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Review Article

Deciphering Riluzole: Illuminating Unexplored Avenues in Cancer Treatment through Mechanistic and Clinical Insights

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ABSTRACT

Drug repositioning is a well-established concept for therapeutic agents with well-recognized safety profiles and thoroughly described pharmacokinetic properties. Despite the advancement in oncology research, repositioning is imperative for anti-neoplastic activities to fill the gap of cancer therapy, the most unmet medical need today. Among various clinically accepted therapeutic agents, riluzole is a drug that has been shown to be effective in various cancers. However, it was primarily developed to treat and control amyotrophic lateral sclerosis, a fatal neurodegenerative disease. Riluzole recently exhibited to either limit cell growth or kill the cell after altering or affecting pathways like growth signaling, glutamate secretion, glutathione synthesis, Ca^{2+} homeostasis, generation of reactive oxygen species, nucleic acid integrity, autophagy, apoptosis, etc. Because of this multifaceted action, riluzole has been found to be effective in some malignancies originating from various tissues, including the nasopharynx, breast, colon, skin, bone, pancreas, and liver.The present review examined mechanistic pathways, effects of riluzole treatments on different types of cancers, pre-clinical evaluation, clinical status, and future perspectives of riluzole as an anticancer agent.

Keywords: Riluzole, Glutamate-dependent signaling, Anticancer, Amyotrophic lateral sclerosis, Glutathione synthesis, Ca^{2+} homeostasis.

INTRODUCTION

Cancer is one of the prominent causes of mortality across the globe and is considered a major public health challenge, with over 9 million deaths and about 20 million new diagnoses in 2020. Overall deaths due to cancer have increased significantly in the time course of 20th century. However, after 1990, the mortality rate due to cancer has decreased by about 1.5% per year, resulting in an about 27% decrease in 2016. Over the last 20 years, the decline in death rates for major malignancies like lung, breast, prostate, and colorectum, is primarily due to the advancements in timely diagnosis, detection and treatment choices, such as chemotherapy, radiation, and surgery. [1] Among various anticancer treatment modalities, chemotherapy has emerged as an important treatment option, increasing the overall survival rate (Fig. 1). Present drug treatment modalities are accompanied by a number of severe side effects, as well as recurrences due to the evolution of drug resistance; thus, saving lives remains a difficult task. These were the primary motives for the growing interest among pharmaceutical chemistry researchers in developing novel chemotherapeutic agents with augmented efficacy and lower toxicity, probably with multi-target activity.[1,2] Despite the latest treatment advancements and cutting-edge technologies in oncology research, cancer remains one of the most unmet medical needs today. To address the gap identification of clinically approved drugs with established safety profiles and pharmacokinetic features for additional indications is necessary for certain well-known disorders. Riluzole is one such drug that is well-known drugs for its action in various malignancies with multi-target activity. Fundamentally, it is a 6-trifluoromethoxy-2-amine derivative of benzothiazole that is 6-(Trifluoromethoxy)-2-benzothiazolamine (Fig. 2).

Primarily, riluzole was developed for the management of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease. Essentially, ALS patients may progressively lose the ability to control the movement of muscles, which might be life-threatening if left untreated.[3-5] It has been demonstrated that riluzole blocks voltage-gated sodium channels (VGSCs) in a dose-dependent manner. [6,7] Although the exact mode of action of riluzole is still not clear, multiple research studies have exhibited that the effects of riluzole result from its capacity to suppress glutamate-dependent signaling by blocking glutamate release and enhancing glutamate absorption. [8] Additionally, riluzole may be useful in various neurological disorders because of overactive glutamate signaling. Apart from its action in neurological disorders, riluzole has been shown to possess fascinating anti-cancer activity, however, still it is exclusively used for the treatment of ALS. It should be noted that glutamate signaling

is also essential for the development and growth of malignancies in many tissues.[9] Because of its efficiency in target drug treatment in various malignancies, *in-vitro* and *in-vivo*, riluzole has gained attention as a possible treatment for a variety of malignancies because of its effectiveness, minimal toxicity, and tolerability.[10,11] Interestingly, riluzole acts against various malignancies without glutamate receptors, as well as malignant cells that contain glutamate receptors. Riluzole exhibited its ability to kill lung cancer cells resistant to cisplatin. Additionally, radiation therapy is more effective against glioma and melanoma with riluzole pretreatment. In combination with chemotherapeutic agents, riluzole has synergistic effects in different types of cancers like glioblastoma, colorectal cancer, melanoma, and triple-negative breast cancer. Riluzole targets different cells through different mechanistic pathways. Understanding molecular targets of riluzole and underlying mechanisms in particular cancer types may enhance the information regarding the drug to treat cancer [10-12].

The present review discourses on the repurposing of riluzole in cancer, mechanism of action of riluzole in ALS, pharmacokinetics, adverse effects, various mechanistic pathways of riluzole implicated in cancer, implications of riluzole in various cancers, clinical status with reference to cancer, and future outlook.

Mechanism of Action: Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis is a neurodegenerative disorder affecting motor neurons in the brain and spinal cord, leading to muscle weakness and paralysis. Riluzole is a drug that has been approved for the management of ALS. Though the precise mechanism of action of riluzole is not well-known, it is supposed to act by decreasing glutamate release and inhibiting glutamate-induced toxicity.[3,4] However, glutamate is considered to be implicated as an important neurotransmitter in the function of the nervous system. In ALS, excessive glutamate release can lead to the overstimulation of motor neurons and subsequent cell death. Basically, riluzole works by decreasing the glutamate release followed by blocking the overstimulation of glutamate receptors, which may slow down the progression of disease (ALS). Furthermore, riluzole has been shown to decrease glutamate release by inhibiting voltage-gated sodium channels, consequently, decreasing the influx of Ca^{2+} ions into the pre-synaptic terminal, which in turn leads to reduced glutamate release (Fig. 2).[6-8] Moreover, riluzole has been exhibited to tune the release of other neurotransmitters, like serotonin, and dopamine which may contribute to its therapeutic effects in other neurological disorders.[13-15] It has been found that riluzole can reduce the release of glutamate by inhibiting voltage-gated sodium channels wherein uptake of glutamate was increased by astrocytes, which possibly contributes to its neuroprotective actions in ALS (Fig. 2). Conclusively, riluzole is a drug of choice in the management of ALS. Further research is required to fully explore the mechanism of action of riluzole and to develop more effective treatments for ALS.

