The Role of Long Non-Coding RNA ZFAS1 in Gliomagenesis: A Scoping Review

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Abstract

Non-coding RNA species play important roles in biological mechanisms that regulate glioma initiation and progression. Recently, evidence suggests that *ZNFX1* antisense RNA 1 (*ZFAS1*) has the ability to act as an oncogene or tumour suppressor, and so plays critical regulatory functions in the development and progression of many types of cancers such as lung, renal and hepatocarcinoma. The roles of *ZFAS1* in glioma cancer are still unclear, and there are numerous potential pathways to explore. The current work provides a scoping review of studies on *ZFAS1*'s functions and underlying molecular mechanisms in the initiation and progression of glioma, as well as a possible field of research to be examined further. A literature search was carried out using Scopus, PubMed, and Web of Science (WoS) using a specified search string, and the data gathered was discussed and reported. This scoping review comprised five original research papers that study *ZFAS1* and its roles in gliomagenesis. *ZFAS1* was found to be highly upregulated in glioma. Tumour-node-metastasis (TNM) stage, lymph node metastases, and overall survival were revealed to be significantly associated with *ZFAS1* status and regulated via several pathways and interactions, such as miRNA signalling, Epithelial-to-Mesenchymal Transition (EMT) and Notch signalling pathway. Furthermore, *ZFAS1* knockdown decreased cell proliferation, migration, and invasion while promoting cell death, implying that *ZFAS1* is involved in the glioma cancer progression. The evaluation of their diagnostic importance and therapeutic potential may aid in the development of novel therapies for glioma cancer.

Introduction

The GLOBOCAN cancer incidence and mortality database estimated that 308,102 new cases of brain cancer and 251,329 deaths from brain cancer occurred in 185 countries in 2020 [1]. Gliomas, which originate in the brain or spinal cord's glial cells, account for more than 80% of all malignant brain tumours and are the leading cause of brain tumour death worldwide [2]. According to the 2007 World Health Organization (WHO) classification of central nervous system (CNS) tumours, gliomas were classified based on their cell type; astrocytoma, oligodendroglioma, ependymoma, or mixed tumour (e.g., oligoastrocytoma). They are divided into four grades based on the degree of malignancy from least (low-grade; I and II) to most aggressive (high-grade; III and IV) [3]. Despite advancements in the treatment of malignant glioma through surgical, radiotherapy, chemotherapy, and a combination of multiple therapies, the prognosis remains poor due to tumour invasion, metastasis, and chemoresistance, with the majority of grade IV glioblastoma patients living for less than two years [4]. Thus, finding possible diagnostic and prognostic molecular markers to aid the development of glioma treatments is crucial. In addition, the dysregulation of lncRNAs can be a potential biomarker in glioma diagnosis, prognosis, and target therapy[5].

There are several reliable biomarkers for glioma, available such as; 1p/19q codeletion, MGMT promoter hypermethylation, IDH mutations, circulating tumor DNA (ctDNA), and circulating tumor RNA (ctRNA) include mRNAs, long non-coding RNAs (lncRNAs), and small non-coding RNAs (snRNAs) [6]. lncRNAs are transcripts with more than 200 nucleotides that lack functional open reading frames [7]. Recently, studies reported that lncRNA plays an important role in modulating comprehensive cellular process by regulating transcriptional gene expression, and post-transcriptional as well as epigenetic modification [8]. In addition, lncRNA is a molecule that has recently been shown to play an important role in cancer signalling. They exert their influence through a variety of different mechanisms of action. The molecular decoy mechanism, also known as competitive endogenous RNA, is frequently described. Using this mechanism, lncRNAs can bind molecules such as microRNA (miRNA) and prevent them from mediating their effect on downstream gene signalling [5]. Besides, the loss of lncRNA expression could potentially alter the expression of many genes and promote tumorigenesis. Reported lncRNAs associated with glioblastoma are*MALAT1, H19* [9] *HOTAIR* , and *GAS5* [6].

In general, these lncRNAs could regulate important oncogenic and tumour suppressive pathways and significantly affect glioma development by metastasis, tumorigenesis, chemoresistance, radioresistance, apoptosis, and angiogenesis through various pathways, such as, PI3K/Akt, Wnt/β-catenin or ERK/MAPK pathways. For example, *MALAT1* suppress apoptosis and increase the cell proliferation and viability of glioblastoma (GBM) stem cell. Baspinar *et al.*[10] reported that downregulation of*MALAT1* decrease the stemness nestin and sex-determining region (SRY) Y-box 2 (SOX2) markers in SHG139S GBM cells and induced cell proliferation through ERK/MAPK pathway. *MALAT1* excision (gene silencing) inhibited glioma stem cell proliferation by increasing miR-129 expression, which lowered glioma stem cell viability and growth by inhibiting *SOX2* expression [10].

H19 has been proposed to play a role in the development of glioblastoma malignancy and the maintenance of glioblastoma stem-cell characteristics. Besides, it can promote angiogenesis, cell invasion, and cell growth in glioma. Chen *et al.*[11] suggested that *H19* target miR-200a to inhibit cancer growth by interacting with CDK6 and ZEB1. Silencing *H19* was reported to increased miR-200a expression and reduce the expression of CDK6, and subsequently inhibiting glioma cell proliferation. [11]. *HOTAIR*acts as a stemness promoter by enhancing cell migration, invasion, and proliferation in glioma. Wang et al. [12] revealed that *HOTAIR* was elevated in GBM and involved in temozolomide (TMZ) resistance. The interaction of *HOTAIR* and miR-526b-3p through the epithelial V-like antigen 1 (*EVA1*) pathway results in TMZ-resistant GBM. Additionally, it was discovered that deleting the *HOTAIR*gene mitigate glioblastoma's progression and reduced TMZ chemoresistance [12].

