREEM trial	UMIN 000021107	Completed	Castration-resistant prostate cancer	Ribavirin, docetaxel	1–2	(46)
Ribavirin for patients with recurrent/metastatic (R/M) human papillomavirus (HPV)-related malignancies	NCT02308241	Active, not recruiting	HPV-Related malignancies	Ribavirin	NA	NA

demonstrated reduced metastasis of breast cancer cells *in vivo* (22). In breast cancer, these eIF4E-mediated effects appear to be more pronounced in reducing metastatic potential rather than primary tumor burden. In another preclinical study examining the effect of ribavirin in murine xenograft models, inhibition of primary tumor growth was modest, while metastatic burden was significantly reduced in the lungs of ribavirin-treated (4%) versus control (18%) mice (23). Mechanistically, this same study found that in addition to inhibition of eIF4E, there was significant reduction in the activity of matrix metalloproteinase-3 (MMP-3) and matrix metalloproteinase-9 (MMP-9). These proteins are integral to mesenchymal cancer cell invasion and epithelial-to-mesenchymal transition (EMT) for metastasis, and these findings have led to proposed treatment schemas for using ribavirin in

combination with other agents that target distinct pathways involved in EMT (23, 24). This has resulted in phase I/II clinical trials assessing the efficacy of ribavirin in metastatic breast cancer and other solid tumors, the results of which have yet to be reported (NCT01056757 and NCT01309490).

Hepatocellular carcinoma

The antiviral effects of ribavirin combined with interferon (IFN) therapy have historically shown a robust clinical effect in hepatitis C patients. This mechanism is thought to involve ribavirin's lethal mutagenic properties specific to viral RNA, an effect that may help explain the agent's broad antiviral spectrum (25). As such, the risk of hepatocellular carcinoma (HCC) development from hepatitis C has been shown to be reduced following viral elimination from ribavirin plus interferon treatment. Several clinical trials continue to use this schema as a standard measure for preventing the development of HCC from chronic hepatitis C (26–29). A recent phase I/II clinical trial demonstrated comparable effects between pegylated-interferon plus ribavirin and direct-antiviral therapy on HCC development after HCV eradication (27).

Given these distinct effects, ribavirin has been used as prophylaxis against HCC through its antiviral effects, while its anticancer effects are also being explored for the direct treatment of HCC in preclinical studies. In a similar manner to its effect on M4/M5 AML, ribavirin inhibits the *in vitro* growth and survival of HCC cells compared with normal hepatocytes with corresponding relatively greater inhibition of eIF4E activation in cancer cells (30). Furthermore, in preclinical models, combination therapy with ribavirin plus doxorubicin, a common chemotherapeutic for HCC, demonstrated greater inhibitory effects *in vitro* as well as *in vivo* on tumor growth (∼80% reduction in tumor burden) compared with either agent alone (∼35%). Interestingly, doxorubicin monotherapy actually induced eIF4E activation; the synergistic effects of ribavirin combined with doxorubicin may be explained by ribavirin's inhibition of the increased eIF4E activation by doxorubicin, thereby sensitizing cancer cells to chemotherapy and potentially overcoming mechanisms of resistance (30).

In addition to ribavirin's effects on eIF4E, studies have also examined ribavirin's anticancer effects on HCC through its inhibition of IMPDH. The synergistic binding of tiazofurin and ribavirin at 2 different sites on IMPDH were examined in hepatoma cells. Clonogenic assays using combination therapy demonstrated significantly greater killing of HCC colonies, compared with ribavirin or tiazofurin alone (31). The effects of ribavirin on IMPDH as an anticancer pathway will be further discussed below.

