RESEARCH ARTICLE

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LncRNA CASC2 Inhibits Progression of Glioblastoma by Regulating the Expression of *AKT* in T98G Cell Line, Treated by TMZ and Thiosemicarbazone Complex

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Abstract

Background: The aim of this study was to evaluate the expression alterations of *CACS2* and its target gene, *AKT,* in T98G cell line treated with Temozolomide and Thiosemicarbazone complex (Ni, Cu) and to compare the results with each other. **Methods:** Temozolomide and Thiosemicarbazone complexes were prepared in different concentrations. Cell culturing of T98G cell line was carried out and was classified into 3 groups based on the incubation time (24, 48, and 72h) with utilized agents, after RNA extraction the expression level of *CACS2* and *AKT* genes were evaluated by Real-time PCR. Ultimately, the results were analyzed by Rest software. **Results:** CASC2 expression under Temozolomide treatment at different concentrations (100, 150, 200, and 250 μM) and different time periods (24, 48, and 72h) was increased. Moreover, its expression was significantly upregulated after treating with Ni at the concentrations of 100.5 and 104 μM after 24h. Furthermore, its expression was augmented after 72 h Cu treatment at the concentrations of 15, 16, 17, and 18 μM. In addition, *AKT* expression after Temozolomide and Thiosemicarbazone complex treatment was significantly decreased ($P < 0.001$). The results showed that the expression alterations of *CASC2* and its target gene, *AKT*, after treatment with Temozolomide and Thiosemicarbazone are highly depended on incubation time and concentration. **Conclusion:** In a conclusion, the studied agents at different concentrations and times showed a high potential to control the expression of the studied *lncRNA* and gene in glioblastoma cells.

Keywords: Glioblastoma- temozolomide- thiosemicarbazone

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Introduction

Glioma is a general word for primary brain tumors and can be categorized into different groups such as astrocytic-related malignancies (Astrocytoma, Glioblastoma and Anaplastic astrocytoma), oligondroglioma, mixed glioma and ependymoma. They are the most prevalent type of central nervous system (CNS) malignancy, accounting for around 80% of all primary malignant brain tumors (Hanif et al., 2017) . More than 60% of all brain tumors in adulthood are caused by glioblastoma. Despite several GBM therapies, the condition remains deadly. Patients generally live for 14 to 15 months after receiving this diagnosis. The locations of the various forms of brain tumors vary, but hemispheres of the brain are the most critical locations for GBM malignancy. 95 percent of these cancers develop in the upper brain area, and there is only minor brain tumors in the cerebellum, brainstem, and spinal cord (Nakada et al., 2011) .

Members of the phosphatidylinositol 3-kinase (PI3K) family like lipid kinases are engaged in a variety of signaling pathways which can regulate proliferation, differentiation, migration, trafficking, and glucose homeostasis in glioma cells. In the EGFR/PI3K signaling pathways, *AKT* is a key participant. *AKT* appears to have an essential role in tumor growth and radio sensitivity, according to evidences (Robinson et al., 2011). Perifosine, one of the most promising *AKT* inhibitors, acts by preventing *AKT* from translocating to the cell membrane. Perifosine is now being investigated in clinical trials for a variety of malignancy treatments (Ghobrial et al., 2010; Yap et al., 2008) .

Patients with glioblastoma can be distinguished from cancer-free people by their *lncRNA* expression levels (Xie et al., 2019). Remarkably, the brain expresses almost 40% of all *lncRNAs,* indicating the brain's cellular and functional complexity (Derrien et al., 2012). In glioblastoma cells, deregulation of numerous *lncRNAs* has also been discovered, resulting in aberrant regulation of cancer-related pathways and cellular functions such as apoptosis, proliferation, and survival. In contrast, numerous research have looked at the relationship between *lncRNA* expression levels and TMZ resistance (Rezaei et

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al., 2021) .