Pharmacokinetics

Riluzole exhibits a maximum of 90% oral absorption, with a mean absolute oral bioavailability of around 60%. It exhibited linear pharmacokinetics in the dosage range of 25 to 100 mg, administered twice a day. Absorption and peak blood levels are reduced by around

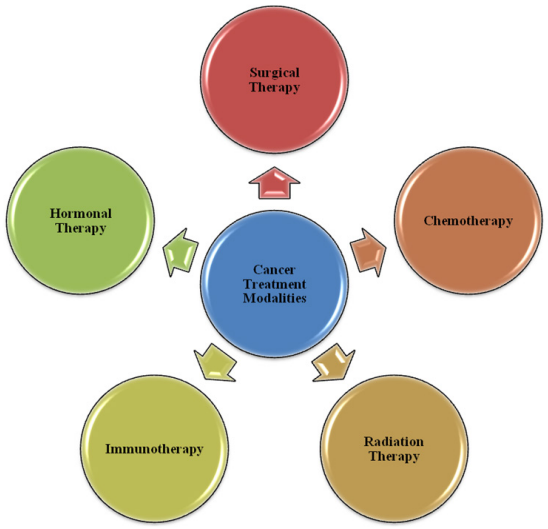

Fig. 1: Diagrammatical representation of various modalities for the treatment of cancer

20, 45%, respectively, if riluzole is administered after a high-fat meal. In a multiple dosages regimen system, riluzole exhibits a mean $t_{1/2}$ (elimination half-life) of 12 hours. Generally, riluzole accumulates in the plasma almost as much as twice and reaches a steady state in less than 5 days, in a multiple-dose treatment. Plasma protein binding of riluzole is about 96%. Riluzole is primarily bound with albumin and lipoproteins in the therapeutic concentration range. Area-under-the-curve (AUC) of the 50 mg marketed tablet was comparable to that of the tablet used in the clinical trials. The C_{max} of the marketed tablet was roughly 30% greater than the tablet used in clinical trials. After a single 50 mg oral dose, the AUC of riluzole was increased approximately 1.7 times in subjects with mild chronic liver insufficiency and approximately 3 times in subjects with moderate chronic liver insufficiency compared to healthy subjects. The pharmacokinetics of riluzole in individuals with severe hepatic impairment have not yet been explored. Furthermore, riluzole undergoes substantial biotransformation, yielding six main and several minor metabolites. Certain metabolites are pharmacologically active evidenced from some *in-vitro* studies. It is mostly biotransformed via cytochrome P450-dependent hydroxylation and glucuronidation, in the liver. Clearance of riluzole varies significantly between individuals, which is most likely due to variations in the activity of CYP 1A2, a primary isozyme implicated in N-hydroxylation. It was found after *in-vitro* investigations that CYP 3A4, CYP 2E1, CYP 2C19, and CYP 2D6, are unlikely to have a substantial role in riluzole metabolism in humans. In human liver microsomes, direct glucuronidation of riluzole using the glucuro-transferase (isoform UGT-HP4), is relatively sluggish. N-hydroxyriluzole is rapidly conjugated at the –NH–OH group, resulting in the synthesis of O- and N- conjugates of glucuronic acid. About 90 and 5% of the radioactivity was recovered in the urine and feces, respectively, after a single 150 mg dose of radiolabeled ^{14}C-riluzole administration to 6 healthy male subjects, during a seven-day period. More than 85% of the metabolites in urine were glucuronides. Only 2% of a riluzole dosage was retrieved as an unaltered compound in the urine [8,16-18].

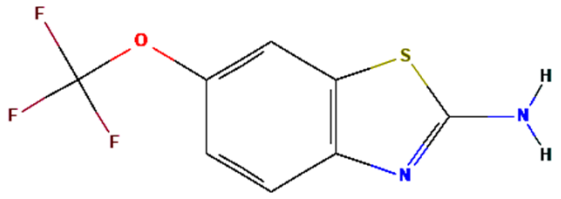

Fig. 2: 2D chemical structure of riluzole [6-(Trifluoromethoxy)-2-benzothiazolamine

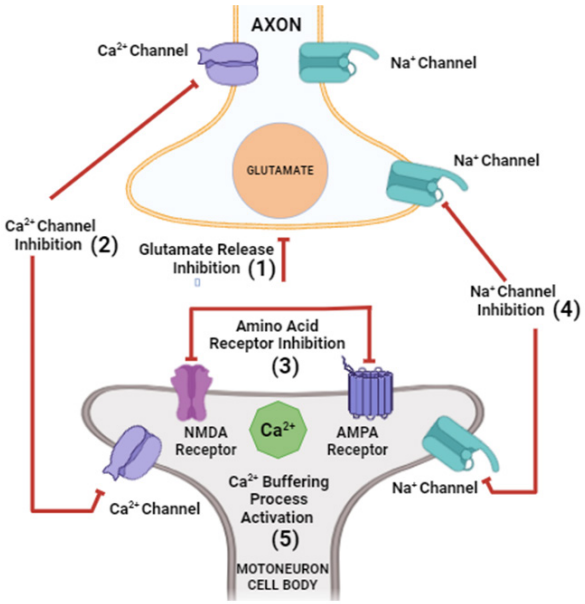

Fig. 3: Mechanism of action of riluzole in amyotrophic lateral sclerosis: (1) glutamate release inhibition; (2) Ca^{2+} channel inhibition; (3) inhibition of excitatory amino acid receptors; (4) voltage-dependent Na^{+} channels blockade; (5) Ca^{2+} buffering processes activation