Malignant tumors: glioblastoma, atypical teratoid/rhabdoid tumor, and others

Ribavirin penetrates the CSF of patients with subacute sclerosing panencephalitis (SSPE) and human immunodeficiency virus (HIV), achieving 74% (ranging 50%–89%) of the concentration in the serum, resulting in clinically achievable and relevant concentrations in the central nervous system (CNS; refs. 32, 33). Following the discovery that gliomas have relatively increased levels of activated eIF4E compared with normal tissue, several studies have examined the effects of ribavirin in preclinical glioblastoma (GBM) models. *In vitro* studies demonstrated that ribavirin inhibits tumor cell growth and migration, and also has direct cytotoxic effects on glioma cells (34–37). Molecular studies suggested that ribavirin also has modulating effects on the eIF4E, EZH2, and ERK pathways (34). Furthermore, in murine xenograft models implanted with GBM cells, ribavirin treatment in combination with temozolomide and/or radiotherapy yielded a significant survival benefit (34). Interestingly, combination therapy with ribavirin, dapsone, and fenofibrate has been proposed as an immunomodulating strategy that can deprive GBM of certain growth factors, including G(M)-CSF; this regimen is hypothesized to help shift the tumor microenvironment of GBM from a granulocytic to lymphocytic population, which in turn may enhance cancer immunotherapy efforts (38). This proof-of-principle for the use of ribavirin in GBM treatment opens the possibility for additional combination therapies that may enhance current regimens.

In regard to other CNS pathologies, a recent study has demonstrated the efficacy of ribavirin in preclinical models of an aggressive pediatric brain tumor, atypical teratoid/rhabdoid tumor (AT/RT; ref. 39). *In vitro* work demonstrated that ribavirin inhibited proliferation, increased apoptosis, and decreased migratory and invasive properties, potentially via modulation of the EZH2 and eIF4E pathways. Furthermore, as monotherapy, ribavirin treatment increased median survival by 49% in an intracranial xenograft model. Taken together, these findings position ribavirin as a promising agent for further investigation in AT/RT (39).

Finally, several preclinical studies have documented the antineoplastic effects of ribavirin in combination with standard chemotherapies against other tumors, including retinoblastoma, renal cell carcinoma (RCC), and prostate cancer (40–43). In RCC, ribavirin potentiated the effect of IFN-α immunotherapy (41). It has also been postulated that ribavirin could further modulate the tumor environment by shifting the immune response from a Th2 to a Th1 phenotype (44, 45). In prostate cancer, recent phase I trials have demonstrated that ribavirin in combination with docetaxel was well tolerated, with promising response rates in patients diagnosed with docetaxel-resistant castration-resistant prostate cancer (UMIN000012521 and UMIN000021107; refs. 43, 46). Furthermore, preclinical studies have demonstrated that inhibition of eIF4E, genetically or with ribavirin, resulted in enhanced radiosensitivity of several tumor cell lines (but not noncancerous cells) suggesting a role for ribavirin as a radiosensitizing agent (47). These studies have laid a framework for further investigations by demonstrating the potential of ribavirin as an enhancer of chemotherapy and radiotherapy.

Ribavirin Antitumor Mechanisms of Action

Although a significant body of evidence has described ribavirin's therapeutic efficacy in a variety of cancers, understanding of its specific mechanism(s) of action continue to grow. Recent studies have better characterized these underlying effects. The most robust preclinical and clinical data suggest that ribavirin's principal target in several cancers is eIF4E, modulated via direct binding and competitive inhibition. Recent studies also demonstrate modulation of several other key pathways, including ERK, EZH2, and IMPDH, in a cancer-specific manner (34, 37, 48). Changes in these pathways following ribavirin treatment may be the result of either indirect effects downstream of eIF4E inhibition or perhaps direct targeting by ribavirin. In the section below, we outline the evidence regarding potential targets and mechanisms of action