There were several challenges in studying these lncRNAs. For example, Baspinar et al. reported that many recent studies of MALAT1's pro-tumorigenic functions were supervised in patient-derived primary GBM cultures rather than using established GBM cell lines such as U87, SHG139, and U251. When compared to patient-derived primary GBM, established cell lines can lose the inherent molecular and pathophysiological features of the native tumour; thus, the conflicting results could be due to cell culture errors. Nevertheless, study of lncRNAs in glioma still merit a big good impact for glioma therapy. Understanding the role of lncRNA in GBM or glioma pathogenesis may aid in the development of nanoformulation and enzyme modification, such as antisense oligonucleotides (ASOs), ribozymes, or deoxyribozymes, which may improve the delivery of lncRNA-targeting agents into brain tumours [10]. Recently studies discovered a new potential lncRNA in glioma development, which is *ZFAS1* .

ZNFX1 antisense RNA 1 (*ZFAS1*) islocated on chromosome 20q13.13 and can be found in both cell nucleus and cytoplasm [13]. Recent research found that*ZFAS1* expression is upregulated in many human cancers, including glioma, lung, colon, liver, ovary, and gastric cancers, but downregulated in breast cancer [13].*ZFAS1* upregulation is associated with clinic pathological features and prognosis, such as tumour-node-metastasis (TNM) stage, lymph node metastasis, and overall survival in various cancers. In addition, *ZFAS1* has the potential to be utilised as a cancer prognostic biomarker due to its vital roles in tumour progression or cell apoptosis in a variety of human malignancies, including cervical cancer (CC), pancreatic cancer (PC), and

glioma, through modulating mRNA, miRNA, or protein/gene expression. [14].

A study by Su *et* al. [14] reported that high *ZFAS1* expression was correlated with chemosensitivity and prognosis of CC. Overexpression of *ZFAS1* inhibits miR-190a-3p by sponging the miR-190a-3p and promoted the expression of Kruppel-like factor 6 (*KLF6)* , which lead to CC cell proliferation. Additionally, *ZFAS1* increases CC tumor growth and cell proliferation by upregulating LIN28 and enhanced CC cell metastasis by sponging miR-647 [14]. Another study by Rao *et al.* , [15] described the interaction of *ZFAS1* with High mobility group protein 2 (HMGA2) and miR-497-5p in pancreatic cancer. HMGA2 upregulation promotes cell cycle entry and inhibits apoptosis, which increases cancer cell proliferation. HMGA2 influences various DNA repair mechanisms and promotes epithelial-to-mesenchymal transition by activating signalling pathways such as MAPK/ERK, TGF/Smad, PI3K/AKT/mTOR, NFkB, and STAT3. *ZFAS1* was suggested to promote HMGA2 expression through sponging miR-497-5p in PC, therefore increase the PC development [15].

Wang *et al.* [16] reported that *ZFAS1* was associated with advanced pathological stages and larger tumour sizes in colorectal cancer (CRC) by regulating the synthesis of fatty acid which can promote the malignant phenotype of CRC. Knockdown of *ZFAS1* downregulate the expression of the implicated genes to steroid biosynthesis and fatty acid metabolism, sterol regulatory element-binding protein 1 (*SREBP1*), fatty acid synthase (*FASN*), and stearoyl CoA desaturase 1 (*SCD*) in CRC. In addition, *ZFAS1* regulates fat metabolism to promote CRC progression by interacting with polyadenylate-binding protein 2 (*PABP2*) to facilitate the interaction of *PABP2* and *SREBP1* , stabilising *SREBP1* mRNA and activating its downstream genes *SCD1* and *FASN* [16]. All of these information are clear evidence that *ZFAS1* play crucial roles in tumorigenesis.

In glioma, *ZFAS1* which acts as an oncogene, with its expression being elevated in glioma cell lines, such as U87MG, U251 and T98G, are reported to be associated with patient outcomes and overall survival [17]. According to Gao et al. [18], *ZFAS1* plays an oncogenic role in glioma by regulating the Epithelial-to-Mesenchymal-Transition (EMT) process and Notch signalling pathways. Knocking down *ZFAS1* inhibited EMT and Notch signalling, as well as glioma cell proliferation, migration, and invasion. The EMT process has been shown to be involved in cancer metastasis [18]. Lv *et al.*[19] confirmed that *ZFAS1* expression promotes the EMT process and they discovered that knocking down *ZFAS1* decreased the expression of Matrix metalloproteinase-2 (*MMP2*), Matrix metalloproteinase-9 (*MMP9*), N-cadherin, Integrin 1, Zinc finger E-Box binding homeobox 1 (*ZEB1*), and significantly increased the expression of E-cadherin in glioma cells [19].

It is currently unclear how exactly *ZFAS1* is involved in regulating gliomagenesis, as there is still a lack of understanding regarding its role in this cancer. However, some progress has been made in studying the mechanisms of *ZFAS1* , although it is still in its early stages in comparison to other types of cancer such as cervical, pancreatic, and colon cancer, where *ZFAS1* 's biological functions have been extensively investigated. Despite the limited understanding of the regulatory mechanisms of *ZFAS1* in glioma, it has been identified as a prognostic marker and potential therapeutic target due to its association with patient survival and glioma development [19]. *ZFAS1* further research is needed to fully understand the role of *ZFAS1* in gliomagenesis and to develop potential therapeutic strategies targeting this lncRNA.

Hence, this scoping review was carried out with the goal of comprehensively outlining the published research on the mechanisms of *ZFAS1* in glioma and identifying any current knowledge gaps. This review will assist our understanding of *ZFAS1* 's role in gliomagenesis, such as its potential as a biomarker in glioma, the importance of *ZFAS1* in glioma development, the pathways and cellular mechanisms that contribute to and affect glioma development, and identify gaps in knowledge that will guide future research in this discipline.