Tolerance

Patients with ALS frequently tolerate riluzole well. Some frequent adverse reactions were more commonly encountered in riluzole-treated patients than in placebo-treated patients like stomach discomfort, pneumonia, asthenia, dizziness, nausea, impaired lung function, diarrhea, vertigo, vomiting, anorexia, somnolence, and circumoral paresthesia.[19,20] In premarketing clinical studies, approximately 14% of the 982 patients with ALS who received riluzole terminated therapy due to an unpleasant event. The most prevalent adverse events reported by individuals who stopped owing to adverse events were nausea, stomach discomfort, constipation, and ALT increases. In an ALS dosage response trial, the rate of riluzole discontinuation for nausea, stomach discomfort, asthenia, and ALT rise were dose-related.[18, 21-23]

Remarkably, toxicities subsided completely when the riluzole drug was discontinued. The human lymphocyte chromosomal aberration assay exhibited that there was an ambiguous clastogenic response, however, not replicated in a second assay done at corresponding or higher dosages. Hence, riluzole was not found to show any clastogenic activity in the human lymphocyte assay. Chromosomal damage due to N-hydroxyriluzole, the principal active metabolite of riluzole, was evaluated in the mouse lymphoma mutation assay and the *in-vitro* mouse micronucleus assay. From the above tests, it was observed that N-hydroxyriluzole was found to exhibit damaging effects on chromosomes. However, when tested in the HPRT gene mutation assay, N-hydroxyriluzole did not show any mutagenic activity, *in-vitro,* in the cell line. Mutagenic activity of N-hydroxyriluzolewas also found negative in the Ames bacterial gene mutation assay, the *in-vitro* UDS assay in rat hepatocytes, and the *in-vivo.*[18,24].

Mechanistic Pathways of Riluzole to Inhibit Cancer

Suppression of glutamate secretion

Glutamate signaling is predominantly found in the CNS and functions as a primary excitatory neurotransmitter. It has been found that glutamate signaling is active in different types of cells and tissues.[25,26] Fundamentally two types of receptors viz. mGluR and GRM are implicated in the glutamate signaling pathway.[9,27] Despite having substantially different structures and mechanisms of action, glutamate receptors typically have comparable roles and engage in cooperative or antagonistic interactions with one another. [28] Intake of Ca^{2+}, an important ion responsible for preserving cellular homeostasis, is generally regulated by ionotropic glutamate receptors (Fig. 3). Various cellular activities, including survival, growth, stress response, and proliferation, are governed by the signal transduction pathways mediated by the G-protein coupled metabotropic glutamate receptor.[9] A glutamate cystine antiporter called as cystine glutamate antiporter (xCT) controls the antioxidant system in cells [29]. Furthermore, it has been shown that cancer cell lines with a variety of tissue origins release glutamate through the xCT transporter [30, 31]. There is an association between xCT levels and *in-vitro* proliferation of cells and *in-vivo* tumor growth. As the level of xCT expression increases, the dependency of these cells to the greater amounts of glutamate through the xCT mechanism increases the responsiveness of the cells towards the riluzole. Fumagalli *et al.*, (2008) found that riluzole increased the excitatory amino acid transporter 1 (EAAT-1) glutamate absorption in EAAT-1 transfected HEK cells, dose-dependently.[32] Riluzole increased the levels of both xCT and EAAT-1 in the sequencing data obtained from two melanoma cell lines. Furthermore, it has been studied that in HEK cells that were transfected with xCT, a considerable concentration of glutamate is released. These findings demonstrate that EAAT-1 plays an important role in glutamate absorption whereas xCT is important in glutamate release to the extracellular environment, implying that EAAT-1 and xCT act in coordination to maintain the glutamate equilibrium in cells of melanoma. Because a fast rise in cytoplasmic glutamate causes cell death in PC12 cell line as a result of an increase in reactive oxygen species (ROS), riluzole-mediated cytoplasmic glutamate elevation may have the same effect on melanoma cells.[33] As a result, cancer cells rapidly stimulate the expression of xCT due to a fast rise in glutamate-mediated oxidative stress (OS).

In fact, melanoma cells exposed to oxidative stress from serum deficiency upregulated xCT protein expression after 2 to 3 hours. By suppressing xCT, riluzole can work as a stimulant of oxidative stress.[34] Riluzole was found to decrease tumor growth markedly

in xenograft mice and *in-vitro* cytotoxicity in GRM3-expressing gliomas [10]**.** Riluzole inhibits the production of cisplatin-resistant small-cell lung cancer cells, *in-vitro* by upregulating the xCT pathway and CD44 variant.[35] Hence, suppression of glutamate secretion could be a potential mechanistic pathway to inhibit tumor growth.