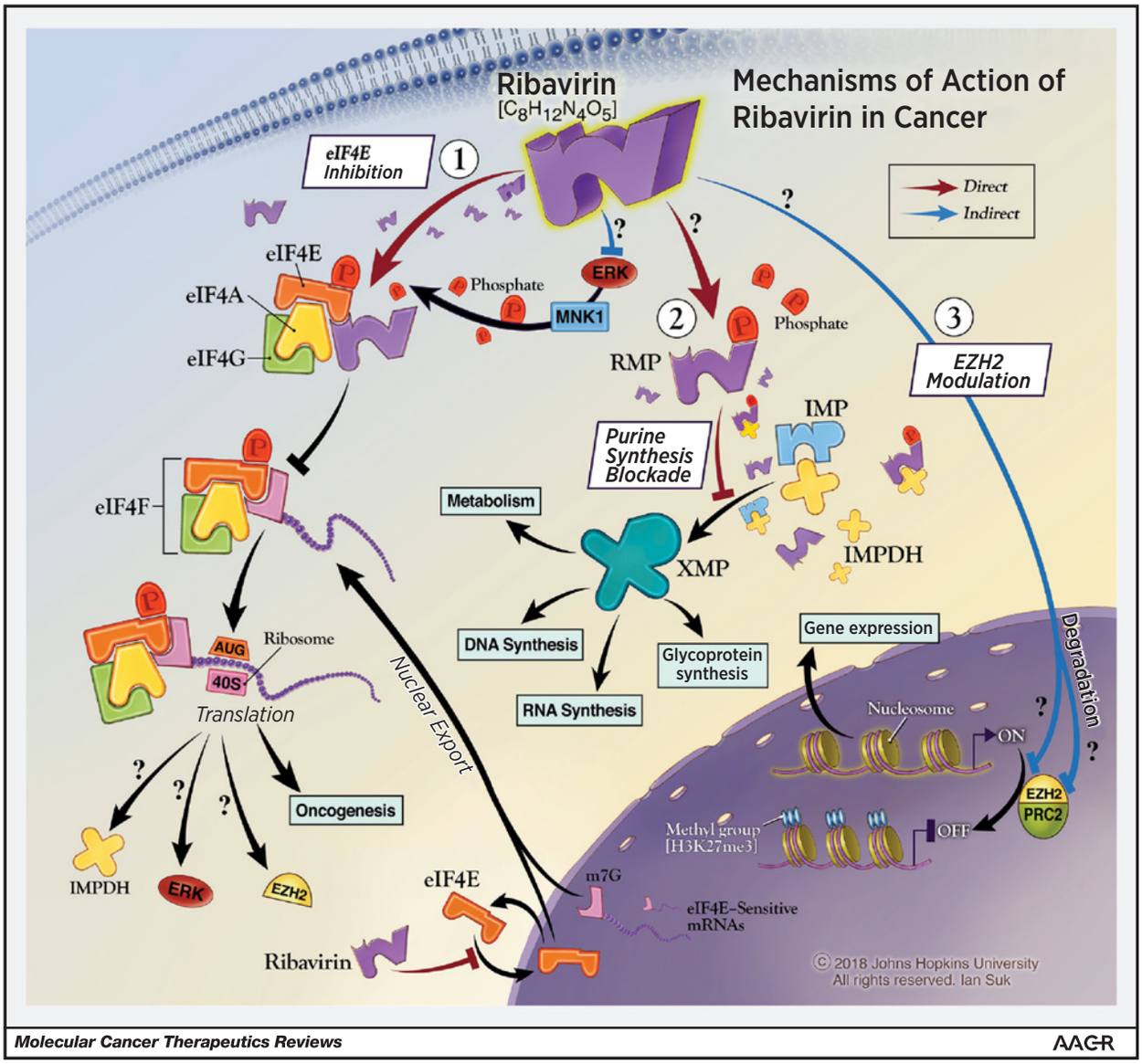


Figure 1.

Potential mechanisms of action for ribavirin's anticancer properties. Schematic representing the various molecular effects of ribavirin treatment in cancer cells. Ribavirin directly inhibits eIF4E and IMPDH, affecting the translation process and cancer cell metabolism, while indirectly affecting cell proliferation, cell-cycle, and gene-expression processes, potentially through the modulation of the MAPK pathway (ERK, MNK1) and the epigenetic regulator EZH2.

of ribavirin, focusing on known direct effects of ribavirin treatment as well as potential indirect effects.