As for the first time, cancer susceptibility candidate 2 (*CASC2*) *lncRNA* was known as a tumor suppressor non-coding RNA in human endometrial cancer (Yu et al., 2018). *CASC2* generates three alternative transcripts: *CASC2*a, *CASC2*b, and *CASC2*c, and in fact, it is the *lncRNA CASC2*a that functions as a tumor suppressor in malignancies like glioma (Palmieri et al., 2017) (Palmieri et al., 2017). *lncRNA CASC2*, on the other hand, is elevated in glioma and promotes carcinogenesis. *CASC2*a and *CASC2*c are both derived from *CASC2*, but only share the first three exons and have different downstream exons. *CASC2*a inhibits glioma development, whereas *CASC2*c functions as an onco-RNA (Liu et al., 2017) . It has been demonstrated that the mediated prohibition of glioma growth, migration, invasion, and cell apoptosis by *CASC2*a is linked to miR-21 dysregulation. By functioning like a miR-101 decoy sponge, *CASC2*c promotes astrocytomic carcinogenesis (Junyang et al., 2018). Moreover, by inhibiting miR-181a expression, *lncRNA CASC2* can inhibit the *AKT*/PI3k signaling pathway. *AKT* activation is one of the most frequent molecular cancer changes (Bellacosa et al., 2005). The *AKT* pathway is thought to be a significant cancer therapy target. Overexpression of *CASC2* can also inhibit cancer development by suppressing EIF4A3 expression and blocking the PI3K/*AKT*/mammalian target of rapamycin (mTOR) pathway (Zhang et al., 2018).

One chemotherapy medication for treatment of glioblastoma is an alkylating agent, termed Temozolomide (TMZ), which can convert imidazole 4 Carboxamide into active 5-(3-methyltriazen 1-yl) metabolite, causing cell cycle arrests and eventually cell death by destroying double DNA strands (Lee, 2017). TMZ transforms into the active metabolite 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide by non-enzymatic chemical transformation, which reason cell cycle arrestment and finally cell death by breaking the DNA double strands (Lee , 2017; Thomas et al.,2017). Even in older glioblastoma patients, the TMZ can be tolerated (Behm et al.,2013). TMZ, which is an alkylating agent with the ability in causing DNA damage in the malignant cells, is increasing median overall survival from 12.1 to 14.6 months in normal postoperative radiation therapy (60 Gy.30 fractions) (Stupp et al., 2005). Thiosemicarbazone's anticancer effects were merely once ascribed to the inhibition of ribonucleotide reductase, a rate-limiting enzyme engaged in the synthesis of DNA. Thiosemicarbazone's capacity to chelate metal ions is now recognized as the primary factor in its anti-proliferative actions. Thiosemicarbazone complexes' redox activity is crucial to their anticancer function, causing oxidative damage and blocking ribonucleotide reductase's activity. In vivo studies show that many Thiosemicarbazones have the potential to be used as chemotherapeutic agents (Kalinowski et al., 2009). The coordination of metal ions such as Cu, Ni, Zn, and Pd can improve the cytotoxic actions of these ligands, which can also increase their lipophilicity and mechanism of action within the cell (Jayakumar et al., 2014).

The primary goal of this study was to examine at the altered expression of the *AKT* gene and *CASC2 lncRNA* after they were treated with the chemotherapeutic drug TMZ and the Ni, Cu complex of Thiosemicarbazones. Furthermore, we investigated the therapeutic efficiency of utilized agents in different incubation times and concentrations. In addition, the effect of TMZ and Thiosemicarbazones complexes of Ni and Cu on changing the expression levels of *AKT* gene and *CASC2 lncRNA* was compared. The relationship between expression alterations of *AKT* and *CASC2* with each other (whether the increased expression level of *CASC2* at the same time and concentrations can terminate to decreased expression level of *AKT* or not) was examined. Last but not least, the optimal time and effective dosage in which utilized agents could alter the expression level of *AKT* and *CASC2* was determined.

Materials and Methods

The primary goal of this study was to examine at the altered expression of the *AKT* gene and *CASC2 lncRNA* after they were treated with the chemotherapeutic drug TMZ and the Ni, Cu complex of Thiosemicarbazones. This study conducted from April to September 2019 in Zanjan Islamic Azad University Research Center for Science. Human glioblastoma cell line T98G was prepared from the Pasteur Institute of Iran in the first cellular passage and at a density of approximately 80% of cells (2×10^5 cell.cm^2).

T98G Glioblastoma Cell line and cell culture

Cell culturing, passaging, cell counting and preparation of different concentrations of the abovementioned drugs were performed. Human Glioblastoma cell line, T98G was first cultured in Dulbecco's Modified Eagle's Medium (RPMI 90%) medium containing 10% FBS, followed by incubation at 37 °C containing 5% carbon dioxide for six days. Subsequently, three passages were applied to the cells at two consecutive intervals and at each passage period, the resulting cells were transmitted into the flask with fresh culture medium. Finally, the fourth cell passages were selected for next investigations. The aggregated cells were counted by trypan blue staining method. After cell counting, the number of cells were 3×10^4 cells per cm^2. Subsequently, the cells were divided into two groups, including the control and under treatment groups.

