

Characterization of ALDH1A1 Positive Cancer Stem Cells in Glioblastoma Multiforme and Their Response to Temozolomide

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Abstract: Glioblastoma multiforme (GBM) is a type of primary brain cancer that is associated with high malignancy and cellular diversity, along with a poor response to traditional treatment approaches. The function of ALDH1A1 has been linked with stem cell-like properties and drug resistance in various types of cancers. This research focuses on studying the stem cell-like properties and sensitivity to temozolomide (TMZ) of ALDH1A1-positive groups of cells in GBM cell lines. Human GBM cell lines, including U87-MG and U251, were grown and divided into ALDH1A1-positive and ALDH1A1-negative fractions by ALDEFLUOR test and flow cytometry. TMZ resistance was determined by means of the MTT test and apoptosis assay. ALDH1A1-positive cells comprised about $33.2 \pm X\%$ of the examined sample ($n = X$). As compared to ALDH1A1-negative cells, ALDH1A1-positive cells displayed increased ability to form spheres, high expression levels of stem-like markers, and decreased susceptibility to the effects of TMZ. At 48 hours of exposure to TMZ, cells expressing ALDH1A1 had a higher level of viability compared to those not expressing it. This shows a relationship between ALDH1A1 and the stem-cell characteristics of the GBM cells. There is a need for further research in this field with the use of patient samples and manipulation of ALDH1A1.

Keywords: Glioblastoma Multiforme, ALDH1A1, Cancer Stem Cells, Temozolomide Resistance, Tumor Heterogeneity, Stemness Markers, Targeted Cancer Therapy.

1. INTRODUCTION

Glioblastoma multiforme (GBM) is considered the most common primary brain tumor, which is characterized by rapid progression, heterogeneity, and poor response to available treatments. GBM is under the focus of numerous researches and is being currently treated through the maximal possible surgical resection and subsequent radiotherapy and temozolomide chemotherapy; however, the tumor tends to recur because of its complexity. The tumor microenvironment in case of GBM is represented by a number of various cells that have diverse proliferative properties and response to available treatment options, which leads to the difficulties of treating GBM. Despite the number of different treatment options for GBM, such as surgical resection, radiotherapy, and

temozolomide chemotherapy, this tumor is frequently recurring because of its complexity [1].

Cancer stem cells (CSCs) are a specialized category of tumor cells that can self-renew, differentiate, and sustain tumor propagation. The cells are thought to mediate tumor formation and prolonged tumor survival in numerous cancers, such as glioblastoma [2]. CSCs exhibit high resistance to traditional therapies due to increased DNA repair, active drug efflux systems, and adaptive metabolic pathways in GBM. These properties allow CSCs to resist chemotherapy and radiation therapy, which later results in tumor relapse. Several molecular markers have been reported to associate with glioblastoma CSC populations, including CD133, SOX2, and certain aldehyde dehydrogenase (ALDH) enzymes. Experimental studies have also shown that CSC-related transcriptional pathways prevent tumor growth and its maintenance through stem-like properties in glioblastoma cells [3].