Change in glutamate-dependent and -independent intracellular signaling

Generally, glutamate receptors are over-expressed in cancers to instigate malignant cell survival and growth.[11] Numerous important intracellular signaling pathways, such as PI3K/Akt/mTOR, and Ras-Raf-MEK-ERK pathways are implicated in tumorigenesis.[36,37] Variations in different pathways discovered by a genetic investigation opined to a possible broad range of malignancy targeting techniques. [38] Among different glutamate receptors, it has been investigated that the glutamate metabolic receptor (GRM1) is over-expressed in melanomas while the GRM3 receptor is over-expressed in gliomas. Thus, agonists mimicking the glutamate activity stimulate glutamate metabotropic receptor 1 in melanomas and glutamate metabotropic receptor 3 in gliomas activate the Ras-Raf-MEK-ERK pathway.[10,39] Moreover, GRM3 is mutated in the cancerous cells, making the Ras-Raf-MEK-ERK pathway hypersensitive (Fig. 3). It has been observed *in-vitro* as well as *in-vivo* that riluzole treatment for melanoma significantly decreased hyperactivity of the mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinases (ERKs) and PI3K/AKT/mTOR pathway, is an intracellular signaling pathway important in regulating the cell cycle, directly related to cellular quiescence, proliferation, cancer, and longevity. [39-42] Riluzole was observed to be promising in treating the tumor. However, it has been shown that the existence of mutation in phosphatase, due to mutations of the oncogenes BRAF and NRAS, present in melanoma, leads to constitutive signaling of the mitogen-activated protein kinase (MAPK) pathway and thereby enhances tumor growth and promotes disease progression [39, 40]. Moreover, riluzole suppressed the cell proliferation in the brain of the human. Brain tumor stem cells (BTSC) are activated as a result of glioblastoma via lowering GLUT3 transporter expression. The BTSC activity mainly depends on aerobic glycolysis, took up less glucose as a result of reduced GLUT3 expression, consequently a riluzole-induced reduction in a GLUT3, which is dependent upon the decline of phosphorylated (p)AKT, in turn, led to reduction of hypoxia-inducible factor (HIF)1 gene activity.[43] Additionally, GLUT3 is also identified as solute carrier family 2, facilitated glucose transporter member 3 (SLC2A3), a protein in humans encoded by the *SLC2A3* gene which is regulated by HIF1. Moreover, the overexpression of DNA (cytosine 5) methyltransferase 1 (DNMT1), causes hypermethylation of tumor suppressor genes, is significant because it affects the prognosis of glioblastoma. Consequently, riluzole was found to decrease the expression of the DNA methyl transferase 1 (DNMT1) gene.[43,44] Since PI3K/AKT/mTOR pathway is implicated in the development of osteosarcoma. Riluzole inhibits the cell proliferation in osteosarcomas, expressing GRM5. Alternatively, riluzole also changes the phosphorylation of ERK1/2, JNK1/2 kinases, and Wnt/β-catenin signaling, controlling the concentration of the transcriptional co-activator catenin, which is another important mechanistic pathway implicated in oncogenesis. Since the down-regulation of beta-catenin plays a significant role in the development of malignant melanoma. Hence, riluzole enhances the levels of WNT3A protein in melanoma, which stimulates the Wnt/β-catenin pathway and promotes melanocyte differentiation. Riluzole therefore inhibits cell growth in glioma, osteosarcoma, and melanoma by targeting several signaling pathways [45, 46, 47].

Control of intracellular Ca^{2+} signaling

Protein folding and trafficking are influenced by the amount of calcium in (ER), whereas mitochondrial permeability helps to control levels of (ROS) and might have an instant impact on mitochondrial-mediated apoptosis influenced by calcium levels [48]. Riluzole is primarily known to inhibit glutamate signaling by inhibiting glutamate release, but some investigations suggested that it may also target Ca^{2+} signaling in the cells (Fig. 3). Riluzole reduced impulsive Ca^{2+} signaling in the immortalized growth hormone-secreting pituitary cell line (GH3). In another study, riluzole was found to be effective in lowering the elevation of Ca^{2+} rise and cell death brought on by thapsigargin, an inhibitor of sarcoplasmic calcium ATPase. In leukemic megakaryoblasts, riluzole was reported to decrease glutamate release, and glutamate-regulated Ca^{2+} entry and promote differentiation by inhibiting cell proliferation. However, the specific mechanism through which riluzole inhibits Ca^{2+} in cancer cells is not yet clear.[49,50]

Riluzole has the opposite effect, increasing ER stress and Ca^{2+} levels in MG63 osteosarcoma cells by an unidentified mechanism after increasing the cytosolic Ca^{2+} levels in prostate cancer cells. [51,52] Thus, these findings suggest that one of the cellular functions in cancer that riluzole can affect is intracellular Ca^{2+} modulation.

Increase in oxidative stress

The cells frequently experience oxidative stress when the amount of ROS like peroxides or ionic species exceeds the amount of antioxidants inside it. Because of disproportionately greater metabolic needs and altered metabolism, tumor cells produce more ROS, which they utilize for survivability, cell movement, growth, and consequently spread of tumors [53, 54]**.** By absorbing an electron from a radical species on ROS, an antioxidant called glutathione (GSH) reduces levels of oxidative stress and ROS.[55] It is imperative to emphasize that glutamate release and GSH synthesis are intimately associated phenomena because glutamate is the organic precursor of GSH.[56] Rise in reactive oxygen species and ER stress induce genetic material damage besides eventual death in normal cells as a result of intracellular glutamate excess and Ca^{2+} upregulation. [57] The mechanisms for producing antioxidants, including those for GSH fusion and salvage, are frequently upregulated.[58,59] The cancer gene xCT, which intakes cysteine for the production of GSH, is a crucial component of GSH biosynthesis.[29] Riluzole was shown to exhibit lower total GSH levels by preventing glutamate release in cancer cells.[35] The decrease in GSH causes a significant decrease in antioxidants and a rise in ROS, which causes apoptosis and ultimately cell death.[10,60] The latest research showed that riluzole encouraged the build-up of ROS, which led to the breakdown of DNA double strands, hence encouraging the prevention of DNA repair in malignancy, consequently leading to cell death (Fig. 3).[61] Additionally, it has been observed that riluzole increases cellular

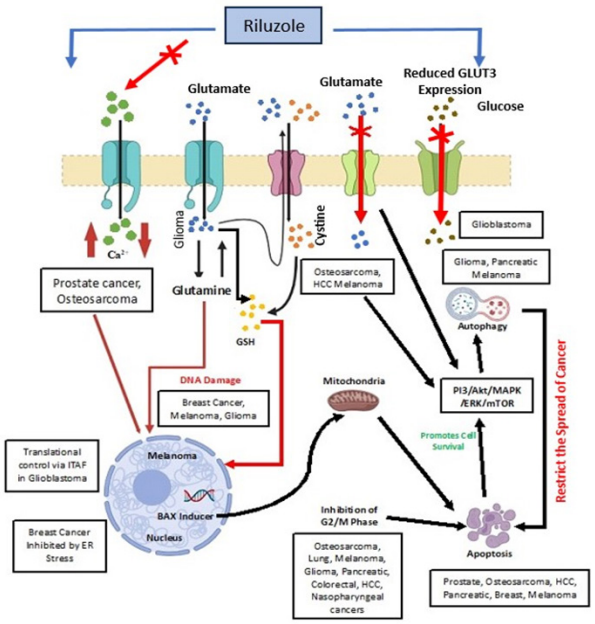

Fig. 4: Various mechanistic pathways of anticancer action of riluzole by glutamate secretion suppression, altering intracellular signaling pathways, increase in oxidative stress, altering protein translation, induced DNA damage, apoptosis and autophagy in cancer cells