TMZ and Thiosemicarbazone complex (Ni, Cu) preparation and drug treatment

In order to prepare the TMZ drug, first 9cc sterile distilled water with 1.5cc DMSO were mixed and 105 μM of this mixture was obtained and 1 tablet of TMZ was dissolved in it. To construct Ni stock, 000.1 g of Ni was dissolved in 1 cc of water. For preparing Cu exactly the same amount of utilized Ni was dissolved in 1 cc of distilled water, and then effective doses of the drugs (TMZ and Thiosemicarbazone complex Ni, Cu) were examined by MTT test.

The detailed information about their concentration and incubation time for prepared drugs is listed in Table 1.

RNA extraction and cDNA synthesis

Following cell treatment according to the groups

listed above, RNA extraction and cDNA synthesis were carried out. After treating the cells with the afore-stated time points (24, 48, 72), in order to evaluate expression of the studied genes after and before treatment, the RNA Extraction (by RNA – Roche-High Pure Nucleic Acid Kit (Gmbh, Manheim, Germany, cat.no 11858874001)) and cDNA synthesis process (Takara Bio, Otsu, Japan, cat.no RR037Q) were performed according to the kit instructions.

Compounds needed for final 10µl volume was contained

5X Primer Script Buffer (1X) (2µl), Primer script RT enzyme Mix1 (0.5µl), Oligo dT (0.5µl), Random hexamers (0.5µl), Total RNA (3.5µl), RNase free dH2O (3µl). The final prepared solution was mixed and cycled for 15 minutes at 37 ° C and then 8-16 seconds at 85 °C and RT-PCR, respectively.

Quantitative real-Time PCR (Q- PCR) and RNA expression profiling

The below-mentioned steps and compounds are required for Real-Time PCR process which were prepared for CACS2 *lncRNA* and the *AKT* target gene: The FASTA sequence of the cDNA library of genes were extracted from the NCBI website. Primer3 software was used for primer designing procedure. Finally, using the software mentioned above, the results were blasted on the NCBI website to confirm specificity and primer binding to all three studied genes. The reactions were adjusted at 25 µl volume. 12.5µl of Cyber Green (1X), 1 µl of forward and reverse primers (0.4µM), 2µl of cDNA, and 8.5µl of deionized water were mixed. The first denaturation schedule was at 95 °C for 10 min and DNA fragments were amplified in 40 cycles (denaturation of 95 °C for five seconds, 51.8 annealing, 30 seconds for the *AKT*, and, *lncRNA CASC2*, and final extension 72 °C, 30 seconds.). Using Rotor-Gene Q real-time PCR cycler (Qiagen, Hilden, Germany) and (Takara Bio, Otsu, Japan, Cat # RR820Q) Sybergreen kit instruction, CACS2 *lncRNA* and *AKT* and *GAPDH* target genes as reference genes were inserted and amplified in real-time PCR. The primer sequences used for the CACS2 *lncRNA*, *AKT* and *GAPDH* target gene is listed in Table 2.

Gene Expression Confirmation and Statistical Analysis

To confirm the presence of the studied genes, PCR products were electrophoresed on 2% agarose gel and the length of the fragments were equal to 125 bp, sequenced and confirmed by Fan Avaran Gene Company (Ebrahimi et al., 2022). After reaction, the difference of target gene CT to the reference gene was calculated as Δct for each sample. Then, 2ΔΔct (fold change) was obtained for each sample. To investigate the expression of CACS2 *lncRNA* and *AKT* target gene by Real Time PCR, LIVAK method (Livak method) and Rest (2002) were used. P <0.05 was considered as the accepted cutoff criteria of significance rate.

Results

Expression changes of lncRNA CASC2 treated with TMZ and Thiosemicarbazone complexes (Cu, Ni)

CASC2 expression in Glioblastoma cells after 24 h treatment with TMZ at different concentrations of 100, 150, 200, and 250µM, showed increased expression level with the rates of (2.41), (2.75), (2.268) and (5.934) respectively. This gene's expression level was also increased after 48h treatment and at the abovementioned concentrations showed (1.304), (1.899), (1.219) and (2.406) expression change level rates. 72 h treatment of the cell line through the same concentrations also showed increased change levels by the rates of (4.075), (1.264), (2.383), and (3.373) respectively (P<0.001) (Figure 1)

The expression changes levels of *CASC2* treated with Thiosemicarbazone is summarized at Table 3 and Figure 2, 3.

