Riluzole: A neuroprotective drug with potential as a novel anti-cancer agent (Review)

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Abstract. Riluzole, a glutamate release inhibitor, has been in use for the treatment of amyotrophic lateral sclerosis for over two decades since its approval by the Food and Drug Administration. Recently, riluzole has been evaluated in cancer cells and indicated to block cell proliferation and/or induce cell death. Riluzole has been proven effective as an anti-neoplastic drug in cancers of various tissue origins, including the skin, breast, pancreas, colon, liver, bone, brain, lung and nasopharynx. While cancer cells expressing glutamate receptors frequently respond to riluzole treatment, numerous types of cancer cell lacking glutamate receptors unexpectedly responded to riluzole treatment as well. Riluzole was demonstrated to interfere with glutamate secretion, growth signaling pathways, Ca^{2+} homeostasis, glutathione synthesis, reactive oxygen species generation and integrity of DNA, as well as autophagic and apoptotic pathways. Of note, riluzole is highly effective in inducing cell death in cisplatin-resistant lung cancer cells. Furthermore, riluzole pretreatment sensitizes glioma and melanoma to radiation therapy. In addition, in triple-negative breast cancer, colorectal cancer, melanoma and glioblastoma, riluzole has synergistic effects in combination with select drugs. In an effort to highlight the therapeutic potential of riluzole, the current study reviewed the effect and outcome of riluzole treatment on numerous cancer types investigated thus far. The mechanism of action and the various molecular pathways affected by riluzole are discussed.

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Contents

1. Introduction

The drug riluzole [2-amino 6 (trifluoromethoxy)benzothiazole], due to its neuroprotective qualities, was Food and Drug Administration-approved in 1995 for the treatment and management of amyotrophic lateral sclerosis (1). Riluzole was indicated to block voltage-dependent sodium channels in a dose-dependent manner (2,3). While the precise mechanism of action of riluzole is still under investigation, numerous studies have demonstrated that the effect of riluzole is derived from its ability to block glutamate release and enhance glutamate reuptake (4), thus leading to the inhibition of glutamate-dependent signaling. Glutamate signaling is hyperactive in numerous neurological diseases in which riluzole may have beneficial effects (5). Of note, cancers of numerous tissues also rely on glutamate signaling to survive and proliferate (6). Consequently, several investigations have explored the efficacy of riluzole treatment in different cancers types, both *in vitro* and *in vivo* (Table I). Due to its efficacy, low toxicity and tolerability, riluzole became a potential treatment for a number of cancer types (7-9). Numerous studies have uncovered multiple mechanisms by which riluzole targets each specific cell type. An understanding of the molecular targets of riluzole and underlying mechanism in specific cancer types may improve the current application of riluzole in cancer treatment. The present review focused on all available data pertaining to the effects of riluzole in cancers of various tissue origins and its potential as a therapeutic agent, as represented in Fig. 1.

2. Mechanisms of action of riluzole

Consequences of blocking glutamate secretion with riluzole. Glutamate signaling is frequently mentioned in the context of

the central nervous system, where it acts as the major excitatory neurotransmitter; however, glutamate signaling is operational in numerous different cell types (10). For instance, there is a strong link between glutamate signaling, cell survival and differentiation of peripheral tissues, including bone (10-12). The key receptors in the glutamate pathway are classified into two broad categories: Ionotropic glutamate receptors and metabotropic-glutamate receptors (mGluR/GRM) (5,13). While the glutamate receptors largely differ in structure and mechanism of action, these receptors frequently share similar functions and interact with each other in a cooperative or non-cooperative manner (14). Ionotropic glutamate receptors are involved in the regulation of ion intake, including certain essential ions such as Ca^{2+}, which is responsible for maintaining cellular homeostasis. The metabotropic glutamate receptors are G protein-coupled receptors, which mediate signal transduction pathways involved in several cellular processes, such as cell stress response, survival, growth and proliferation (5).

Cystine-glutamate antiporter (xCT) is a glutamate cystine antiporter that regulates the antioxidant system in cells that contributes to growth, metastasis and invasion of cancer cells (15). In this context, cancer cell lines of multiple tissue origin have been indicated to secrete glutamate via the xCT transporter (16,17). Of note, gliomas increase glutamate levels in the extracellular space by mislocalized excitatory amino acid transporter 1 (EAAT1) to the nucleus and decreasing the levels of EAAT2, while simultaneously increasing glutamate secretion via the xCT transporter (18). Excessive secretion of glutamate by glioma causes neurotoxicity to facilitate glioma growth (19). The silencing of the xCT by small interfering RNA in glioma decreased glutamate secretion, neurodegeneration and brain edema (20). Of note, riluzole induced cytotoxicity in GRM3-expressing glioma *in vitro* and reduced tumor size in xenograft mice (7). In melanoma, riluzole dose-dependently decreased cell proliferation *in vitro*. It is noteworthy that downregulation of the xCT was observed in xenograft-bearing animals that were treated with riluzole, suggesting that xCT is a possible molecular target of riluzole (21). Another remarkable study suggested riluzole's growth inhibitory effect on cisplatin-resistant small cell lung cancer cells *in vitro* via the upregulation of both the xCT and CD44 variant, of which CD44 is known to stabilize xCT (22). Although riluzole reduced tumor size in vivo, the effects of riluzole were indicated to be independent of glutamate signaling (22). Thus, blocking xCT and subsequent inhibition of glutamate receptors may be one of the mechanisms by which riluzole prevents cell growth.