glutamate levels in hepatocellular carcinoma (HCC), which resulted in a decrease in GSH synthesis due to the reduction in cysteine absorption by the cells. Apoptosis was subsequently brought on by the build-up of ROS as a result of the reduction in GSH synthesis.[60-62] Riluzole enhanced ROS generation in osteosarcoma, activating cAbl kinase, which takes part in processes of cell death.[61] It has been reported that riluzole effectively caused cell death by enhancing the elevated ROS in cisplatin-resistant lung cancer cells.[35] Interestingly, riluzole by inhibiting xCT in cancer cells, where glutamate secretion occurs via xCT, may increase intracellular glutamate levels to produce glutamate-induced peroxidation and reduce GSH formation due to cystine deficiency.[34] Riluzole inhibits the glutamate release and increases the generation of ROS in a number of cancers (Fig. 4).

Protein synthesis

Overcoming drug resistance in cancer basically depends on the mechanistic pathways of riluzole for its anticancer activity and the chemotherapeutic agents implicated in the treatment. It has been demonstrated that mTOR kinase inhibitors make glioblastoma more resistant to mTOR inhibitors by increasing the internal ribosome entry site (IRES) dependent protein synthesis of critical cell cycle regulator (Fig. 3). It was observed that IRES-dependent translation was essential for the growth and resistance of tumors. It has been demonstrated that hnRNA A1 regulates the c-Myc and cyclin D1, genes' translation.[63] In a recent study, it was shown that riluzole inhibits ITAF, hnRNP A1 action to reduce the translations of the genes c-Myc and Cyclin D1, which helps to overcome glioblastoma resistance to mTOR inhibitor.[64] Thus, riluzole may function by interacting directly with proteins that are not involved in the glutamate metabolic and signaling pathway, including ITAF, hnRNP A1.

DNA damage

A cell may be able to withstand DNA damage from external stimuli like UV radiation and chemical carcinogens as compared to internal stimuli like oxidative stress and replication mistakes [65]**.** The damage response to single-stranded breaks (SSBs) and double-stranded breaks (DSBs) in DNA is largely an autonomous process requiring various signaling pathways and protein components, but there are many overlapping aspects (Fig. 3). Both, SSB and DSB can activate the p53 protein, which subsequently governs the damage response in transcriptional and non-transcriptional regulation**.** Cells, in general, employ a number of methods to repair DNA damage, like nucleotide excision repair (NER) and mismatch repair (MMR) pathways are two special repair strategies for errors that arise at a specific nucleotide. MMR is a DNA repair method that converts one component of a mismatched pair of bases to the typically matched base, whereas NER is the principal pathway utilized by mammals to eliminate bulky DNA lesions generally caused by UV exposure. Moreover, cells conduct MMR immediately after DNA synthesis, whereas NER takes place when DNA damage already happened. Generally, MMR replaces mismatches and insertion/deletion loops that are not corrected by proofreading, whereas NER replaces UV radiation or chemical-induced DNA damage.[66, 67]

Several malignancies frequently exhibit DNA damage repair dysregulation, which is frequently correlated with mutations in certain proteins. For instance, a p53 mutation enables the injured cell to proliferate in spite of not meeting the requirements of a checkpoint, allowing injured cells to pass through the checkpoint of the cell cycle. Radiation or chemotherapy typically makes cells with DNA damage response abnormalities less sensitive**.** Experimentally, it has been proved that riluzole accumulates inside the injured cells at cell cycle checkpoints, potentially causing apoptosis. There are two indications, namely, poly (ADP-ribose) polymerases (PARP), a critical enzyme involved in DNA repair and many other cellular processes such as transcription and chromatin structure modulation, and H2AX, a variant of the H2A protein family, a component of the histone octamer in nucleosomes, are typically present in DNA damage and are routinely used to evaluate the efficacy of certain chemotherapeutic agents. Histone H2AX is known to be phosphorylated by kinases like ataxia telangiectasia mutated (ATM) and ATM-Rad3-related (ATR) in the PI3K pathway in response to DSBs, and PARP cleavage linked to the stimulation of SSB repair.[67-69] Another indication that the production of ROS brought on by riluzole therapy is the primary factor producing DNA damage in these cancer cells is the correlation between the build-up of DNA damage and a decline in glutamate release and GSH levels.[10,70] A comparable rise in H2AX phosphorylation was observed in a phase II clinical study for metastatic melanoma.[21] These results clearly imply that riluzole exhibits a damaging effect on DNA in cancerous cells.

Cell Cycle Arrest

Riluzole has been shown to produce G2/M cell cycle arrest in melanoma cells (Fig. 3). After being exposed to riluzole, G2/M cell cycle arrest was exhibited in the hepatocellular carcinoma in Huh 7 and SNU 449 cell line. Moreover, riluzole was shown to suppress the G2/M cell cycle by decreasing the expression of phospho-cdc2

and p21 as well as a rise in cyclin B1 level [45]. Additionally, riluzole caused G2/M phase arrest in the PANC1 and ASPC1 cell lines of pancreatic cancer by decreasing CDK1 level [71]. Riluzole produced DNA damage in an *in-vivo* brain metastasis experiment utilising human melanoma cells that expressed the GRM1 protein. It also enhanced the radio sensitivity, which led to the higher cell damage and decreased metastases in animal models [72]. Moreover, riluzole caused cell cycle arrest in glioma, colorectal adenocarcinoma, and A549 cells (lung cancer) [73]. Riluzole also caused G2/M arrest and apoptosis in human nasopharyngeal cancers [74].