Methodology

This scoping review was accomplished following the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist and PRISMA-ScR Tip Sheets by Trico et al. [20]. The review was conducted in five stages; (1) Determine the research question; (2) Identify

relevant study; (3) Study selection; (4) Data charting process; and v. Collating, summarizing, and reporting results [20]. The research question was developed in accordance with Arksey and O'Malley's guideline which is to begin with a broad review area to determine what is available before narrowing the search [21]. The following research question was examined: What roles does *ZFAS1* play in the development of glioma cancer?

Literature Search

A literature search was conducted using Scopus, PubMed, and Web of Science (WoS) with the search string "Gliomagenesis" OR "Brain cancer" OR "Glioma") AND ("*ZFAS1* " OR "*ZNFX1* antisense RNA" in August 2022. The search did not include any additional filters. To ensure that relevant articles were included, the cited references from included articles were traced. All original research articles on the roles, functions, importance, and mechanisms of*ZFAS1* in gliomagenesis were included. Articles that were not related to the roles of *ZFAS1* , did not include a sub-analysis on glioma cancer, and were not written in English were excluded. Reviews with no primary data were omitted. Abstracts and proceeding papers were also removed to avoid study duplication.

Mendeley Desktop version 1.19.8 (London, United Kingdom) was used to sort out the literature and identify the articles' duplication. EMAEOA and AAR independently filtered out the titles and abstracts of the articles, and then rectified the full-text for detailed analysis based on inclusion and exclusion criteria. Data extraction was accomplished by EMAEOA and AAR, and data were extracted including authors, years, major findings, study design, and limitations. Discussion with the third author, MFMU and EMAEOA resolved any discrepancies in article inclusion. SHAK, KI and NJO contribute in reviewing and revising the grammatical error.

Results

The primary search yielded 32 articles, 13 from Scopus, eight from PubMed, and 11 from WoS. 14 duplicates were removed, resulting in 18 articles that were identified and screened further. A total of 13 articles were eliminated (eight were excluded based on article type, four articles excluded were not related to the topic, and one was not in English). This scoping review contained five original research articles for analysis in the final screening (Figure 1).

Discussion

Scoping review analysis revealed five original articles published between 2017 and 2020, focusing on long non-coding RNA *ZFAS1* and their roles in glioma. *ZFAS1* levels are higher in grade III and IV glioblastoma than in lower grade glioma, such as type I and II. Silencing or knocking down *ZFAS1* in glioma cell lines, altered various cellular processes including the cell cycle activity, invasion, migration, and specific signalling pathways such as EMT, Notch signalling pathway, as well as miRNA signalling as described in Table 1. Table 1 summarises the analyses and results from the five selected articles. This suggests the relevance of *ZFAS1* as a potential target for glioma.

miRNA Signaling

MicroRNAs (miRNA) physiological role is vital in controlling the cell cycle, cell proliferation, differentiation, and apoptosis in normal cells. Most tumor neoplasms such as, gastric, ovarian and uveal melanoma cancers showed high levels of aberrant miRNAs expression. MiRNAs can act as oncogenic miRNAs (onco-miRs) during tumor development and progression based on their level of expression and their main targets are oncogenes and tumor suppressor genes. As an example, in human glioblastoma (GBM) cells, an endogenous miR-7 target the epidermal growth factor receptor (EGFR) and independently inhibit this signalling pathway. High expression of miR-7 in the GBM cell can activate the Akt signalling pathway, thus enhance the invasiveness and viability of GBM cells [22].

Specific lncRNAs may act as competing endogenous RNAs (ceRNAs) to control miRNAs and subsequently affect the expression of their target genes by absorbing or sponging the miRNAs in the cytoplasm [23]. The ceRNAs are lncRNAs that bind to miRNAs and prevent miRNAs from interacting with their target by

decoying or sponging [24]. For example, *ZFAS1* has been shown to sponge miRNAs, and influence tumor growth in melanoma cancer by sponging miR-150-5p [25], microRNA-200b-3p in gastric cancer [26], miR-10a in clear cell renal cell carcinoma [27], miR-329 in bladder cancer [28], and miR-484 in colorectal cancer [29]. In glioma, studies show that *ZFAS1* acts as a molecular sponge for miRNAs such as miR-1271-5p[17], miR-150-5p [30], and miR-432-5p [31]. *ZFAS1* is found primarily in the cytoplasm of glioma cells, implying that it may play a role in the ceRNA network, where *ZFAS1* regulates target gene expression via miRNA sponging [17].

Through three online bioinformatic databases prediction, starBase V3, DIANA and miR code; miR-150-5p was predicted as *ZFAS1* 's potential target, and following that, this prediction was confirmed by luciferase reporter and RNA pull-down analysis, where *ZFAS1* was observed to act as a sponge for miR-150-5p [30]. Furthermore, results revealed that miR-150-5p's expression level in glioma was lower than in Normal Human Astrocytes (NHA) [30]. Pearson's correlation coefficient analysis showed a negative correlation between miR-150-5p's expression and *ZFAS1* 's expression in glioma tissues. The glioma tissues originated from patients who endured tumor resection (ten grade IV, ten grade III, and seven grade II patients). [30]. Moreover, the expression of an endoplasmic reticulum 4-transmembrane protein, proteolipid protein 2 (PLP2), is found to be associated with glioma cell proliferation, migration and invasion [32].

PLP2 protein was discovered to be a functional target mRNA of miR-150-5p [30]. The expression level of PLP2 in glioma cell lines and tissues was higher than in normal brain tissue (NBT), and this protein is implicated in glioma progression regulated by miR-150-5p to inhibit tumor progression and decrease drug-resistance in glioma cells. The same study revealed that *ZFAS1* functions as a miR-150-5p sponge to regulate PLP2 expression in promoting the proliferation, migration and invasion while increasing resistance to TMZ in glioma cells *in vitro* and *in vivo* , via immunodeficient male nude mice [30]. In other studies, miR-150-5p has been implicated in suppressing the progression of several human cancers, such as melanoma cancer and epithelial ovarian cancer (EOC) [33].