Riluzole modulates glutamate-dependent and glutamate-independent intracellular signaling pathways. In numerous cancer cell types, glutamate receptors are overexpressed to enhance cancer cell survival and proliferation (9). Tumorigenesis is linked to several major intracellular signaling pathways, including the PI3K/Akt/mTOR, Ras-MAP-ERK and MAPK/ERK pathways (23-26). A genomic study revealed a high frequency of mutations across pathways, suggesting potential broad cancer-targeting strategies (27). For instance, GRM1 is overexpressed in melanoma cells, whereas the GRM3 receptor is expressed in gliomas (7,28). Stimulation of GRM1 in melanomas and GRM3 in glioma cells by agonists leads to the activation of MAPK signaling, more specifically to the phosphorylation of ERK (7,28). In malignant melanoma, GRM3 is mutated, resulting in hypersensitivity of the MEK-MAPK pathway, and overexpression of GRM5 increased the activation of the MAPK pathway (29,30). In melanoma, treatment with riluzole effectively suppressed MAPK/ERK and PI3K/AKT pathway hyperactivity and related cellular processes, including cell proliferation *in vitro*, as well as in a phase 0 clinical trial (28,31). While the efficacy of riluzole was observed in melanoma, it appears that the presence of mutations in N-Ras, B-Raf or phosphatase and tensin homolog (PTEN) was able to hinder the effect of the drug (31,32). Of note, riluzole in combination with a drug for mTOR, a downstream effector of PI3K/AKT, improved the effect of riluzole on melanoma cells with these mutations (32).

In human brain tumor-like stem cells (BTSCs) derived from glioblastoma, riluzole inhibited cell growth by decreasing GLUT3 transporter expression. Decreased GLUT3 expression resulted in lower uptake of glucose by the BTSCs, which heavily rely on aerobic glycolysis. Consequently, riluzole-induced decrease in GLUT3 was indicated to depend on the decrease in phosphorylated (p-)AKT, leading to a decrease in the induction of hypoxia-inducible factor (HIF)1α expression (33). HIF1α is a transcriptional regulator of the SLC2A3 gene, which encodes for GLUT3 glucose transporter. Therefore, riluzole modulates metabolic activity of cells by altering phosphorylation of AKT. Of note, poor prognosis of glioblastoma is dependent on the overexpression of DNA (cytosine-5-)-methyltransferase 1 (DNMT1), which causes hypermethylation of tumor suppressor genes (34). Riluzole was indicated to decrease DNMT1 gene expression as a consequence of decreased GLUT3 expression and reduced tumor size in mice (33). Therefore, riluzole inhibited cell growth by altering AKT phosphorylation to control glucose metabolism in BTSCs and indirectly altering the expression of tumor suppressor genes to inhibit growth in glioblastoma cells. Furthermore, in osteosarcoma expressing GRM5, riluzole blocked cell proliferation by altering phosphorylation of AKT (at both T308 and S473) and p70 S6 kinase at threonine 389, a hallmark of mTOR activation, suggesting the activation of the PI3K/AKT/mTOR pathway in osteosarcoma growth. In addition, riluzole altered phosphorylation at ERK1/2and JNK1/2 kinases in osteosarcoma (35). Another key pathway linked to oncogenesis is Wnt/β-catenin signaling, which regulates the amount of the transcriptional co-activator β-catenin (36). In malignant melanoma, downregulation of β-catenin has an important role in disease progression and contributes to poor prognosis (37). Of note, in melanoma, riluzole increased the levels of WNT3A protein involved in the stimulation of the Wnt/β-catenin pathway and melanocyte differentiation, subsequently leading to decreased cell proliferation both *in vivo* and *in vitro* (38). Thus, riluzole targets multiple signaling pathways to block cell proliferation in glioma, osteosarcoma and melanoma.

Riluzole regulates intracellular Ca^{2+} in both cancerous and non-cancerous cells. In the endoplasmic reticulum (ER), calcium levels influence protein folding and trafficking, whereas in mitochondria, calcium influences mitochondrial permeability, which contributes to the modulation of

Table I. Effects of riluzole on cancer cells.