Autophagy

Autophagy, commonly referred to as "self-digesting," is a mechanism used to get rid of damaged organelles and improperly folded proteins. It is distinguished by the formation of autophagosomes around the components and their fusion with lysosomes for destruction [75]. It should be noted that ROS and intracellular Ca^{2+} levels are also necessary for controlling autophagy. Basal autophagy regulation of normal cells helps to maintain homeostasis; however, autophagy dysregulation in cancer is associated with unchecked cell proliferation and growth (Fig. 3). In dose-dependent manner, drug increased cell death and an autophagy precursor, p62, as shown in pancreatic cell line studies [75, 76, 77]. In another study, after the administration of riluzole, the androgen receptors were found to be reduced by autophagy a castrate-resistant prostate cancer cell line that expressed GRM1. Since riluzole rise the Ca^{2+} levels, subsequently causes to enhance the level of autophagy markers like p62, Beclin1 and LC3AII [52]. Additionally, riluzole reduced the expression of GLUT3 and PI3K/Akt signaling in gliomas, which further resulted in autophagic cell death [43].

Induction of Apoptosis

Normally, activated AKT, mTOR, ERK, RAS, and BRAF proteins that down regulate pro-apoptotic proteins while activating anti-apoptotic proteins contribute to cancer survival through increased activity of MAPK/ERK and PI3K/AKT pathways (Fig. 3). An interaction of ligand to the cell receptor through the extrinsic apoptotic route or the intrinsic apoptotic pathway causes apoptosis to occur. Both processes include successive caspase cleavage, but with different enzymes. Cells that are under oxidative stress or that have suffered significant, irreparable damage to their DNA typically initiate the intrinsic apoptotic pathway. Pro-apoptotic proteins control the process. The apoptosome, which is composed of Apaf 1 and cytochrome c as well as pro caspase 9, is generated when the apoptotic process is started. When this complex is formed, caspase 9 is then released from this complex and activated, which in turn activates caspases 3 to 7 and causes apoptotic cell death [78].

Apoptosis suppression and abnormal down-regulation of apoptotic proteins is usual in cancer cells. In malignancies like pancreatic, hepato-cellular, prostate, and breast cancer, riluzole treatment was observed to cause apoptotic cell death by altering a number of cellular processes, such as causing oxidative stress, preventing autophagy, and decreased expression of intracellular signaling pathways. It has been observed that the treatment with riluzole cleaved the caspase 3 and 9 as well as PARP in HCC. Moreover, caspase 3 was found to be up-regulated in pancreatic cancer cells [76, 77, 78, 79]. Additionally, after receiving radiation along with the riluzole, it was observed that the quantity of cleaved PARP and caspase 3 increased in melanomas [80]. Riluzole reduced the cell viability in prostate cancer cell line by activating caspase 3, 8, and 9, regardless of whether they were androgen-dependent [81]. It has been reported that riluzole supplied in combination with iron oxide nanocages caused osteosarcoma cells to undergo apoptosis and hence, reduced osteosarcoma tumors in a xenograft animal model [82, 83].

Pre-clinical Outlook

It is a well-known fact that cancer cells usually mutate across a variety of mechanisms to promote uncontrolled cell proliferation. Hence, enhanced knowledge of cancer biology of control of crucial elements and mechanisms promoting cell growth and metastasis brought the advancement in the cancer treatment. As a result of improved knowledge more potent combinatorial medicines are gradually replacing the conventional treatment modalities. In addition to conventional chemotherapy and radiation therapy, innovative cutting-edge cancer treatment approaches are gaining ground. Among the most promising are CRISPR/Cas9 gene therapy, nanoparticles, and immunotherapy [84, 85].

In some preclinical settings, it was studied that riluzole caused a substantial decrease in osteosarcoma tumor size in naked mice xenografts when administered through iron oxide nanocage as the drug delivery system [83]. Currently, riluzole is being investigated pre-clinically in conjunction with other drugs to augment the anti-cancer effects bring the drug into clinical trials. It has been investigated that riluzole works in combination with other drugs to reduce cell viability and proliferation while increasing apoptosis [78, 79]. Furthermore, these effects may be amplified by specific drug combinations in various cancer cell lines. For instance, it has been shown that riluzole in conjunction with paclitaxel, cisplatin and sorafenib, exhibited a synergistic effect in colorectal cancer, triple-negative breast cancer, and melanoma [86, 87]. Moreover, riluzole combined with AKT or mTOR inhibitors demonstrated better outcomes in xenograft and *in-vitro* experiments in glioblastoma and melanoma [43, 63]. As evidenced by the plethora of research findings in cell lines and pre-clinical settings, these investigations, however, shed light on the various mechanistic pathways by which riluzole works and further pave the way for future clinical trials and translation of riluzole alone or in combination with other anti-neoplastic agents from lab to bed side.

A radiosensitizer, also known as a radio sensitizing agent, is a pharmaceutical product that increases the impact and toxicity of radiation treatment. Basically, radiation treatment shrinks tumors by damaging the nucleic acid using different external energy sources such as X-rays and protons. Some radio-sensitizers are directly toxic on their own, whereas others only become effective when exposed to radiation, and improve the radiation therapy in cancers [88]. Riluzole can be used as a radio sensitizer in addition to being combined with other drugs to help combat cancer. Riluzole with radiation treatment showed a synergistic impact on melanoma and glioma, both *in-vivo* and *in-vitro* [10, 80]. In another preclinical study, riluzole combined with radiation therapy significantly reduced the tumor volume. An *in-vitro* investigation on brain tumor stem cells showed an unpredictably more growth suppression with a lower dosage of riluzole combined

with radiation treatment. However, radiation treatment combined with a greater dosage of riluzole was more successful *in-vivo*.[43, 71] Additionally, *in-vivo* and *in-vitro*, riluzole enhanced cytotoxicity as compared to treated groups with riluzole alone, more sensitive to radiation via activating the ATM/P53 signaling pathway [73].