Expression changes of AKT treated with TMZ and Thiosemicarbazone complex (Cu, Ni)

AKT gene expression was significantly reduced in

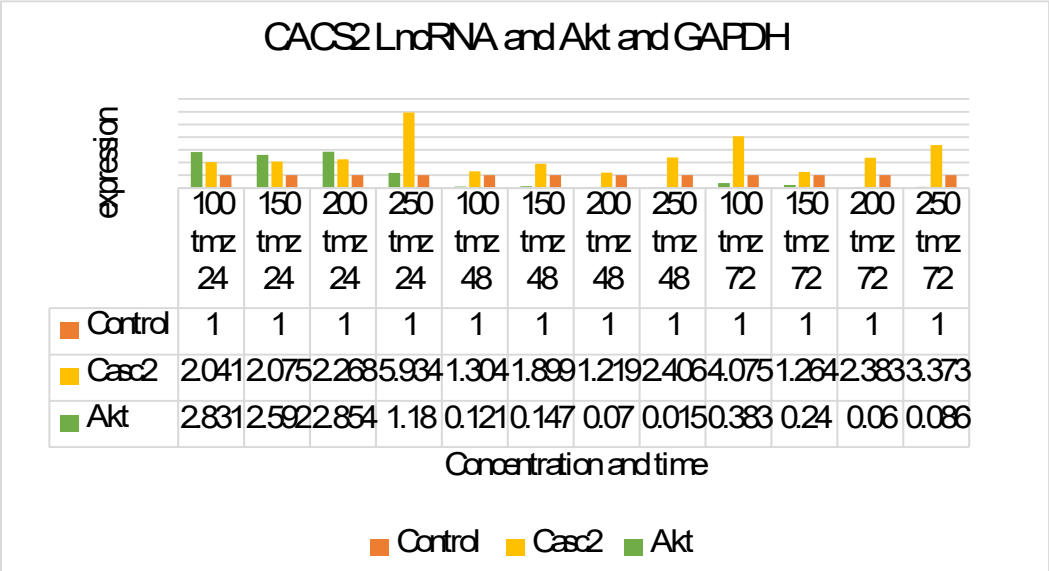

Figure 1. Results of CACS2 LncRNA and AKT and *GAPDH* Reference Gene Expression Treated with TMZ.

Table 1. Drug Names, Incubation Time and Concentrations

Drug name	Incubation time	Concentrations
Temozolomide	24, 48, and 72 h	100, 150, 200, and 250 µM
Thiosemicarbazone complex (Cu)	24 h	17.5, 20, 22.5, and 25 µM
Thiosemicarbazone complex (Cu)	48 h	15.5, 17, 18.5, and 20 µM
Thiosemicarbazone complex (Cu)	72 h	15, 16, 17, and 18 µM
Thiosemicarbazone complex (Ni)	24 h	100.5, 104, 107.5, and111 µM
Thiosemicarbazone complex (Ni)	48 h	57, 61, 65, and 69 µM
Thiosemicarbazone complex (Ni)	72 h	46, 48, 50, and 52 µM