Cancer type/cell lines	Mechanism	(Refs.)
Pancreatic cancer		
PANC1, SW1990, BXPC3, ASPC1	Autophagy G2/M cell cycle arrest Apoptosis	(67)
Colorectal cancer		
HCT116, H630, HCT8, CACO2 and HT29	Sensitizes cells to cisplatin reduces cell viability *in vitro* and polyp development *in vivo* G2/M arrest, apoptosis	(77,92)
Hepatocellular carcinoma		
SNU449, Huh-7	G2/M cell cycle arrest Apoptosis	(52)
Melanoma		
SKMEL2, C8161, UACC903 and 1205Lu	G2/M cell cycle arrest MAPK/PI3K/AKT signaling DNA damage Apoptosis	(28,32,38,53)
Prostate cancer		
LNCaP-androgen-dependent C4-2-androgen-independent 22Rv1 VCaP CWR1-R1ca	ER stress Autophagy Apoptosis	(44,71)
Breast cancer		
SUM149 SUM102 SUM229	Apoptosis ER stress	(79,91)
Glioblastoma		
LN229, T98G, short term PDX patient-derived line GBM6	Translational control	(57)
U87MG glioblastoma		
Neuroblastoma		
Neuron-neuroblastoma hybrid (NSC-34D), IM32 neuroblastoma cells	Calcium levels	(41)
Lung cancer		
A549	G2/M arrest, apoptosis	(63)
Glioma		
U87MG glioma cells, U118MG & LN229	Cytotoxicity, tumor suppression, DNA damage	(7,33)
Brain tumor stem-like cell lines used: 11SP and 64SP	Autophagy Sensitizes to radiation	
C6 cells	G2/M arrest, apoptosis	(63)
Human nasopharyngeal carcinoma		
CNE1, CNE2 and HNE1	ATM/P53 G2/M arrest Sensitizes to radiation	(64)
Osteosarcoma	Inhibits cell proliferation	(35)
LM7 and OS482	Apoptosis	
LM7	cAbl kinase activation YAP phosphorylation at Y357, binding to p73 and Bax promoter activation	(55)

ER, endoplasmic reticulum; YAP, YES-associated protein.