Direct effects of ribavirin

Biochemically, only two known targets have been demonstrated to bind ribavirin directly in purified biochemical assays: eIF4E and IMPDH (13, 49). Furthermore, the abovementioned AML studies demonstrated the ability of ribavirin to modulate eIF4E activity directly in clinical studies (7).

Eukaryotic translation initiation factor 4E (eIF4E). eIF4E is a cap-binding subunit that combines with eIF4G and eIF4A to form the eukaryotic translation initiation factor 4F (eIF4F) complex (Fig. 1). Translation initiation in eukaryotes begins with the binding of the eIF4F complex to the 5-prime methyl-7 guanosine (m^7G) cap of mRNA transcripts, which is mediated by eIF4E. eIF4E modulates translation, and thus expression, of a subset of mRNAs, many of which are implicated in proliferation, survival, and oncogenesis. These include *cyclins D1* and *D3*, c-*MYC*, *MDM2*, *VEGF*, *survivin*, and *Bcl-2* (50). It has been estimated that some 30% of tumors overexpress eIF4E, and its overexpression has been associated with poor prognosis, malignant transformation, and drug resistance (4, 50, 51).

Currently, ribavirin is the only direct inhibitor of eIF4E to be studied in clinical trials. Ribavirin has been shown to bind eIF4E at the functional site used to bind the m^7G cap of mRNA transcripts *in vitro*. Ribavirin competes with target mRNAs for binding, subsequent translocation to the cytoplasm, and ultimately, translation (Fig. 1; refs. 13, 49). Indeed, Volpon and colleagues showed that addition of either ribavirin triphosphate (RTP) or m^7GTP to purified human eIF4E resulted in conformational changes in its peptide backbone formation. These findings were observed working with clinically achievable ribavirin concentrations ranging from 20 to 40 μmol/L (52). Furthermore, tiazofurin, a ribavirin analogue unable to bind eIF4E, does not impair eIF4E activity, suggesting that only analogues that mimic the m^7G cap have efficacy in inhibiting eIF4E (53).

Some of the most convincing evidence regarding ribavirin's potential to target eIF4E in malignancy comes from molecular and translational studies examining ribavirin's effects in AML (6, 7, 13). Many subsets of AML have been shown to have upregulated eIF4E and associated increased *cyclin D1* mRNA transport. Initial preclinical studies demonstrated that ribavirin strongly inhibited colony formation of primary M5 AML specimens, many of which express upregulated eIF4E. Further molecular analysis showed reduction of eIF4E-dependent proteins, and a reduction in AKT phosphorylation. Additionally, eIF4E relocalization from the nucleus (where it is found in 80% of patient cells prior to treatment) to the cytoplasm, as well as reduced eIF4E levels, was associated with clinical response. Interestingly, both lack of clinical response and relapse coincided with either continued nuclear localization or reentry of eIF4E into the nucleus, respectively (6). Conversely, ribavirin failed to suppress colony formation of samples taken from primary M1 AML patients, which have normal levels of eIF4E and nondysregulated *cyclin D1* mRNA transport (13). These studies provided the foundation for a phase I trial to understand the safety of ribavirin and low-dose cytarabine for treating relapsed or refractory AML with elevated eIF4E (7). In this study, authors once again correlated clinical response with molecular targeting of eIF4E. Six patients who had this molecular response (evidenced via changes in *eIF4E* mRNA and eIF4E relocalization) included patients who clinically had a complete remission (CR), partial remission (PR), or blast remission (BR). No targeting of eIF4E was observed in patients with progressive disease. Additionally, 3 patients who achieved a full or partial molecular response but did not achieve a CR, PR, or BR, all displayed a decreased blast count or evidence of hematologic improvement. Furthermore, upon relapse, all 6 patients with complete molecular response showed eIF4E relocalization back to the nucleus and increased eIF4E levels consistent with loss of clinical activity (7).