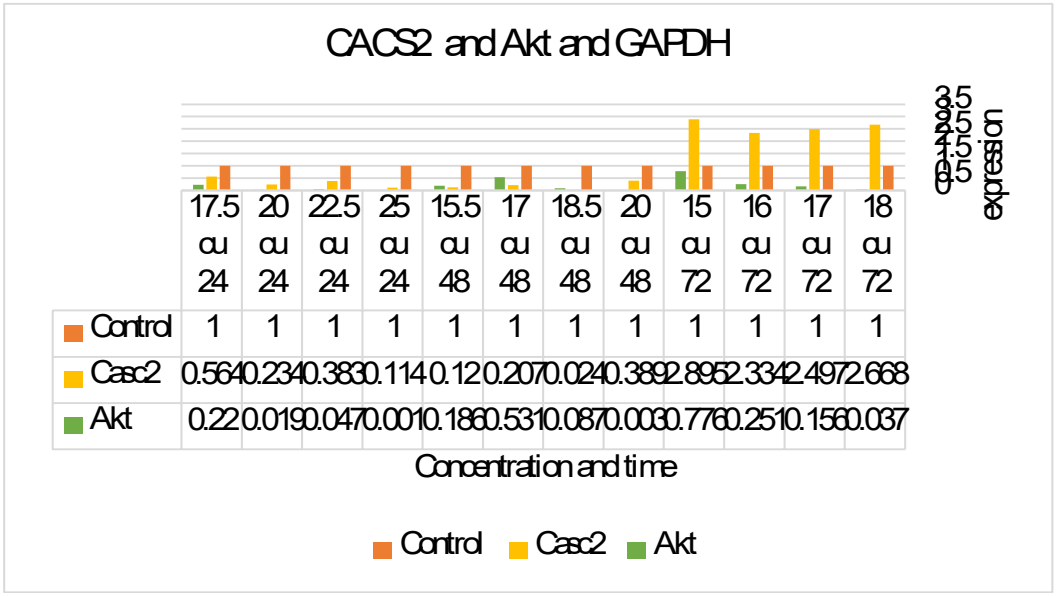

Figure 2. Results of CACS2 LncRNA and AKT and *GAPDH* Reference Gene Expression Treated with Thiosemicar Bazone Complex (Cu).

glioblastoma cells treated with TMZ after 48 hours at all doses of 100 µM (0.121), 150 µM (0.147), 200 µM (0.07), and 250 µM (0.015). After 72 hours, all doses of 100 µM (0.383), 150 µM (0.24), 200 µM (0.06), and 250 µM showed a substantial reduction in expression too (0.086) (P<0.001).

At all doses, *AKT* expression was significantly reduced by Thiosemicarbazone complex Cu after 24, 48, and 72-hour treatment. At 24h, the reduction in expression was 17.5 µM (0.22), 20 µM (0.019), 22.5 µM (0.047), and 25 µM (0.001). At 48h, the values were 15.5 µM (0.186), 17 µM (0.531), 18.5 µ M (0.087), and 20 µM (0.003), whereas at 72h, the values were 15 µM (0.776), 16 µM (0.251), 17 µM (0.156), and 18 µM (0.37), respectively (P0.001). *AKT* was significantly reduced in expression after 48 hours of treatment with Thiosemicarbazone complex (Ni) at all investigated doses of 57 µM (0.001), 61 µM (0), 65 µM (0.005), and 69 µM (0). After 72-hour treatment, these expression changes were 46 µM (0.539), 48 µM (0.33), 50 µM (0.362), and 52 µM (0.76). (P<0.001) (Figures 1,2,3).

Results of changes in CASC2 and AKT expression at common times and concentrations treated with TMZ and Thiosemicarbazone complex (Cu, Ni)

CASC2 and *AKT* expression alterations were significant after 48 hours of treatment with TMZ at doses of 100 µM (1.304), 150 µM (1.899), 200 µM (1.219), 250 µM (2.406), and 100 µM (0.121), 150 µM (0.147), 200 µM (0.07), and 250 µM (0.015). In addition, following 72 hours of cell line treatment, a substantial increase in expression was found at all doses of 100 µM (4.075), 150 µM (1.264), 200 µM (2.383), and 250 µM (3.373). After 72 hours, the expression of *AKT* reduced in all concentrations of 100 µM (0.383), 150 µM (0.24), 200 µM (0.06), and 250 µM. (0.086). (P<0.001).

CASC2 expression increased significantly 72 hours after Cu treatment at all investigated doses of 15 µM

Table 2. Primer Sequences of *CASC2, AKT* and *GAPDH* Genes

Name	Forward sequence	Reverse sequence
CASC2	5'-GCACATTGGACGGTGTTTCC-3'	5'-CCCAGTCCTTCACAGGTCAC-3'
AKT	5'-TCTATGGCGCTGAGATTGTG-3'	5'-CTTAATGTGCCCGTCCTTGT-3'
GAPDH	5'-TCTTCACCACCATGGAGAAGGCT-3'	5'-CATGCCAGTGAGCTTCCCGTTCA-3'

Table 3. Gene Expression Alternation of CASC2, AKT and *GAPDH* Genes under Treatment with Thiosemicarbasone (Ni and Cu) and TMZ, in Different Concentrations and Times.