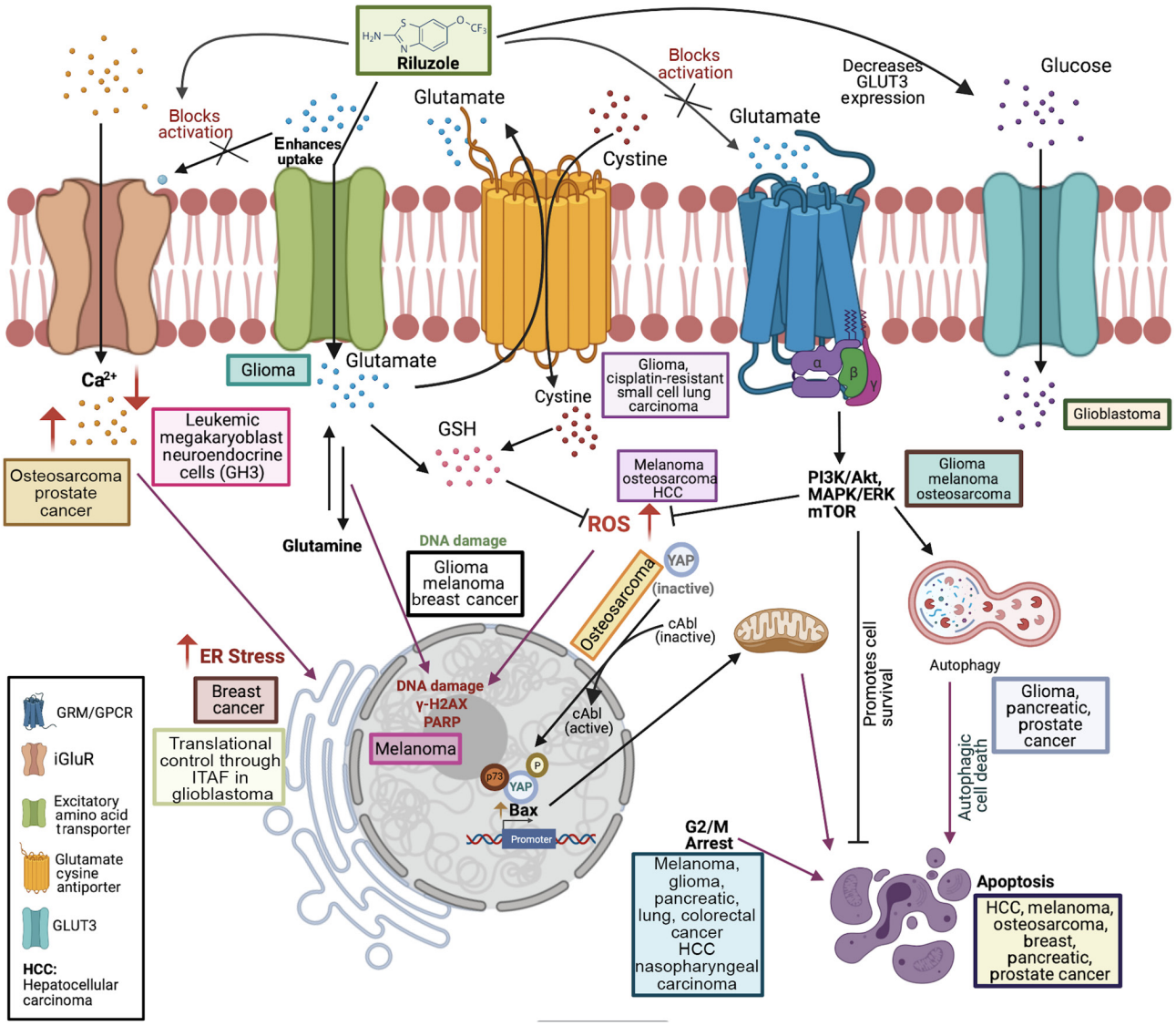

Figure 1. Schematic representation of receptors and pathways targeted by riluzole. Riluzole was indicated to increase Ca^{2+} levels in osteosarcoma and prostate cancer, while decreasing Ca^{2+} levels in leukemic megakaryoblast neuroendocrine cells (GH3). Increased Ca^{2+} levels contribute to ER stress, as observed in breast cancer. Riluzole is known to block protein translation in glioblastoma through ITAF, IRES trans-acting factor. Riluzole is thought to inhibit glutamate release by blocking the voltage-dependent sodium channels (not shown) and enhances glutamate uptake through excitatory amino acid transporter, which regulates extracellular glutamate levels. Glioma cells lack a functional glutamate uptake system, leading to excessive extracellular glutamate. Riluzole blocks glutamate cystine antiporter in glioma and cisplatin-resistant small cell lung carcinoma. Inhibition of glutamate cystine antiporter by riluzole reduces cystine import, thereby decreasing GSH synthesis, which in turn leads to increase in ROS, as observed in melanoma, osteosarcoma and HCC. Increases in ROS lead to DNA damage as reported in glioma, melanoma and breast cancer. In melanoma cells, riluzole elevates γ-H2AX levels and increases PARP cleavage. DNA damage caused by riluzole leads to cell cycle arrest in G2/M phase, as observed in melanoma, pancreatic cancer, HCC and nasopharyngeal carcinoma. Increased ROS may contribute to phosphorylation of YAP by cAbl kinase to promote apoptosis in osteosarcoma. Inhibition of glutamate release by riluzole prevents activation of GRM and signaling through these receptors, as reported in glioma, melanoma and osteosarcoma. Blockage of these pathways by riluzole induces autophagic death in glioma, pancreatic cancer and prostate cancer. Riluzole induces apoptosis in breast cancer, melanoma, HCC, prostate cancer, pancreatic cancer and osteosarcoma. Riluzole was also indicated to decrease glucose transporter GLUT3 levels, thereby decreasing glucose import in glioblastoma. Thus, riluzole targets numerous types of receptors/transporters and associated signaling pathways to cause cell death in various cancer types. The figure was rendered using Biorender.com. ROS, reactive oxygen species; HCC, hepatocellular carcinoma; ER, endoplasmic reticulum; GSH, glutathione; GRM, metabotropic glutamate receptors; GPCR, G protein-coupled receptor; YAP, YES-associated protein; PARP, poly (adenosine diphosphate ribose) polymerase; iGluR, ionotropic glutamate receptors; ITAF, IRES trans-acting factor.

intracellular reactive oxygen species (ROS) levels and may have a direct effect on mitochondrial-mediated apoptosis inside the cell (39). Although riluzole is known to inhibit glutamate release and hence block glutamate signaling, certain studies reported its potential for targeting Ca^{2+} signaling in cells. In one of these studies, riluzole inhibited spontaneous Ca^{2+} signaling in the immortalized growth hormone-secreting pituitary cell line GH3 (40). In another study using the neuroblastoma-spinal motor neuron fusion cell line NSC-34D (non-cancerous), riluzole counteracted the upregulation of Ca^{2+} increase and cell death induced by thapsigargin, a known inhibitor of sarcoplasmic calcium ATPase (41). While the exact mechanism for Ca^{2+} inhibition by riluzole still remains to be determined in cancer cells, riluzole was reported to block glutamate release and glutamate regulated Ca^{2+} entry in leukemic megakaryoblasts and promoted differentiation by

blocking cell proliferation (42). Contrary to this effect, riluzole increased cytosolic Ca^{2+} increase in prostate cancer cells that increased ER stress, and increased Ca^{2+} levels in MG63 osteosarcoma cells through an unidentified pathway (43,44). These results suggested that intracellular Ca^{2+} regulation is one of the cellular processes in cancer that may be affected by riluzole.

Riluzole increases oxidative stress. Typically, the cell is thought to be under oxidative stress when the intracellular levels of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and ion species (O_2^-, OH^-), exceed the levels of antioxidants. Tumor cells have comparatively higher metabolic requirements and altered metabolism; consequently, they generate increased ROS, which are employed by the cells for survival, cell motility and metastasis, tumor progression and angiogenesis (45,46). Glutathione (GSH) is a well-known antioxidant that reduces oxidative stress and ROS levels by accepting an electron from a free radical on ROS (47). Of note, GSH synthesis is directly linked to glutamate release, since glutamate serves as one of the precursor molecules of GSH (48). In normal cells, the overload of intracellular glutamate and upregulation of Ca^{2+} leads to increases in ROS and ER stress, resulting in both DNA damage and subsequent apoptosis (49). On the other hand, cancer cells have a number of mechanisms in place to resist apoptosis. The antioxidant production pathways are typically upregulated, which include GSH synthesis and salvage pathways (50,51). A key player in GSH production is xCT, which imports cystine for GSH synthesis and is overexpressed in cancer (15).