For instance, Liang *et al.*[25] found that miR-150-5p acts as a tumor suppressor in melanoma cells by negatively regulating the expression of Ras-related protein Rab9A (*RAB9A*). The elevated expression of *ZFAS1* in melanoma tissues and cells promoted melanogenesis, including proliferation, migration, and invasion, while suppressing apoptosis via the *ZFAS1* /miR-150-5p/RAB9A axis [25]. Xia *et al.*[33] also reported that miR-150-5p is involved in EOC, where this miRNA suppressed the malignancy and reduced chemoresistance in EOC cells by targeting the expression of specificity protein 1 (*Sp1*) . They found that *ZFAS1* 's expression was overexpressed in EOC tissues and cell lines, and *ZFAS1* directly regulated miR-150-5p to promote the progression and chemoresistance in EOC by regulating *Sp1* [33].

ZFAS1 enhanced gliomagenesis through modulating the miR-1271-5p/HK2 axis, where miR-1271-5p was suggested to be*ZFAS1* 's potential target and Hexokinase 2 (HK2) was miR-1271-5p's target. *ZFAS1* enhanced the expression level of HK2 in glioma cells through sponging activity of miR-1271-5p, and subsequently promoting the development of glioma. MiR-1271-5p was found to be downregulated in glioma tissues, and a dual-luciferase reporter assay confirmed the existence of an interaction between miR-1271-5p and ZFAS1. It was reported that their interactions were negatively proportional with each other [17]. Upregulation of miR-1271-5p suppressed gliomagenesis by increasing apoptosis and reducing glioma cell proliferation, migration, and invasion which is consistent with earlier research findings [34]. Additionally, the study reported that ZFAS1 and HK2 protein expression in glioma tissues exhibited a positive correlation, whereas HK2 and miR-1271-5p protein expression exhibited an inverse correlation [17]. HK2 was found to be the key enzyme that contributes significantly to the Warburg effect. Warburg effect is a term to indicate cancer cells' preference to metabolise glucose anaerobically. High expression of HK2 can lead to chemo- or radiation resistance of glioma cells via the aberrant of the apoptosis pathway [35].

Apart from miR-150-5p and miR-1271-5p, *ZFAS1* can also sponge miR-432-5p. A study has shown that *ZFAS1* and miR-432-5p had a significant negative association where miR-432-5p was a direct target of*ZFAS1* to modulate glioma cells viability and cisplatin resistance [31]. miR-432-5p expression was found to be down-regulated in glioma tissues and cell lines, while overexpression of this miRNA lowered glioma cell viability

and enhanced cisplatin-induced glioma cell death [36]. In breast cancer tissues, miR-432-5p expression level was found to be downregulated as compared to neighbouring healthy tissues [37].

Epithelial-to-Mesenchymal Transition

EMT is a biological process that transforms epithelial cells into mesenchymal cells by losing cell polarity and junctions while gaining the ability to migrate, invade, proliferate, and differentiate into a particular cell types [38]. EMT also can make epithelial tumour cells migrate and invade without impairing their viability [38]. EMT can be classified into three subtypes based on the context in which it occurs: EMT type 1, EMT type 2, and EMT type 3, with EMT type 3 being associated with subsets of tumour cells that undergo phenotypic changes to promote migration, invasion, and metastasis[39]. In EMT type 3, the formation of metastatic tumour nodules occurs at distant sites as a result of some tumour cells with a transitional mesenchymal phenotype that undergoes mesenchymal-epithelial transition (MET), a reverse conversion of EMT [40].

The EMT process activation by tumour microenvironment (TME) stimuli has been considered a crucial process for tumour cells to gain their highly malignant phenotypes[41]. Once the formation of TME is established, Unfolded Protein Response (UPR) aids tumour cells in epithelial to EMT by overcoming the stress of cell detachment, and also increases EMT transcription factor expression and reduces cell–cell junction markers, promoting metastasis [42]. EMT is highly related with glioma malignancy[43], and lncRNAs have been identified to coordinate various cellular processes in many tumour cells, including glioma cells, through EMT regulation[44].

Key EMT protein markers were reported to be increased by overexpression of *ZFAS1* which in turn promoted the development of glioma [45]. Several key EMT markers, including N-cadherin, *MMP2, MMP9, ZEB1* , Integrin β1, Twist, and Snail, exhibited a significant reduction in expression when *ZFAS1* was knocked down, whereas E-cadherin expression increased [45]. These results were similar to Gao *et al.* [18] findings, where suppression of *ZFAS1* led to a significant decrease in the expression level of EMT-related proteins (N-cadherin and Snail), and an increase in E-cadherin [46]. Lv *et al.* [19] investigated whether *ZFAS1* influenced EMT in 69 glioma tissues by using Pearson's correlation analysis to examine the link between *ZFAS1* expression and EMT markers. The findings showed a positive correlation between several EMT markers (N-cadherin, *ZEB1* , Integrin β1 and Twist) and *ZFAS1* 's expression, while a negative correlation between E-cadherin and *ZFAS1* 's expression was observed. Thus, these findings suggested *ZFAS1* could enhance malignant glioma's migration and invasion by inducing the EMT process [45].