Drug name	Gene/ LncRNA	Concentration	Incubation time	Expression change level
Thiosemicarbazone (Cu)	CASC2	17.5	24h	0.564
		20	24h	234
		22.5	24h	0.383
		25	24h	0.114
		15.5	48h	0.12
		17	48h	0.207
		18.5	48h	0.024
		20	48h	0.389
		15	72h	2.895
		16	72h	2.334
		17	72h	2.497
		18	72h	2.668
Thiosemicarbazone (Ni)	CASC2	100.5	24h	2.283
		104	24h	2.634
		107.5	24h	0.067
		111	24h	0.037
		57	48h	0.616
		61	48h	0.051
		65	48h	0.121
		69	48h	0.001
		46	72h	0.465
		48	72h	0.032
		50	72h	0.665
		52	72h	0.654
TMZ	CASC2	100	24h	2.041
		150	24h	2.075
		200	24h	2.268
		250	24h	5.934
		100	48h	1.304
		150	48h	1.899
		200	48h	1.219
		250	48h	2.406
		100	72h	4.075
		150	72h	1.264
		200	72h	2.383
		250	72h	3.373
Thiosemicarbazone (Cu)	AKT	17.5	24h	0.22
		20	24h	0.019
		22.5	24h	0.047
		25	24h	0.001
		15.5	48h	0.186
		17	48h	0.531
		18.5	48h	0.087
		20	48h	0.003
		15	72h	0.776
		16	72h	0.251
		17	72h	0.156
		18	72h	0.037
Thiosemicarbazone (Ni)	AKT	100.5	24h	4.452
		104	24h	3.9
		107.5	24h	3.669

Table 3. Continued

Drug name	Gene/ LncRNA	Concentration	Incubation time	Expression change level
Thiosemicarbazone (Ni)		111	24h	4.335
		57	48h	0.001
		61	48h	0
		65	48h	0.005
		69	48h	0
		46	72h	0.539
		48	72h	0.33
		50	72h	0.362
		52	72h	0.755
TMZ	AKT	100	24h	2.831
		150	24h	2.592
		200	24h	2.854
		250	24h	1.18
		100	48h	0.121
		150	48h	0.147
		200	48h	0.07
		250	48h	0.015
		100	72h	0.383
		150	72h	0.24
		200	72h	0.06
		250	72h	0.086

(2.895), 16 µM (2.334), 17 µM (2.497), and 18 µM (2.668). Moreover, the expression of *AKT* reduced after 72 hours in all concentrations: 15 µM (0.776), 16 µM (0.251), 17 µM (0.156), and 18 µM (0.37). ($P<0.001$). At standard durations and concentrations, there was no increase in *CASC2* expression and no decrease in *AKT* expression when treated with Ni.

Discussion

Gliomas constitute the most common primary brain tumors and the most lethal central nervous system cancers (Chen et al., 2013; Shen et al., 2019). Many studies have found a relationship between *lncRNA*s and glioma. A research, discovered that a variety of *lncRNAs* are implicated in the beginning of glioma (Li et al., 2019). The goal of this work was to evaluate the expression alteration of *lncRNA CASC2* in glioma cell line impacted by TMZ and Thiosemicarbazone Complexes.

In the present study, we showed that the alteration in the expression of *lncRNA CASC2* and *AKT* after TMZ and Thiosemicarbazone complex of Cu/Ni treatment differs with each other (Figure 4). We also demonstrated that the Thiosemicarbazone complex Cu had the largest effect on boosting *lncRNA CASC2* expression in contrast to TMZ and performed better and less effectively than TMZ. In addition, Ni, when compared to TMZ, had an impact at lower concentrations and for a shorter period of time. The comparison of the effects of TMZ, Ni, and Cu on *AKT* expression revealed that the Thiosemicarbazones complex Cu had the highest effect on increasing *AKT* expression in comparison with TMZ and performed better at lower concentrations and faster time than the TMZ medication.