Effectiveness of Riluzole in Various Cancers

Fundamentally, riluzole has been shown to block voltage-gated Na^+ channels in a dose-dependent manner [6, 7]. However, the exact mechanism of action of riluzole is not yet clearly explained, a number of studies have shown that the effects of riluzole are attributed to its capacity to inhibit glutamate release and improve glutamate reuptake, resulting in the blockade of glutamate-dependent signaling [8]. Glutamate signaling is overactive in several neurological conditions where riluzole can be employed as a drug of choice for the treatment [89]. Furthermore, it has been investigated that glutamate signaling is implicated in various types of tumors and plays a significant role in the growth and development of tumors [9]. Various studies have been conducted to evaluate the efficacy of riluzole therapy in various types of cancers, both *in-vitro* as well as *in-vivo* (Table 1). Implementation of riluzole for the treatment of a variety of cancers due to its effectiveness, minimal toxicity, and tolerance could be a viable approach to treat cancer.[10, 11, 91] A plethora of research findings have shown various mechanistic pathways by which riluzole targets different types of cancer cells (Table 1). Understanding the precise targets of riluzole and its fundamental mechanistic pathway in certain malignancies may further enhance the role of riluzole in the treatment of cancer.

Recently, riluzole was tested in various cancer cells and shown to inhibit cell growth and tempt cell death. It is exhibited to be effective as an anticancer drug in malignancies of the skin, breast, brain, nasopharynx, colon, pancreas, liver, bone, and lung.[43,45,78] Essentially, cancerous tissues expressing glutamate receptors are usually found to respond to riluzole therapy, however, surprisingly a number of cancer tissues do not possess the glutamate receptors but respond to the riluzole as an anticancer agent. The activity of riluzole as as a potential anti-cancer agent is primarily due to its multifaceted mechanistic pathways such as inhibition of glutamate release, growth signaling pathways, glutathione synthesis, reactive oxygen species formation, DNA integrity, Ca^{2+} homeostasis, autophagic and apoptotic processes.[51,76] Moreover, in combination with other drugs, riluzole shows synergistic benefits in triple-negative breast cancer, melanoma, colorectal cancer, and glioblastoma.[69,85,86] The effects and results of riluzole treatment on different cancers explored thus far in order to highlight the therapeutic prospects of riluzole are promising and encouraging.

Table 1: Mechanism and effectiveness of riluzole in different types of cancer

Type of Cancer	*Cell-line*	*Mechanism of Action*	*References*
HCC	Huh-7, SNU449	Apoptosis- G2/M cell cycle arrest	[78]
Breast cancer	SUM102 SUM149 SUM229	Apoptosis ER stress	[86]
Osteosarcoma	LM7 and OS482 LM7	Inhibits cell proliferation Apoptosis	[45]
Pancreatic	ASPC1, SW1990, PANC1, BXPC3	Apoptosis- G2/M cell cycle arrest Autophagy	[76]
Colorectal cancer	ASPC1, H630, HT29, CACO2 HCT116 and	*In-vitro* decrease in cell viability and *in-vivo* polyp growth by sensitising cells to cisplatin. Arrest in G2/M, apoptosis	[86]
Melanoma cancer	UACC90 ,1205Lu, SKMEL2and C8161	Cell cycle arrest in G2/M phase, MAPK/PI3K/AKT signaling DNA damage, apoptosis	[39, 40, 69, 89]
Neuroblastoma	IM32 neuroblastoma cells, Neuron-neuroblastoma hybrid (NSC-34D),	Calcium levels	[49]
Glioblastoma	Glioblastoma GBM6 U87MG, short term PDX patient-derived line, T98G, LN229	Protein synthesis control	[63]
Lung cancer	A549	Apoptosis- G2/M cell cycle arrest	[72]
Glioma	U118MG & LN229, U87MG glioma cells	DNA damage, tumor suppression, Cytotoxicity	[10, 43]
Human nasopharyngeal carcinoma	HNE1, CNE1 and CNE2	G2/M arrest Sensitizes to radiation, ATM/P53	[73]
Brain tumor stem-like	64SP,11SP C6 cells	G2/M arrest, apoptosis Autophagy Sensitizes to radiation	[72]
Prostate cancer	C4-2-androgen-independent 22Rv1 LNCaP-androgen-dependent CWR1-R1ca VCaP	ER stress Autophagy Apoptosis	[52]

Table 2: Clinical status of riluzole in the management of various malignancies

Type of Cancer	*Phase*	*Objective*	*Masking*	*References*
Breast Cancer	I	To estimate the impact of inhibition of glutamate receptors with riluzole on the development of breast cancer in the women with stage I to IIIA.	Open-label	[98]
Metastatic Colorectal cancer	I	To find out the optimum dose, advantages, and/or side effects of riluzole and its effects in combination with standard of care mFOLFOX6 and bevacizumab in metastatic colorectal cancer subjects.	Open-label	[98]
Brain Metastasis	I	To estimate the optimum safe dose of riluzole that can be taken concurrently with standard whole-brain radiotherapy in the subjects with multiple brain metastasis	Open-label	[98]
Advanced Solid Tumors and Melanoma	I	To explain the safe and effective dose of sorafenib tosylate in combination with riluzole for the treatment of subjects with all types of solid tumors which are refractory to conventional therapy or for those malignancies for which no typical therapies exist.	Open-label	[98]
Advanced Melanoma	II	In an initial phase I clinical trial, action of riluzole were studies in the subjects with stage III and/or stage IV melanoma that can be surgically removed.	Open-label	[99]
Stage III and IV Melanoma (Resectable)	0	To estimate the daily dose of riluzole to observe the shrinkage in advanced melanoma in patients. Furthermore, long-term toxicity of riluzole was assessed when administered, and a comparison of the survival of these subjects with historical data.	Open-label	[42]