Notch Signaling Pathway

The Notch signalling pathway is an evolutionary conserved intercellular pathway that governs various physiological and developmental processes such as tissue homeostasis, stem cell maintenance, proliferation, apoptosis, and cell fate decision [47]. Several studies have shown a positive correlation between Notch signaling pathway and lncRNA involved in various tumor progressions whereby the lncRNA regulates this pathway by acting as an oncogene or tumor suppressor [48]. For instance, Wu *et al.* (2021) discovered that the lncRNA, HOXA cluster antisense RNA 2 (*HOXA-AS2*) plays a tumour-promoting role in cell proliferation and migration of cervical cancer by activating Notch pathway [49]. LncRNA, FAM83H antisense RNA 1 (*FAM83H-AS1*) promotes cell proliferation of colorectal carcinoma and is linked to poor prognosis by regulating the Notch pathway [50]. In a study by Liu *et al.*[37] they found that the lncRNA, small nucleolar RNA host gene 1 (*SNHG1*), enhances progression of laryngeal cancer in cell proliferation by modulating Notch1 pathway [35].

In glioma, overexpression of Notch receptors occurred, and activation of the Notch pathway plays a critical oncogenic role by contributing to brain tumours' growth, survival, invasion and recurrence [51]. The most frequent somatically mutated gene in more than 2900 tumors; Endometrial cancer (n = 248), prostate cancer (n = 112), large intestine (n = 224), esophageal cancer (n = 146), lung cancer (n = 230), glioblastoma (n = 291), ovarian cancer (n = 316), skin cancer (n = 121), liver cancer (n = 231), pancreatic cancer (n = 99), breast cancer (n = 507) and renal cancer (n = 424) are as follows; Notch-1 (7.3%), Notch-2 (7.0%), Notch-3 (5.9%) and Notch-4 (5.6%) [52]. Research by Wang *et al.* (2018) reported that upregulation of

prostate cancer-up-regulated long non-coding RNA 1 (PlncRNA-1) might promote glioma development and progression by activating the Notch pathway through modulation of several Notch signal-related protein expressions, including Notch-1, Jagged 1 and HES family bHLH transcription factor 1 (Hes-1) [48]. As for the correlation between lncRNA *ZFAS1* and Notch pathway in glioma, Gao *et al.* [18] discovered the inhibition of *ZFAS1* significantly reduced the expression of two Notch signal-relate proteins, Hes-1 and notch intracellular domain (NICD) [18], where both proteins have crucial roles in Notch pathway [53]. This result suggested that *ZFAS1* could be another key activator for the Notch pathway, facilitating glioma progression. However, the exact mechanism by which *ZFAS1* activates the Notch pathway is still unknown, whether directly or indirectly [13].

The general outcome of ZFAS1 upregulation in glioma cells is depicted in Figure 2. Figure 3 shows how high ZFAS1 expression in gliomas can affect the activities of glioma cells, with miRNA sponging inhibiting cell apoptosis and cisplastin cytotoxicity, and decreasing patient survival. Additionally, it summarises the impact of ZFAS1 expression on glioma cell metabolisms. For instance, it increases glucose uptake by glioma cells, increases cell migration, invasion, and proliferation, and increases resistance to chemotherapy drugs like TMZ. Furthermore, by regulating the expression of a protein that's essential to the development of gliomas, high ZFAS1 expression can promote the development of gliomas through the EMT and Notch signalling pathways.

Limitations

There are some limitations to this scoping review. Most studies used the same knockdown approach and technology, which was the RNAi silencing technique. As a consequence, there may be some bias and a lack of variability in the study samples. In line with the advancement in biotechnology, the researcher might apply the deep sequencing technique, such as next generation sequencing (NGS) platform to explore novel RNA targets that interact with *ZFAS1* in gliomagenesis [54]. Besides, CRISPR-CAS9 is also a good technology for genome editing as it is faster, cheaper, more accurate, and more efficient than other genome editing methods to study functional regulatory actions by *ZFAS1* . The study included in this review used small sample sizes, for example just 15 patients were included to characterize *ZFAS1* expression in glioma grade III and IV, while only 10 patients for grade I and II glioma. Furthermore, the study did not analyze the other potential miRNA, like miR-124-3p, miR-186 and miR-146b-5p, and *ZFAS1* related target genes in gliomagenesis, as example p53. Finally, there were very few original articles on *ZFAS1* and glioma compared to other cancer types indicating that further studies using different cell lines such as U343-MG and LN18 for molecular analysis and uncovering novel cancer pathways including Phosphatase and tensin homolog (*PTEN*) since *PTEN* mutations are strongly associated with shorter survival in glioma patients, implying that *PTEN* status strongly correlates with patient prognosis and *PTEN* is a prominent tumor suppressor [55] or Receptor Tyrosine Kinase (RTK) as RTK is well-known in glioma initiation and progression as it serve as docking sites for cytoplasmic signaling effectors once phosphorylated. However, in cancer, RTKs are abnormally activated and thus contribute to the oncogenic phenotype by (1) encouraging cancer cell overproduction of growth factors; (2) overexpression and/or amplification of the RTK itself, enabling hypersensitivity to low ligand concentrations; (3) acquiring mutations in their ligand-binding or kinase domains; (4) fusion of kinase domains with motifs of other, unrelated proteins; or (5) chromosomal translocation, giving rise to a chimeric product with enhanced kinase activity [56].