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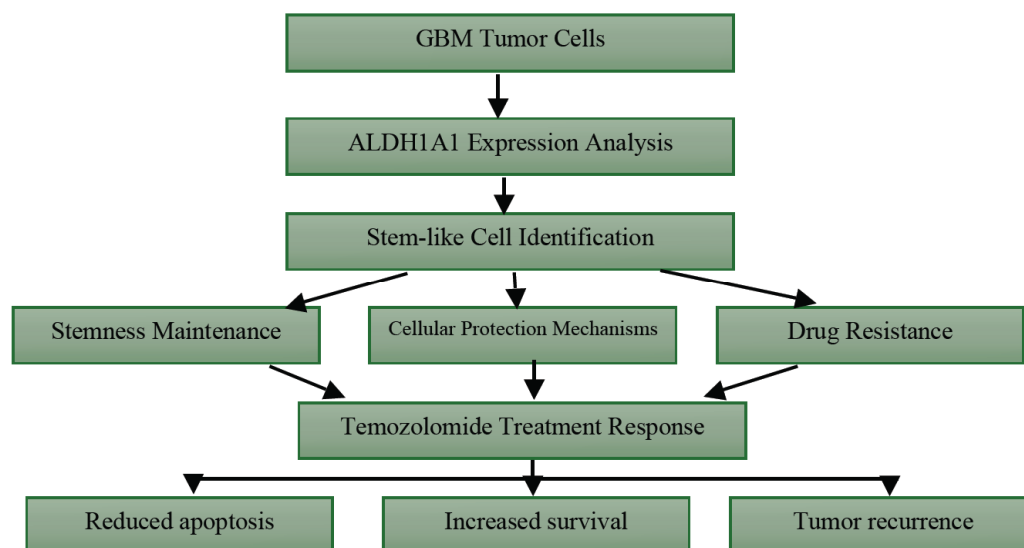


Figure 1: Conceptual Model of ALDH1A1-Enriched Stem-like Characteristics and TMZ Response in Glioblastoma.

Figure 1 presents a conceptual model explaining how ALDH1A1 expression affects chemoresistance in glioblastoma cells. The procedure starts with analyzing GBM tumor cells for ALDH1A1 expression, which allows identification of cancer stem cell populations in the tumor. Some of the biological processes triggered by these stem-like cells include stemness maintenance, cellular protective mechanisms, and drug resistance pathways. This helps the cells become more resistant to the stress imposed by temozolomide therapy. Therefore, the incidence of cell death is rare and the survival rate is high in such resistant cells, which eventually results in tumor recurrence and further disease progression. This can be used to explain the assumed connection between the overexpression of ALDH1A1 and the stem cell-like features and sensitivity to TMZ.

Aldehyde dehydrogenases (ALDH) are important in terms of detoxification, oxidative stress, and retinoic acid signaling. The ALDH1A1 variant has proved to be a critical functional marker for stem-like phenotype in tumor cells and a sign of increased tumorigenicity. Elevated activity of ALDH enzymes has been reported in many cancer types and is often linked with the aggressiveness of tumors and drug resistance [4]. Processes related to ALDH metabolism contribute to tumor cell migration, proliferation, and microenvironment interaction in glioblastoma. Recent studies also suggest that ALDH-mediated signaling cascades can increase or decrease GBM cell sensitivity to temozolomide by regulating cell stress responses and survival. Since temozolomide remains the first-line chemotherapeutic agent for GBM, the relationship between ALDH-positive cancer stem cells

and chemotherapy response has become a significant field of inquiry [5]. Feature selection can help determine significant glioblastoma stem cell biomarkers [6].

Despite extensive research on CSC biomarkers for GBM, the role of ALDH1A1-enriched cells in stemness features and temozolomide susceptibility is poorly characterized. This paper examines the features of ALDH1A1-positive stem-like cells in glioblastoma and assesses their response to temozolomide therapy, thereby helping identify potential molecular targets to improve therapeutic efficacy and guide the development of therapeutic strategies.

The rest of this paper will be structured as follows. Section II is a review of the literature pertinent to the research on the following topics: glioblastoma cancer stem cells, ALDH-associated markers, and chemoresistance mechanisms. The experimental methods employed for the study are documented in section III and include cell cultures, detection of ALDH1A1 positive cells and temozolomide treatments. The analysis and outcomes of the experimental work conducted on stem cell attributes and chemotherapy reactions are explained in section IV. Section V highlights the significance of the findings from a biological perspective and explores the potential applications in the design of therapies. Section VI provides a summary of the conclusions along with potential avenues for future research.