By inhibiting glutamate release in cancer cells, riluzole has been indicated to decrease the overall GSH levels (22). The reduction in GSH leads to a marked reduction in antioxidants and increased ROS, which induces cell cycle arrest in G2/M phase and eventual apoptosis (7,22,52,53). In one of the most recent studies on DNA damage repair inhibition in melanoma, riluzole promoted the accumulation of ROS that triggered DNA double-strand breaks (54). However, this effect was observed in both GluR1-positive and -negative melanoma cells, which further added to the complexity of the mechanisms of riluzole causing oxidative stress (54). Of note, in hepatocellular carcinoma (HCC), riluzole treatment was indicated to cause an increase in cellular glutamate content, which led to a decrease in GSH production as a consequence of a decrease in cysteine uptake by the cells. This further resulted in the accumulation of ROS, leading to apoptosis. In addition, administration of riluzole inhibited growth in HCC xenografts, and reduced GSH and increased ROS levels in the tumors (52). In osteosarcoma, riluzole increased ROS production, leading to the activation of cAbl kinase, which participates in events leading to apoptosis (55). Of note, the increased ROS in cisplatin-resistant lung cancer cells were further enhanced by riluzole to induce cell death, making it the only agent so far to successfully kill cisplatin-resistant cells (22). It is noteworthy that in cancer cells where glutamate secretion occurs via xCT, riluzole may increase intracellular levels of glutamate to cause glutamate-induced oxidative stress while simultaneously blocking GSH synthesis due to deficiency of cystine by blocking xCT (21). While the mechanism remains to be fully elucidated, the available studies suggested that in numerous cancer types, glutamate release inhibition by riluzole contributes to ROS production.

Riluzole and protein translation. In glioblastoma, inhibitors of mTOR kinase have been indicated to enhance IRES-dependent protein synthesis of key regulators of the cell cycle, leading to resistance to the mTOR inhibitors. Furthermore, IRES-dependent translation was reported to have a key role in tumor growth and resistance. One of the significant proteins involved in response to the accumulation of misfolded protein is IRES trans-acting factor (ITAF) heterogenous nuclear ribonucleoprotein A1 (hnRNP A1). hnRNA A1 has been indicated to regulate IRES-dependent translation of c-myc and cyclin D1 genes (56). In a recent study using surface plasmon resonance imaging, riluzole was indicated to directly bind to hnRNP A1 through its specific binding site to prevent IRES RNA binding. This was the first report to demonstrate the interaction of riluzole with a protein directly to regulate its activity (57). In a recent study, riluzole was indicated to downregulate ITAF hnRNP A1 activity to decrease translation of c-myc and cyclin D1 genes, and as a result, to assist in offsetting the resistance to mTOR inhibitor in glioblastoma (57). Thus, riluzole may exert its effect through direct interactions with proteins outside of the glutamate metabolic and signaling pathway, such as ITAF hnRNP A1.

Riluzole induces DNA damage. A cell may be able to sustain DNA damage from intracellular factors such as oxidative stress and replication errors to extracellular factors such as ultraviolet radiation and chemical carcinogens (58). There are two prominent classifications of DNA damage: Single-strand breaks (SSBs) and double-strand breaks (DSBs) (59). It is well known that cells have adopted several strategies to repair DNA damage; for instance, SSBs are repaired through a base excision repair pathway, whereas for DSBs, the homologous recombination repair or the non-homologous end-joining pathways are utilized. Damages that arise at a particular nucleotide also have specialized repair mechanisms, such as the nucleotide excision repair and mismatch repair pathways. Cell cycle checkpoints serve as a protective mechanism against DNA damage. Together, these pathways comprise the cellular DNA damage response (DDR) (59,60). Dysregulation of DDR is a common finding in various cancers and is typically associated with mutations of specific proteins in a given pathway. For instance, mutation in p53 allows the damaged cell to pass the cell cycle checkpoint and proliferate despite not satisfying the checkpoint requirements. Cells with DDR defects frequently become desensitized to radiation and chemotherapy (59).

Multiple lines of evidence suggest that riluzole causes damaged cells to accumulate at cell cycle checkpoints, eventually triggering apoptosis. Specifically, riluzole treatment in glioma and melanoma cells produce increased levels of DDR proteins, such as poly(adenosine diphosphate ribose) polymerase (PARP) and H2AX (7,53). These two biomarkers are frequently used to assess drug efficacy, since both are frequently present when DNA is damaged. The DSBs are known to induce the phosphorylation of histone H2AX, while PARP cleavage is associated with activation of SSB repair (59). Furthermore, the accumulation of DNA damage is also associated with a reduction in glutamate release and GSH levels,

suggesting that the production of ROS stimulated by riluzole treatment is the main contributor to the DNA damage in these cancer cells (7,53). A similar increase in phosphorylation of H2AX was also observed in a phase II clinical trial for advanced melanoma (61). These studies strongly suggest that riluzole may cause DNA damage, most likely due to induction of ROS in cancer cells.

Riluzole causes G2/M cell cycle arrest. In numerous studies, riluzole has been indicated to cause G2/M cell cycle arrest in cancer cells. For instance, the HCC cell lines SNU 449 and Huh-7 exhibited cell cycle arrest in G2/M phase upon exposure to riluzole. Riluzole was indicated to elevate the expression of cyclin B1 and depress the expression of p21 and p-cdc2, leading to G2/M cell cycle inhibition in these cells (35). Riluzole also induced G2/M phase arrest in the pancreatic cancer cell lines PANC1 and ASPC1 in a dose-dependent manner, while concomitantly decreasing the levels of the regulatory protein cyclin-dependent kinase 1 (40). In an *in vivo* brain metastasis study using GRM1-expressing human melanoma cells, riluzole caused G2/M phase arrest and DNA damage. In addition, Riluzole increased radiosensitivity, resulting in enhanced DNA damage and reduced metastases in an animal model (62). Riluzole also induced cell cycle arrest in A549 cells (lung cancer), as well as glioma and colorectal adenocarcinoma cells (63). Furthermore, in studies involving human nasopharyngeal carcinoma, Riluzole induced G2/M arrest and apoptosis (64). In summary, these studies provide evidence that riluzole causes cell cycle arrest in G2/M phase in HCC, pancreatic cancer, melanoma and nasopharyngeal carcinoma cells.