Conclusion

With the technological advance of next-generation sequencing and bioinformatic analysis, lncRNAs have been identified as potential oncogenes or tumor suppressor genes that play important roles in tumorigenesis. *ZFAS1* , a newly discovered lncRNA, was highly upregulated in glioma. The aberrant expression of lncRNAs *ZFAS1*in glioma contributes to a better understanding of the molecular mechanisms and pathways in glioma as lncRNAs have now been demonstrated to be a critical cellular regulatory network in a variety of cancers, including glioma. In short, amplified *ZFAS1* was found to be significantly associated with a variety of clinicopathological features and prognoses, including TNM stage, lymph node metastasis, and overall survival. *In vitro* , *ZFAS1* knockdown inhibited cell proliferation, cell migration and invasion, and

promoted cell apoptosis in glioma, implying that *ZFAS1* played a role in tumorigenesis and progression. *ZFAS1* molecular mechanisms implicated in gliomagenesis, such as competing endogenous RNA (ceRNA) and sponging for certain microRNAs, have been studied. These mechanisms can affect *ZFAS1* gene expression and the signalling protein in gliomagenesis. Furthermore, *ZFAS1* is involved in several signalling pathways that lead to gliomagenesis, such as the EMT and Notch signalling pathways. Each mechanism or pathway is not independent, but rather interacts with others. Overall, a deep understanding of *ZFAS1* mechanisms in glioma is crucial for better innovation, targeted therapies, and translation of novel anticancer treatments to precision medicine which may improve the clinical outcomes. Future studies to produce further uncover the mechanism of *ZFAS1* are necessary to enhance the prognosis of glioma patients.

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Authors' contributions

NAM and AAR have made contribution on the draft and the major contributor on writing the manuscript. MFMU contribute in discussion. SHAK, KI and NJO contribute in reviewing and revising the grammatical error. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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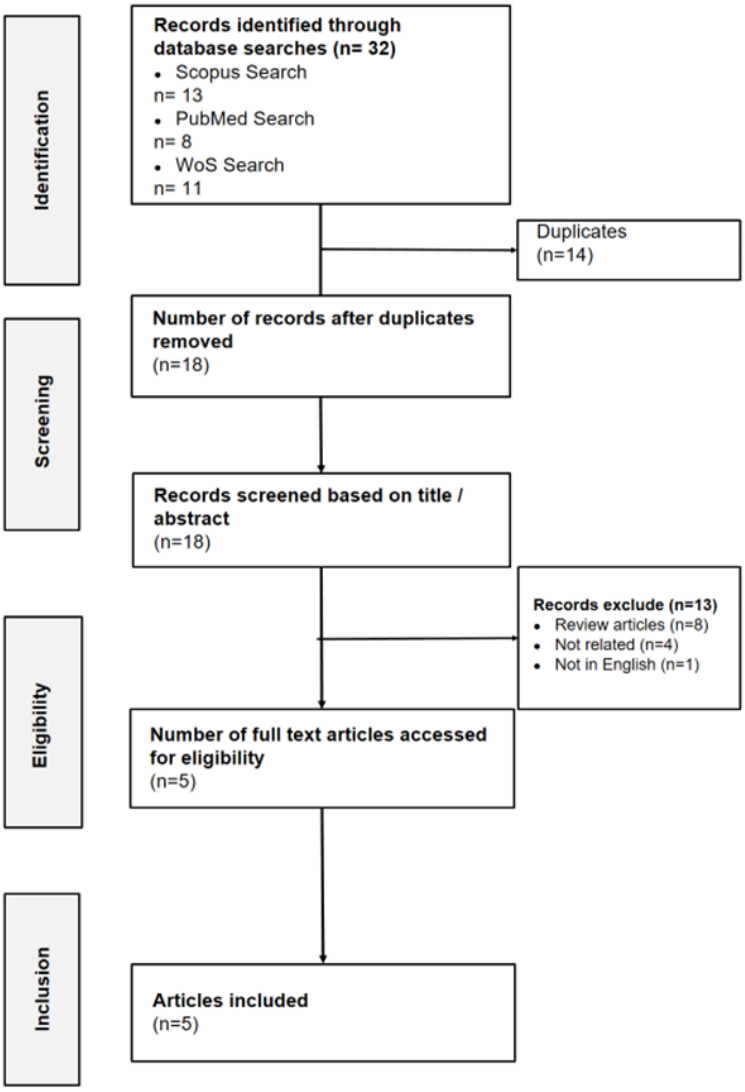