Further molecular studies identified Importin 8 as a factor that directly binds to eIF4E and imports it into the nucleus (54). This trafficking was only observed for cap-free eIF4E, indicating that selectivity is reduced upon binding of m^7cap analogues, such as ribavirin. Ultimately, considering the importance and association of relocalization of eIF4E in the response to ribavirin treatment in AML, the Importin 8–eIF4E complex could potentially serve as a novel therapeutic target and avenue for increasing the efficacy of eIF4E inhibition, and overcoming acquired resistance (54).

Breast cancer studies have also provided insight into the potential mechanistic roles of ribavirin (18). Petterson and colleagues first assessed the efficacy of ribavirin against breast cancers overexpressing eIF4E and showed inhibition of proliferation and clonogenic potential across several breast cancer cell lines. This growth inhibition was suppressed via the siRNA knockdown of eIF4E, indicating eIF4E dependence of ribavirin inhibition. Cytoplasmic levels of eIF4E-specific mRNA targets were found to be decreased, suggesting inhibition of eIF4E-mediated mRNA transport, similar to previously discussed leukemia studies. Total mRNA levels were not affected; however, protein levels of targets NBS1 and cyclin D1 were decreased. Another mRNA target of eIF4E, *VEGF*, was found to have no difference in its cytoplasmic to nuclear mRNA ratio. However, a decrease was evident at the protein level. Overall, these findings suggest that ribavirin plays a role in modulating eIF4E in both the cytoplasm and nucleus. Furthermore, both phosphorylated AKT and phosphorylated 4EBP1, a target of mTOR downstream of AKT, were decreased, indicating suppression of the AKT pathway (18).

Additional recent studies have supported ribavirin's modulation of eIF4E in multiple cancers. Studies involving oral tongue squamous cell carcinoma, brain tumors, pancreatic cancer, Ph^+ CML, RCC, and retinoblastoma demonstrated that treatment with ribavirin, alone or in combination with paclitaxel or radiation, resulted in decreased proliferation *in vitro* and *in vivo* (40, 42, 55, 56). At the molecular level, ribavirin treatment resulted in suppression of phosphorylation of eIF4E, mTOR, 4EBP1, and AKT, and in decreased protein expression of eIF4E mRNA targets, including cyclin D1, c-MYC, and VEGF.

Given that many patients demonstrating an initial clinical response to ribavirin ultimately relapsed, recent work has focused on exploring mechanisms of resistance to ribavirin (9). At least two forms of drug resistance may exist: one characterized by decreased levels of adenosine kinase, which is important for retention of ribavirin inside the cell, and another characterized by increased expression of the sonic hedgehog factor (SHH), glioma-associated protein 1 (GLI1), and drug-metabolizing UGT1A enzymes, and glucuronidation of both Ara-C and ribavirin, ultimately modulating drug activity and inhibiting ribavirin's ability to bind eIF4E. Indeed, this increased expression correlated with response to and even relapse following therapy (9). Genetic and pharmacologic inhibition of GLI1 and SHH, respectively, restores sensitivity to said therapies and are the rationale for a phase II trial examining ribavirin treatment in combination with SHH inhibition in AML (NCT02073838).

Inosine-5′-monophosphate dehydrogenase (IMPDH). IMPDH is a biosynthetic enzyme involved in the conversion of inosine monosphosphate (IMP) to xanthosine monophosphate (XMP; Fig. 1), the rate-limiting step in *de novo* synthesis of guanine nucleotides (57). It is a key determinant of cellular guanine levels and is subsequently critical for DNA and RNA synthesis, G-protein–dependent intracellular signaling, and protein transport. Two isoforms of IMPDH exist: one expressed in normal cells (IMPDH1) and another that is linked to malignant transformation and elevated in proliferating cells and tumors (IMPDH2; ref. 58, 59). IMPDH has therefore been considered as a potential chemotherapeutic target (60).