Autophagy. Autophagy, also known as 'self-digestion', is a process of elimination of misfolded proteins and damaged organelles. It is characterized by the autophagosome formation around the components and the fusion of autophagosome with the lysosome for degradation (65). Of note, intracellular calcium and ROS levels are also implicated in autophagy regulation. In normal cells, basal regulation of autophagy contributes to homeostasis; however, in cancer, autophagy dysregulation is linked to uncontrolled cell growth and proliferation, making it a desirable target for cancer therapy (65,66). As observed in pancreatic cell lines, riluzole resulted in the upregulation of an autophagy substrate, p62, as well as cell death in a dose-dependent manner (67). In another study using a castrate-resistant prostate cancer cell line expressing GRM1, riluzole upregulated intracellular Ca^{2+} levels and increased the expression of autophagy markers such as Beclin 1, LC3AII (microtubule associated protein1 light chain 3AII) and p62, leading to autophagy-mediated degradation of androgen receptor (44). Furthermore, in glioma, riluzole caused downregulation of PI3K/Akt signaling and GLUT3 expression, which led to autophagic cell death (33). Together, these studies indicated that riluzole interferes with autophagic pathways to induce cell death in various cancers types.

Riluzole induces apoptosis. Typically, upregulated PI3K/Akt and MAPK/ERK pathways contribute to cancer survival via activated Akt, mTOR, ERK, Ras and Braf proteins that downregulate pro-apoptotic proteins while activating anti-apoptotic proteins. Apoptosis is induced via the intrinsic apoptotic pathway or externally by ligand binding to the cell receptor via the extrinsic apoptotic pathway (68). Both pathways include sequential caspase cleavage, with slight differences in caspases involved in the process. The intrinsic apoptotic pathway is generally triggered in cells under oxidative stress or when DNA sustains serious unrepairable damage. The process is regulated by pro-apoptotic proteins such as Bax and Bak, and anti-apoptotic proteins like Bcl-2 and Bcl-xl. When the apoptotic processes are initiated, the apoptosome is formed, which is made up of Apaf-1 and cytochrome *c* (released from mitochondria), as well as pro-caspase-9. The formation of this complex then cleaves and activates caspase 9 to further activate downstream caspases (caspases3/6/7) to induce apoptotic cell death (68). In cancer cells, aberrant downregulation of apoptotic proteins and inhibition of apoptosis is common.

In certain studies on melanoma, HCC, pancreatic cancer, prostate cancer and breast cancer, riluzole treatment was indicated to induce apoptotic cell death by alteration of different cellular processes, ranging from oxidative stress induction, autophagy inhibition, and downregulation of survival intracellular signaling pathways (7,52,67,69). In a number of these studies, apoptosis was assessed by detecting caspase-3 and caspase-9 levels, which are common apoptotic markers. In HCC, cleaved caspase-3 and -9, as well as PARP, were increased with riluzole treatment (52). Similarly, pancreatic cancer cells also exhibited an increase in caspase-3 (67). Furthermore, in melanomas, both GRM1-positive and GRM1-negative, an increased amount of cleaved PARP and caspase-3 was observed following treatment with riluzole and radiation (70). In prostate cancer cells lines, independent of their androgen-dependent status, riluzole decreased cell viability by activation of caspase-3, -8 and -9 (71). In addition, riluzole was also indicated to induce apoptosis following cell cycle arrest via the activation of the ATM/p53 pathway in nasopharyngeal carcinoma (64). A recent study by our group suggested that in osteosarcoma, riluzole activated c-Abl kinase, which is typically activated during DNA damage response. Activated c-Abl kinase was indicated to phosphorylate YES-associated protein (60), a transcription coactivator, to facilitate its interaction with P73, a homolog of P53. Riluzole-mediated YAP and P73 complex was reported to activate Bax promoter to regulate pro-apoptotic activity (55). Previous studies by our group also suggested that iron oxide nanoparticle-delivered riluzole induced apoptosis *in vitro* in osteosarcoma cells and shrunk osteosarcoma tumors in a xenograft mouse model (72,73). Since apoptosis ultimately leads to the elimination of cancer cells, a better understanding of the induction of riluzole-mediated cell death, the effects of riluzole on different cellular processes and how they are related to apoptosis may further improve the use of riluzole in different cancer types.

3. Riluzole in cancer treatment

Mono- and combined therapy. A better understanding of cancer biology, particularly the regulation of key factors and processes fueling cell growth and metastasis, will bring advancements in cancer treatment. Cancer cells are known to harbor multiple mutations across different pathways to promote cell proliferation and metastasis (74). While monotherapy with single drugs

Table II. Combination therapy with riluzole.