Clinical Status of Riluzole in Cancer

Clinical trials assist scientists in comparing and contrasting the drugs for better results which provide the platform for the future development of new drugs. Hence, the main aim of a scientist is to gather data to establish the potential effects and important properties of an investigational drug**.** Clinical trials for regulatory clearance must establish the therapeutic advantages of a drug in a specific populace. Systematic clinical studies are required to develop a drug after evaluating efficacy, and safety, determining the recommended dose administration, and pharmacokinetics in an exploratory manner and clinical advantages are compared with conventional therapies. Clinical development of drugs is typically accomplished in three stages viz. phase I, phase II, and phase III trials. Usually, drugs employed in the treatment of cancer have been clinically developed after the evaluation of safety and efficacy in various phases of clinical trials. The pharmacological actions and adverse effects of anticancer drugs differ from those of traditional therapeutic agents and molecularly targeted treatments, necessitating an update of facts to consider in clinical research.[92,93] Chemotherapy is the most often used cancer treatment approach. For better clinical outcomes of anticancer agents, it is critical to know which techniques work best together. The ideal cancer combination therapy may be designed, after knowing the precise processes by which anticancer agents eliminate tumors. [94,95]

Because chemotherapy is the most promising modality for the treatment of cancer, riluzole can be used to increase the anticancer efficacy of conventional chemotherapy, immunotherapy, and targeted immunotherapy. Apart from being a drug of choice in the treatment of ALS, riluzole has been demonstrated to have intriguing anti-cancer properties.[23-25] It should be emphasized that glutamate signaling is also required for the growth and development of tumors in a variety of tissues [9]**.** Multiple studies have been carried out to investigate the efficacy of target drug therapy in the treatment of various cancers *in-vitro* as well as *in-vivo*. Riluzole has attracted attention as a potential arm for the treatment of a number of cancers due to its efficacy, safety and tolerability [95, 96]. Furthermore, riluzole demonstrated the capacity to destroy cisplatin-resistant lung cancer cells. Moreover, the combination of riluzole radiation therapy and riluzole was shown to be more effective against glioma and melanoma. Furthermore, riluzole has synergistic benefits with several drugs in glioblastoma, colorectal cancer, melanoma, and triple-negative breast cancer [35, 86]. For its anticancer properties, riluzole has been used in clinical studies at various stages (Table 2). In one of the clinical trials, glutamate receptor blockade with riluzole affects the signaling through the mGluR1 signaling pathway in pre- and post-treatment tumor subjects [7]**.** In another study, the highest allowable dose of riluzole may be given simultaneously with conventional whole-brain radiation in patients with multiple brain metastases**.** Riluzole in combination with modified leucovorin calcium, fluorouracil, and oxaliplatin, 6/bevacizumab was tested clinically to characterize the safety, and toxicity and determine the suggested phase II dose in patients with metastatic colorectal cancer**.** The adverse events and optimal sorafenib tosylate dose in combination with riluzole were studied in the subjects with solid tumors or melanoma that have extended to other body parts and cannot normally be treated with conventional therapy. Moreover, riluzole has the potential to inhibit or slow tumor cell development. Sorafenib tosylate may inhibit tumor cell development by inhibiting some of the enzymes required for cell proliferation. Combining sorafenib tosylate with riluzole resulted in increased tumor cell death. In resectable stage III and IV melanoma, it has been found that daily dosage of riluzole causes tumor shrinking in patients with advanced melanoma as judged by RECIST criteria. Furthermore, long-term toxicity of riluzole was assessed when administered, and a comparison of the survival of these subjects with historical data [98]**.** The promising effects of glutamate receptor blockage on cellular pathways were crucial in the growth and development of melanoma and were investigated in the subjects with stage III or IV melanoma following surgical procedures. Changes in the expression of activated PLC and ERK in lysates from tumor tissue biopsies were studied clinically [7]. The latest updates

on clinical trials conducted on riluzole as a novel anticancer agent are encouraging the development of riluzole as a therapeutic agent to cure various malignancies.

Future Scope

Over the last many years, it has been comprehended that the advancements of modalities in cancer treatment are growing rapidly. The ultimate goal of anticancer research is its application in the clinic. Significant research in this direction is still being conducted. Despite the substantial research and advancements in the treatment of cancer, current anticancer strategies are still not sufficient. Riluzole exhibited good results as an anti-cancer agent but has not yet been comprehensively explored for anticancer activity. However, substantial research has been published for preclinical investigation, and recently from various clinical trials promising developments for riluzole have been discussed.

Treatment of cancer using a chemotherapeutic approach is different as compared to radiotherapy or surgical removal. Generally, chemotherapy is the widely used and most preferred treatment modality for cancer management. The future of management of cancers will include the various potential combinations of current treatment strategies. Hence, to get the maximum possible anticancer effects, it is imperative to decide which strategy will work in combination effectively. The definitive combination treatment strategy can be designed to manage the cancer, after understanding the precise mechanistic pathway by which drug(s) subside the tumor. Since, the most promising anticancer strategy is chemotherapy; hence, riluzole can be explored to improve the therapeutic efficiency of conventional chemotherapy, immunotherapy, and targeted immunotherapy.

Since, traditional chemotherapy has certain marked limitations like severe adverse effects, resistance etc., therefore more effective and safe anticancer agents and their combinations are under investigation. Initially, the concept of targeted therapy was introduced with the inception of hormone replacement therapy, which was then developed into more advanced therapeutic anticancer agents like monoclonal antibodies and TKIs because they target certain ligands and receptors present in the tumor cells. Hence, using the same strategy, hormone replacement therapy in combination with riluzole could be a promising approach in the management of various cancers. Riluzole could also be studied to target the over-expressed receptor-based strategies applicable to specific tumor cells.

CONCLUSION

The present review provides current knowledge and new insights into the mechanisms of action of riluzole in cancer. It has been found that riluzole affects several biological processes, such as apoptosis, survival, and responses to stress. Riluzole has been shown to have cytotoxic effects in triple-negative breast cancer cells when paired with paclitaxel, cisplatin-resistant lung cancer cells, radiation sensitization impact upon melanoma, nasopharyngeal, gliomas and carcinoma, m-TOR inhibitor-resistant glioblastoma. Thus, riluzole exhibits significant potential for the treatment of malignancies that are difficult to treat. The effectiveness of riluzole may be increased, and its translation into clinical use may be made easier with further knowledge of the precise mechanisms by which it works both on its own and in conjunction with other drugs.

CONFLICT OF INTERESTS

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