Ribavirin is known to be a competitive inhibitor of IMPDH that binds directly to IMPDH (Fig. 1). Its 5-monophosphate

metabolite (RMP) blocks the IMP-XMP attachment site, leading to depletion of guanylate pools (31, 61). RMP binds to purified IMPDH from rat hepatocytes with a reported K_i of 0.8 μmol/L (61). However, this does not always correlate with cytotoxicity and may be dependent on cell-specific abilities to produce RMP (vs. RTP which is not active against IMPDH; refs. 61–63). Multiple reports discuss the use of ribavirin and other IMPDH inhibitors as potential chemotherapy agents. In the breast cancer cell line MCF-7, ribavirin treatment caused growth inhibition comparable with siRNA against IMPDH, while combining ribavirin with siRNA did not lead to a difference in cell viability compared with either ribavirin or siRNA alone (48). Ribavirin treatment of leukemic cells resulted in a significant decrease in *IMPDH2* at the mRNA level and upregulated other pathways involved in purine biosynthesis (64). Additionally, leukemic cells undergo necrosis after treatment with mycophenolic acid, a compound structurally similar to ribavirin and a known inhibitor of IMPDH. It has been speculated that ribavirin could have similar effects via its inhibition of IMPDH (65).

Ribavirin inhibition of IMPDH has also been shown to play a role in the induction of autophagy. Ribavirin treatment of glioma cells resulted in apoptosis as well as an associated induction of autophagy, which played a role in competing with and inhibiting said induction of apoptosis. Treatment also resulted in decreased activity of mTORC1, a known suppressor of autophagy, and decreased mTORC1-activating Src/AKT signaling. Bypassing IMPDH with the addition of guanine inhibited autophagy induction. Conversely, substituting ribavirin with the IMPDH inhibitor tiazofurin replicated the results obtained with ribavirin. Meanwhile, suppression of autophagy resulted in the sensitization of glioma cells to ribavirin-induced apoptosis. These findings uniquely demonstrate autophagy induction as a response to ribavirin treatment. Additionally, these results suggest that inhibition of autophagy may sensitize glioma cells to IMPDH inhibition (37).

Indirect effects of ribavirin

In addition to its direct effects on the eIF4E and IMPDH pathways, ribavirin treatment also appears to modulate other pathways central to carcinogenesis, including the EZH2 and MAPK/ERK pathways (Fig. 1). These effects may occur via direct binding and modulation or perhaps via upstream modulation of eIF4E. Indeed, eIF4E is responsible for the translation of over 3,000 mRNA transcripts (10, 66, 67). These include factors important for cell survival and growth such as *AKT* and epigenetic regulators such as *HDAC*. Furthermore, recent work has demonstrated that the DEAD box RNA helicase DDX6 functions to maintain self-renewal potential by associating and binding *EZH2* mRNA transcripts and recruiting them to eIF4E for translation (68). Thus, these various effects observed after ribavirin treatment may be the result of direct or indirect effects. Available evidence is discussed below.

The histone-lysine N-methyltransferase, enhancer of zeste homolog 2 (EZH2). EZH2, a histone methyltransferase, is the catalytic subunit of the polycomb repressive complex 2 and is responsible for transcriptional silencing via methylation at lysine 27 (K27) of histone H3 (Fig. 1; ref. 69). Recent literature has suggested that EZH2 could function as a master regulator of transcription, and strong evidence has demonstrated links between EZH2 and cancer. For example, EZH2 is essential for cancer cell line proliferation and cancer stem cell maintenance in breast cancer and GBM (70, 71). Many reports have shown gain-of-function, oncogenic mutations as well as overexpression of EZH2 in various cancers, while acquired resistance to standard chemotherapies has also been associated with EZH2 overexpression (72).