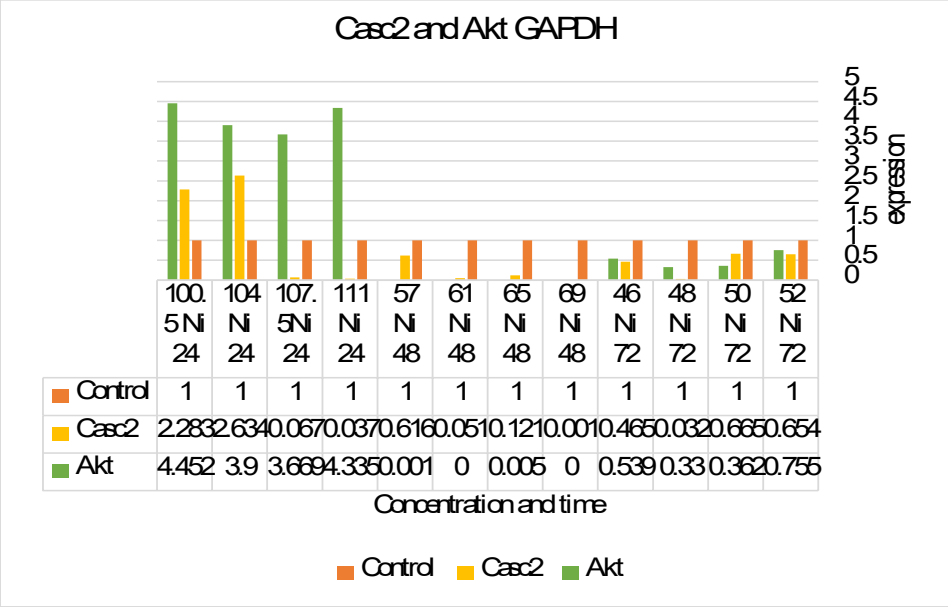

Figure 3. Results of CACS2 LncRNA and AKT and *GAPDH* Reference Gene Expression Treated with ThiosemicarBazone Complex (Ni).

Ni also needed lower concentrations and a shorter half-life than TMZ. As a result, it can be concluded that the Ni and Cu were more potent than TMZ in changing *AKT* expression.

CASC2 and mir21 *lncRNA*s have been found to have an inhibitory impact on each other's expression in studies. *CASC2* promotes gene transcription and cell survival by decreasing mir21 expression via the mir21 / PTEN / *AKT* / mTOR axis and the NF- κB signaling pathway (Feng et al., 2017). Furthermore, *CASC2* inhibits the mir-18 / PIAS3 / STAT3 axis, which promotes cell survival (Huang et al., 2016). *CASC2*, on the other hand, promotes cell death by suppressing mir21 expression via the pdcd4/p21 axis. Controlling variations in *CASC2* expression and its connection to *AKT* expression via several pathways might therefore influence Glioblastoma development.

CASC2 expression was shown to be significantly lower in cisplatin-resistant NSCLC tissues and cell lines, according to XIAO and colleagues in 2020. MiR-18a, which was upregulated as *CASC2* expression decreased, lowered the protein content of IRF-2 in NSCLC cells. Furthermore, the transcription factor ELF1 was discovered to be a *CASC2* promoter and to enhance its expression levels in cisplatin-resistant NSCLC cells (S-YH, 2020). Zhu & al. reported in 2019 that *CASC2* over-expression improved the cisplatin's inhibitory impact on cell viability and increased the release and apoptosis of cisplatin-induced LDH. They discovered