Subjects/samples	Cancer type	Therapeutic agents combined with riluzole	Mechanism	Observed effects with riluzole	(Refs.)
Primary HCC from 4 patients	HCC	Sorafenib	Multikinase inhibitor targets angiogenesis (Raf-1, b-Raf) target proliferation (VEGF, PDGFB receptors)	Additive effect on cell growth inhibition	(52)
MDA-MB-231, SUM149, SUM229 *in vitro* and in xenograft	Triple-negative breast cancer	Paclitaxel	Inhibitor of tubulin, inhibit mitotic spindle assembly involved in chromosome segregation and cell division, induced apoptosis	Synergistic cell growth inhibition, induced apoptosis	(79)
HCT116 with knocked down hERG expression	Colorectal cancer	Cisplatin	Binds to DNA and inhibits replication, promotes DNA damage, inhibits mitosis	Synergistic effect in reduced viability of cisplatin-resistant cells due to hERG1 overexpression	(77)
$TREK^{+/+}$/C7/BL6 mice	Not cancerous	Oxaliplatin	Inhibits DNA synthesis, DNA replication and transcription, induces apoptosis. Neurotoxic side effects (elevated glutamate release)	Reduced neurotoxic side effects due to TREK-1 potassium channel	(92)
Melanoma cell lines for *In vitro* C8161 (WT BRAF & NRAS), UACC903 UACC930, HT144 (BRAF & PTEN mt) SKMEL2 (NRAS mt) For *in vivo* C8161 UACC903 All cell lines are GRM1-positive except UACC930	Melanoma	Rapamycin	mTOR inhibitor Combination therapy was more effective than with individual agent	Decreased anchorage-independent growth and tumor growth in xenograft. Combination therapy effective regardless of BRAF mutation and PI	(32)
Melanoma cell line expressing GRM1: UAC903, 1205Lu, C8161 with either B-RAF WT or mt, *in vitro* and xenograft	Melanoma	Sorafenib	Multikinase inhibitor targets angiogenesis (Raf-1, b-Raf) target proliferation (VEGF, PDGFB receptors)	Synergistic effect Reduced PI3K/Akt signaling, reduced cell proliferation on C8161, additive effect on UAC903 and 1205Lu	(78)
Melanoma cell line expressing GRM1: UAC903, 1205Lu, C8161 with either B-RAF WT or mt, *In vitro* and xenograft	Melanoma	PLX4720	Inhibit B-RafV600E	Synergistic effect but less efficacy compares to with sorafenib	(78)
Intracranially injected melanoma C8161-luc^{+}	Melanoma	Radiation	Increased apoptosis	Enhanced the effect of radiation	(62)
GRM3 expressing cell line U87, and T98G cell line, patients' primary samples with detectable GRM3 expression	Glioma	Radiation	ROS, DNA damage, apoptosis	Enhanced ROS accumulation, reduced PI3K/Akt and MAPK/ERK signaling and DNA damage and apoptosis induction	(7)

Table II. Continued.

Subjects/samples	Cancer type	Therapeutic agents combined with riluzole	Mechanism	Observed effects with riluzole	(Refs.)
LN229 and T98G cell lines	Glioblastoma	pp242	mTOR inhibitor	Synergistic effect on proliferation inhibition, enhanced cell cycle arrest and apoptosis induction	(57)
T98G and UG87	Glioblastoma	TMZ	TMZ-induced O^6-methylguanine DNA methyltransferase (MGMT expression)	Synergistic effect in T98G cells but not UG87 Suppressed intracranial tumor growth	(93)
LM7	Osteosarcoma	Iron oxide nanocage	Apoptosis	Iron oxide nanocage-delivered riluzole was most effective on inducing apoptosis both *in vitro* and *in vivo*	(72,73)

HCC, hepatocellular carcinoma; TMZ, temozolomide; ROS, reactive oxygen species; WT, wild-type; mt, mutant type; GRM1, metabotropic glutamate receptor 1; ERG1, ether-a-go-go-related 1 ion channel; PDGFB, platelet-derived growth factor B; NRAS, neuroblastoma RAS; B-RAF, B-raf proto-oncogene; PTEN, phosphatase and tensin-like protein.

is still in use, it is slowly being replaced by newer and more efficient combinatorial treatments. In addition to traditional radiation and chemotherapy, newer advanced methods of cancer treatment are gaining momentum. Immunotherapy, nanoparticles and gene therapy using the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9) gene editing system are among the most promising (75,76). According to studies by our group, when delivered via iron oxide nanocage particles, riluzole caused the highest reduction in osteosarcoma tumor size in nude mouse xenografts (72,73).