Fig. 1 Article selection process

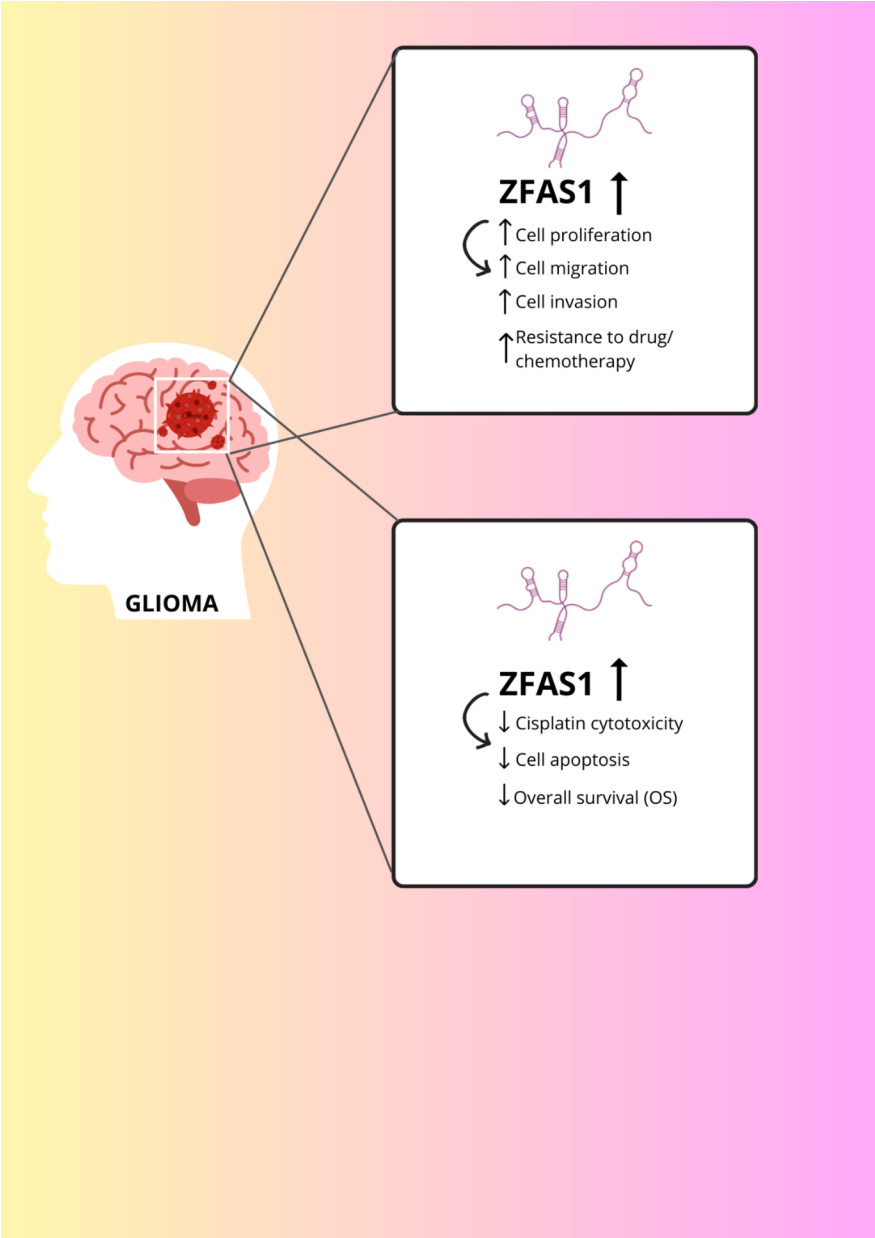

Fig. 2 Common consequences of high ZFAS1 expression in glioma cell

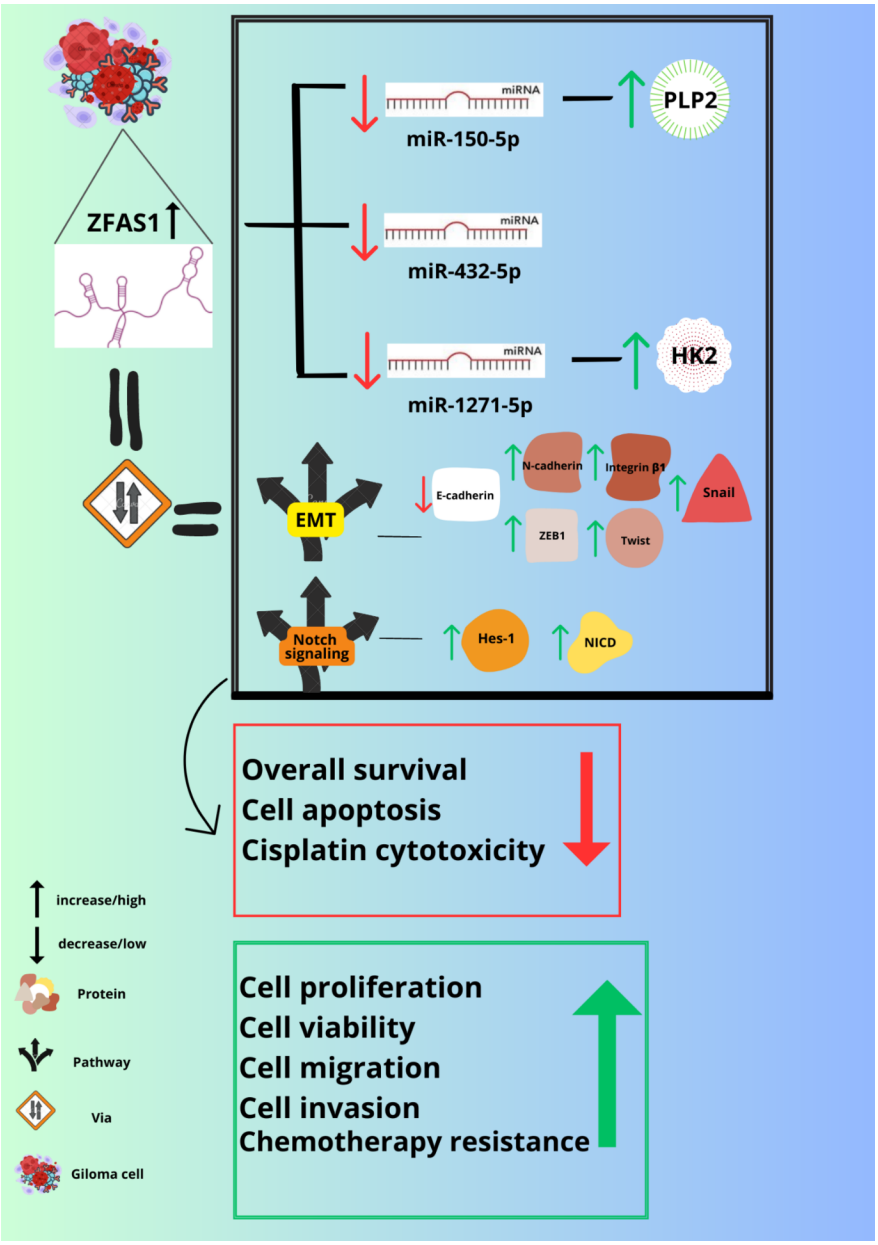

Fig. 3 The interactions between miRNA and upregulated ZFAS1, and their effect on protein expression and activities in glioma cell

Table 1 Summary of findings on the mechanism of action of*ZFAS1* in glioma cancer