Recent evidence suggests a possible link between ribavirin and EZH2 as an important mediator of antitumor effects (Fig. 1), though these appear to be cell-type specific. In one study, the introduction of ribavirin led to a profound decrease in *EZH2* mRNA in breast cancer cells and a modest decrease in prostate cancer cells (48). At the protein level, ribavirin treatment resulted in a decrease in EZH2 protein expression in both cell lines. siRNA against EZH2 caused a similar inhibitory effect as ribavirin, and the concurrent use of siRNA and ribavirin did not result in greater inhibition versus either treatment alone. Disruption of the EZH2 pathway was further validated via decreased histone methyltransferase activity and decreased trimethylation of its target, H3K27. Furthermore, ribavirin has recently been reported to show promise in neuro-oncology in tumors known to overexpress EZH2. In a study demonstrating ribavirin's effects against CNS tumors, ribavirin reduced protein expression of EZH2, among other related proteins, in several glioma and atypical teratoid/rhabdoid tumor cell lines (34, 39). These mechanistic data are supported by systematic computational searches and comparisons *in silico*, demonstrating high structural similarity between ribavirin and 3-deazaneplanocin-A (DZNep), a compound known to target EZH2 for degradation and cause apoptosis in breast cancer (48). These findings support ribavirin's role in modulating EZH2 and should be further investigated as the epigenetic and translational implications are numerous.

The MAPK pathway, ERK, MNK, PDGFRA. MAPK pathways are well-studied signaling cascades involved in development, proliferation, and survival. One such pathway implicated in cancer pathogenesis involves PDGFR, Ras, Raf, MEK1/2, ERK, MNK1/2, and eIF4E. In addition to its direct inhibition of eIF4E, ribavirin has also been shown to modulate other members of this signaling cascade (Fig. 1). One such study sought to assess changes in gene expression in response to ribavirin treatment via microarray. The authors showed that ribavirin sensitivity of cell lines correlated strongly with positive differential gene expression of *PDGFRA*, the cell-surface receptor for platelet-derived growth factor. PDGFRA is important for physiologic signaling and also implicated in the pathogenesis of various cancers, thus representing a potential therapeutic target (35).

In another study utilizing the melanoma cell line IGR39, ribavirin reduced the association between Ras and Raf proteins. The authors demonstrated these findings using immunoprecipitation and immunoblots and showed that this reduced association occurred via a ribavirin-induced decrease in Ras affinity for Raf. The study also showed that ribavirin treatment resulted in decreased expression of phosphorylated ERK but not total ERK (73). It is possible that this effect on ERK activity occurred via upstream targeting of Raf or perhaps also via direct targeting of ERK, or even via the modulation of eIF4E.

As mentioned above, ribavirin is an effective therapeutic in leukemia. In a study investigating therapy for ALL and CML with ribavirin and imatinib, ribavirin treatment (at concentrations ranging from 30 to 60 μmol/L) decreased the phosphorylated forms of eIF4E, MEK, ERK, and MNK1 in the ALL cell line SUP-B15. Similar effects were demonstrated in patient-derived

ALL primary blasts, albeit at a significantly higher dose of ribavirin (500 μmol/L), further highlighting the effect of ribavirin on the MAPK pathway (55).

Conclusions

The FDA-approved antiviral drug ribavirin has demonstrated efficacy in the treatment of numerous human cancers in preclinical studies. Furthermore, ribavirin has been shown to be safe and effective in early clinical trials, including AML and HNSCC (Table 2). Additionally, ribavirin has not only been effective as monotherapy, but has also been shown to enhance the effects of common chemotherapies and radiotherapy. These have important clinical implications in the push to repurpose current drugs, drive down the cost of medical care, optimize existing therapies, and overcome mechanisms of resistance. Though it is not yet clear how and to what extent ribavirin affects its molecular targets, accumulating data continue to shed light on not only possible mechanisms, but also consequently on critical pathways in distinct cancers. Further investigations should continue to explore these mechanisms of action that contribute to ribavirin's anticancer properties. Such work would improve our treatment options for various cancers, and exemplify the benefits of drug repurposing in order to encourage researchers to continue the search for clinically effective and cost-efficient candidates already in safe use today.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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