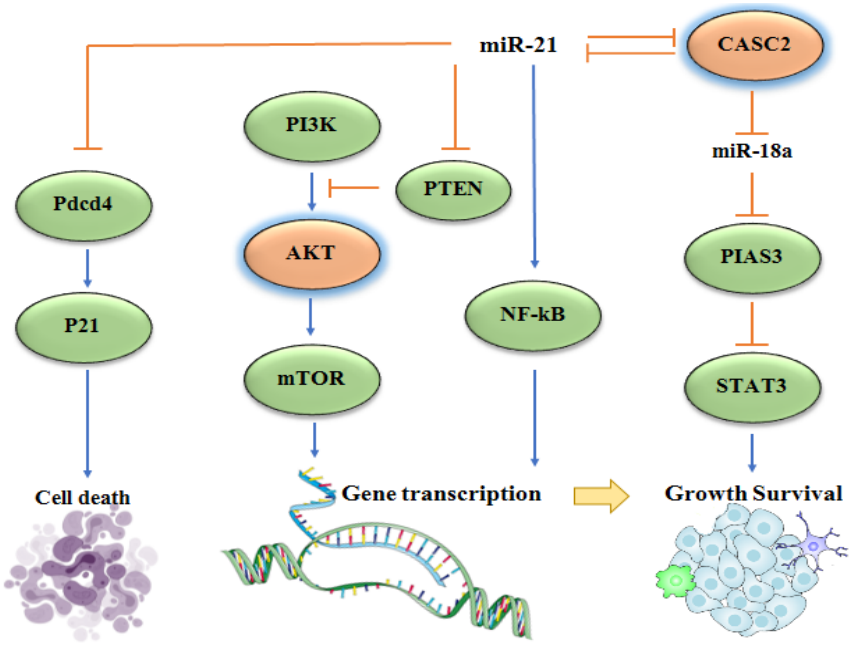

Figure 4. Relationship between *LncRNA CACS2* and *AKT* Gene with PI3K/AKT/mTOR Signaling Pathway in Glioblastoma

that *CASC2* inhibited the *AKT* pathway via suppressing miR-181a expression (Dengyan et al ., 2019) . In 2018, Jin and coworkers discovered a *CASC2*/miR-24/miR-221 axis that influences TRAIL resistance in hepatocellular carcinoma (Xiaoxin et al., 2018). Wang and colleagues stated in 2017 that they were researching *CASC2* RNA as a glioma suppressor in the Wnt/-catenin signaling pathway. *CASC2* overexpression significantly reduced glioma cell proliferation, migration, and invasion by inhibiting the Wnt/-catenin signaling pathway (Ronglin et al., 2017) . Their findings suggest that activating the *AKT*/mTOR pathway inhibits cisplatin-induced apoptosis, resulting in cisplatin resistance. As a result, their findings show that cisplatin resistance in human ovarian cancer cells can be reversed by targeting the *AKT*/mTOR survival pathway (Peng et al., 2010). In 2005, Li and colleagues published a study and demonstrated that MCF7, MDA468 and T47D cells show a drug-dose-dependent rise in phosphorylated *AKT* levels within 24 hours after doxorubicin treatment. Phosphorylation of *AKT* by doxorubicin was linked to enhanced kinase activity and was reliant on phosphoinositide 3-kinase (PI3-K) (Xinqun et al., 2005) .

The results of a study on the changes in the expression of the *CASC2* tumor suppressor *lncRNA* and the *AKT* oncogene treated with the mentioned drugs, as well as a comparison of the findings with previous studies, revealed the importance of the *AKT* oncogene in the PI3K/*AKT* signaling pathway and changes in the expression of the *CASC2* tumor suppressor *lncRNA* in proliferation and apoptosis of all cancer types. The findings highlight the significance of increasing in the expression of the *AKT* oncogene and decreasing the expression of the *CASC2* tumor suppressor gene in glioblastoma patients treated with Thiosemicarbazones complex Ni and Cu. We discovered that these drug compounds had a positive effect on changes in the expression of these genes.

Finally, *lncRNA CASC2* is one of the key role players involved in tumor suppression in glioblastoma. In terms of effectiveness and influence on changes in the expression of the investigated genes, evidence revealed that the Thiosemicarbazones complex Ni and Cu, as well as the standard medication TMZ, depended on time and concentration. Increases in the duration and concentration of the cell line's treatment with the indicated medicines, on the other hand, resulted in significant alterations in the expression of the *AKT* gene. In compared to the control groups, the outcomes of the medication combinations exhibited two types of rising and lowering impacts. *CASC2* tumor suppressor *lncRNA* and *AKT* oncogenes expression changes had a beneficial influence on regulating the expression of the genes examined. These alterations suggested that the investigated pharmacological compounds had a non-random and focused influence on gene expression changes. These effects might vary depending on the concentration and duration. Therefore, the studied drugs, including Thiosemicarbazone complex Cu and Ni, had good potential to control changes in the expression of the studied genes, which confirms the effectiveness of these compounds in controlling changes in gene expression due to mutations, treatment, and reduction of glioblastoma progression associated with changes in the expression of suppressor and oncogene genes including *CASC2* and *AKT*.

Author Contribution Statement

Study concept and design: G. A.T; Acquisition of data: Sh. Sh; Analysis and interpretation of data: G. A.T; Drafting of the manuscript: N. Z; Critical revision of the manuscript for important intellectual content: S.M, G. A.T; Administrative, technical, and material support: Sh. Sh.

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Ethics

The present study was approved by the institutional ethics committee (IR.IAU.Z.REC.1399.038).

Conflict of Interest

The authors declare that they have no conflict of interest.

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