References	Experimental Model	Major Findings
Zhang *et al.*[17]	This study aims to determine the role of ZFAS1 in the regulation of miR-1271-5p/HK2. *In vitro* model: U251, T98G, A172, LN229 and HS683 glioma cell lines *In vivo* model: Xenograft nude mice injected with U251 cells transfected with lentivirus vectors containing sh*ZFAS1.* Human Glioma tissues (n=59) from Tongling People's Hospital, China: Grade I (n=11), Grade II (n=22), Grade III (n=17) and Grade IV (n=9)	Human glioma samples Grades III and IV have higher expression of ZFAS1 than glioma grade I and II. U251 and T98G cell lines have the highest ZFAS1 expression compared to A172, LN229 and HS683. Si-ZFAS1 of U251 and T98G cell lines decreased cell proliferation, while increasing apoptosis and inhibiting cell invasion. Similarly, silencing of ZFAS1 reduced tumor growth in xenograft nude mice. miR-1271-5p expression which was lower in glioma tissues and glioma cell lines compared to normal brain tissues and NHA, were increased in si-ZFAS1 transfected cells than in si-NC transfected cells. ZFAS1 increased the expression of Hexokinase 2 (HK2) in glioma cells by targeting miR-1271-5p. This is evident where ZFAS1 inhibition suppressed HK2 expression, and the suppressive effect could be eliminated by combining it with a miR-1271-5p inhibitor. *ZFAS1* promoted the development of glioma through regulating miR-1271-5p/HK2 axis by acting as the miR-1271-5p sponge to increase HK2 expression.

References	Experimental Model	Major Findings
Li *et al.*[57]	This study aims to determine the role of ZFAS1 in regulating miR1505p / PLP2. *In vitro* model: U87, U251, LN229, and T98G cell lines *In vivo* model: Xenograft model-U87 cells expressing sh-*ZFAS1* were injected into the mice. Human glioma tissues (n=27) from Nanjing First Hospital, China: Grade II (n=7), Grade III (n= 10), Grade IV (n=10)	ZFAS1 expression is highest in grade IV gliomas. U87 and U251 cell lines have higher *ZFAS1* expression than T98G and LN229 cell lines. Knockdown of *ZFAS1* increased the susceptibility of U87 and U251 to temozolomide. miR-150-5p expression was lower in glioma tissues than in normal brain tissues and was shown to be negatively correlated to *ZFAS1* expression in glioma tissue. *ZFAS1* promoted proliferation, migration and invasion and increased resistance to temozolomide in glioma cells by sponging miR-150-5p to regulate Proteolipid protein 2 (PLP2). *ZFAS1* knockdown reduced PLP2 expression, and the inhibitory effect was partially reversed by miR-150-5p inhibitors. sh-ZFAS1 significantly inhibited tumour growth in xenograft nude mice as evident in the reduction of average volume and weight of the tumours.

References	Experimental Model	Major Findings
Yang *et al.*[36]	This study aims to determine the regulation of miR-432-5p by ZFAS1. *In vitro* model: U87, U251, A172 and LN299 glioma cell lines Human glioma tissue (n=25) from Shanxian Central Hospital, China: Lower grade glioma; I and II (n=10), High-grade glioma; III and IV (n=15)	U251 and LN229 cells have the highest *ZFAS1* expression compared to U87 and A172. High ZFAS1 expression was correlated with advanced tumor stage and shorter overall survival in glioma. miR-432-5p expression was down-regulated in grade III-IV gliomas compared to grade I-II gliomas. Similarly, miR-432-5p expression was decreased in glioma cell lines. miR-432-5p was suggested to be a target of *ZFAS1*, where silencing of ZFAS1 increases the miR-432-5p expression, reduced cell viability, improves cisplatin cytotoxicity while increasing the apoptosis of U251 and LN229 cells. This is further confirmed by the downregulation of miR4325p, which lowered the effects of ZFAS1 knockdown on cisplatin cytotoxicity and cell viability in glioma cells.

References	Experimental Model	Major Findings
Lv *et al.*[19]	This study aims to elucidate the molecular mechanisms of *ZFAS1* in regulating the epithelial–mesenchymal transition (EMT) in glioma cancer. *In vitro* model: HS683, T98G, U87, and U251 human glioma cell lines Human glioma tissues (n=69) from First Affiliated Hospital of Nanchang University, China: Astrocytomas, n=45; oligodendrogliomas, n=7; ependymomas, n=3; choroid plexus tumours, n=2, and others, n=8	Patients with high ZFAS1 expression exhibited a lower overall survival time than patients with low ZFAS1 expression, suggesting that ZFAS1 overexpression may serve as an independent prognostic marker for glioma patients. *ZFAS1* expression level was higher in high grade glioblastoma cell lines (T98G, U87, and U251) compared with the grade glioma cell line (HS683) Si-ZFAS1 suppressed cell proliferation, decreased colony formation, induced apoptosis, impeded cell migration, and cell invasion in U87 and U251 cells. ZFAS-1 silencing increased the G0/G1 phase of the cell cycle while decreasing the S phase. ZFAS1 was proposed to enhance glioma migration and invasion by promoting EMT progression. This is demonstrated by the negative correlation between the level of E-cadherin and ZFAS1 expression, as opposed to other EMT markers, N-cadherin, Integrin β1, ZEB1, and Twist, which are associated with ZFAS1 expression.
Gao *et al.*[18]	This study aims to determine the regulation of epithelial–mesenchymal transition (EMT) and Notch signaling pathway by ZFAS1. *In vitro* model: U87 and U251 glioma cell lines Human glioma tissues (n=46) from Affiliated Hospital of Hebei University of Engineering, China.	*ZFAS1* expression was upregulated in high grade (III–IV) glioma compared to low grade (I–II). *ZFAS1* inhibition suppressed the cells proliferation, migration and invasion, and induced apoptosis in U87 and U251 cells. ZFAS1 was found to promote glioma development via the EMT and Notch signalling pathways, where the expression of EMT-related proteins, E-cadherin, was increased and the expression of proteins N-cadherin and Snail was decreased in si-ZFAS1 transfected glioma cells, while Notch signal-related proteins Hes-1 and NICD were decreased.