In keeping with its promising potency and efficacy, riluzole is being tested in combination with other drugs to enhance its actions. Riluzole is frequently observed to have synergistic effects with other drugs to decrease cell proliferation and viability while also promoting apoptosis. Select drug combinations may amplify these effects in different cancer cell lines. For instance, riluzole in combination with paclitaxel in triple-negative breast cancer, or cisplatin in colorectal cancer, or sorafenib in melanoma, was demonstrated to have synergistic effects (77-79). Combinatorial treatment with riluzole and GRM3 antagonist LY341495 blocked the MAPK signaling pathway in glioma, sensitized glioma to radiation and decreased anchorage-independent colony growth (7). In an *in vivo* study in mice with intracranially injected melanoma cells, combinatorial treatment led to a significant decrease in tumor volume (62). In melanoma, a combination of sorafenib with riluzole was tested in comparison to another potent inhibitor, PLX4720, with riluzole. While both combinations had synergistic effects, the inhibitory effects of sorafenib and riluzole on cell proliferation were the strongest (78). In HCC, riluzole with sorafenib had an additive effect in decreasing cell proliferation (52). Melanomas harboring PTEN and NRAS mutations exhibit resistance to the action of riluzole after prolonged treatment (19). Of note, riluzole in combination with mTOR or AKT inhibitors had promising results *in vitro* and in xenograft studies in melanoma with these mutations, and in glioblastoma (32,57). Table II provides a summary of noteworthy experiments that include riluzole in combination with other drugs. While the majority of these studies are limited and confined to animal experiments at the most, they may provide information on mechanisms of action of riluzole or possibly lead to clinical trials in the future.

Riluzole as a radiosensitizer. Aside from using riluzole in combination with other drugs, it may be used as a radiosensitizer to produce favorable effects against cancer. The use of riluzole and radiation therapy revealed a synergistic effect *in vitro* and *in vivo* in both melanoma and glioma cell lines (7,70). Malignant melanomas, which typically metastasize into the lungs and brain, are particularly dangerous. Despite several scientific advancements, the prognosis for advanced-stage melanoma patients remains bleak. According to the American Cancer Society, the 5-year relative survival rate for metastatic melanoma patients is only 27% and these tumors are frequently resistant to chemotherapy and radiation treatment, which makes them even more dangerous and prone to reoccurrence. In an *in vivo* study in mice injected intracranially with melanoma cells, riluzole in combination with radiation led to a significant decrease in tumor volume and GRM1-expressing melanoma exhibited increased apoptosis (62). In another *in vitro* study on BTSCs, a lower dose of riluzole in combination with radiation therapy unexpectedly resulted in enhanced growth inhibition compared to a higher dose of the drug (33). However, a higher dose of riluzole with radiation therapy proved to be more effective *in vivo* (33). In addition, riluzole sensitized human nasopharyngeal carcinoma

cells to radiation through the ATM/P53 signaling pathway and cytotoxicity was enhanced compared to groups treated with riluzole alone both *in vivo* and *in vitro* (64).

Toxicity and adverse effects of riluzole. Riluzole was observed to have low toxicity in patients with amyotrophic lateral sclerosis (ALS) treated with a daily oral dose of ~50 mg (80). The area under the curve for the serum concentration at 24 h was ~2,000 ng/ml (81,82). Riluzole is well tolerated, with the most common adverse effect observed being headache (83). Other significant side effects were nausea, vertigo, somnolence and asthenia, which were indicated to be dose-related (84,85). Elevation of alanine aminotransferase was also commonly observed in patients (86). Rare and less frequently reported side effects are acute hepatitis, leukopenia and methemoglobinemia (87). Much rarer cases of hypersensitivity pneumonitis and multi-organ autoimmune syndrome were also reported (88). More recently, interstitial pneumonia was observed in 21% of 92 patients enrolled (89). In patients with ALS, riluzole prolonged tracheostomy-free survival by 3-6 months; however, the long-term toxicity of riluzole requires to be determined for cancer patients considering their longer survival expectancy. In a study performed in Europe and North America, riluzole was reported to be well-tolerated for up to 7 years (90). Further studies require to be performed to assess the long-term tolerability and side effects of riluzole.

Limitations of riluzole. In breast cancer cells, riluzole-induced DNA damage is independent of mGluR1 expression or ER status (8,91). Of note, riluzole-induced DNA damage was not observed in the breast cancer cell line MCF-7, which expresses wild-type P53, whereas other cell lines expressing mutant P53 exhibited riluzole-induced DNA damage (8). On the contrary, in melanoma, riluzole induced DNA damage in an mGluR-dependent manner (53). However, it remains to be determined whether cell line-specific differences in riluzole sensitivity, as observed in breast cancer, are dependent on replicative stress induced by other mutations in cell lines besides mutations in P53 (8). In any particular cancer type, it requires to be determined whether the response to riluzole is dependent on the mutational profile of the cell line, particularly those mutations that predispose the cells to oxidative or replicative stress.

4. Conclusion

The current review presents up-to-date information of the mechanisms of action of riluzole. Riluzole has been reported to interfere with diverse cellular processes, such as stress-related response, survival and apoptosis. Various noteworthy studies have demonstrated the cytotoxic effects of riluzole in the following scenarios: i) Cisplatin-resistant lung cancer cells; ii) mTOR inhibitor-resistant glioblastoma; iii) triple-negative breast cancer cells in combination with paclitaxel; iv) sensitizing effect on melanoma, gliomas and nasopharyngeal carcinoma to radiation. Riluzole thus holds great promise for cancers that are challenging to treat effectively. Investigations into the efficacy of riluzole in cancers exhibiting drug resistance may be an effective strategy for circumventing drug resistance in cancer. A deeper insight into the specific mechanisms of riluzole alone and in combination with other drugs in a cell type-dependent manner may improve the efficacy of riluzole and facilitate translation into clinical use.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

All authors (AB, SL, CT, IT, TT, SSM) performed literature searches and wrote and edited the article. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

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Not applicable.

Competing interests

The authors have no competing interests to declare.

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