2. LITERATURE REVIEW

Cancer stem cells (CSCs) have become an important aspect of tumors with regard to their formation and cancer pathogenesis in malignant

cancers, including glioblastoma [7]. These cells are self-renewable and able to differentiate, and they can keep the growth of the tumour and the ability to regenerate heterogeneous populations of tumours. Initial studies on the biology of CSCs have placed an emphasis on the connection between the cell-of-origin and the appearance of the stem-like cancer cell that persistently retains the malignant properties into later disease development [8]. Later studies have revealed that the CSC populations of tumors are not homogeneous but show a great deal of functional heterogeneity, which affects treatment response and disease outcome [9]. There are different experimental methods that have been devised to isolate and identify CSCs in brain tumors. Sphere-formation assays, fluorescence-activated cell sorting, and gene expression profiling are popular techniques that can be used to describe stem-like cells and assess their biological properties [10]. The approaches enable scientists to explore the molecular pathways that preserve stemness and tumor propagation. Clinical evidence suggests that tumors enriched with cancer stem cells (CSCs) exhibit aggressive growth, increased resistance to conventional therapies, and a higher risk of recurrence, highlighting the importance of targeting CSC populations to improve treatment efficacy and achieve durable therapeutic outcomes [11].

The aldehyde dehydrogenase enzymes among the biomarkers related to cancer stem cells have received a lot of attention due to their effects on detoxification and metabolism of cells. Increased expression of isoforms of ALDH1 has been observed in various cancers and often correlates with stem-like phenotypes and increased cancerogenicity [12]. Increased cell survival and resistance of tumor cells to programmed cell death are also associated with high ALDH activity and can be used to offer functional understanding of the metabolic activity of stem-like cells in comparison to other CSC markers such as CD133 [13]. The molecular signatures of tumor progression and immune interactions with the tumor microenvironment have been demonstrated to be affected by specific molecular signatures of CSC-related genes [14]. The results lend credence to the fact that the ALDH-related markers could be useful in assessing the aggressive tumor behavior and as a therapeutic target. Research on the tumor microenvironment investigates the resistance mechanisms of glioblastoma cancer stem cells [15]. Uniform clinical data systems help assess the treatment response in glioblastoma research [16].

One of the greatest challenges to the effective treatment of glioblastoma is resistance to

chemotherapy. The most common chemotherapy drug applied in the treatment of GBM is temozolomide (TMZ); nevertheless, this drug is eventually resisted by most tumors. The CSC populations cause this resistance by different means, which include increased DNA repair, disturbed cell cycle re-establishment, and the activation of survival signaling pathways [11]. Other molecular regulators, such as CD133-dependent pathways, have been demonstrated to mediate resistance to chemotherapy and radiotherapy in cancer stem cells [17]. Other cellular pathways, like autophagy and non-coding RNA regulation, participate in the survival of CSC populations and their survival to therapeutic stress [18]. Other signaling pathways, such as the p38 MAP kinase cascade, enhance maintenance of stem cells and metastatic capacities, which enhances the resilience of the tumor against chemotherapy [19]. All these mechanisms prove the fact that CSC-related pathways are a significant factor in mediating therapeutic resistance in glioblastoma.

The literature reviewed shows that the populations of cancer stem cells play a powerful role in the progression of glioblastoma and its resistance to treatment. Past research has established that CSCs have distinctive biological features that enable them to maintain tumor cell growth, avoid cell death, and overcome chemotherapy. The enzymes related to ALDH are some of the biomarkers most commonly associated with CSCs and have been extensively associated with the stem-like nature as well as tumor aggressiveness. Moreover, several pathways associated with autophagy, cell cycles, and signaling via kinases also cause CSCs' resistance to temozolomide therapy. These results suggest that the characterization and the discovery of ALDH-related stem cancer cells would be valuable in the understanding of the biology of glioblastoma and in the development of specific therapeutic approaches that would overcome chemoresistance.

3. METHODOLOGY

3.1. Cell Culture and Identification of ALDH1A1-Enriched Populations

In order to examine ALDH1A1-related stem cell characteristics and temozolomide sensitivity *in vitro*, human glioblastoma cell lines, U87-MG and U251, were selected. Cultivation of cells was carried out in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin at 37°C and 5% CO₂.

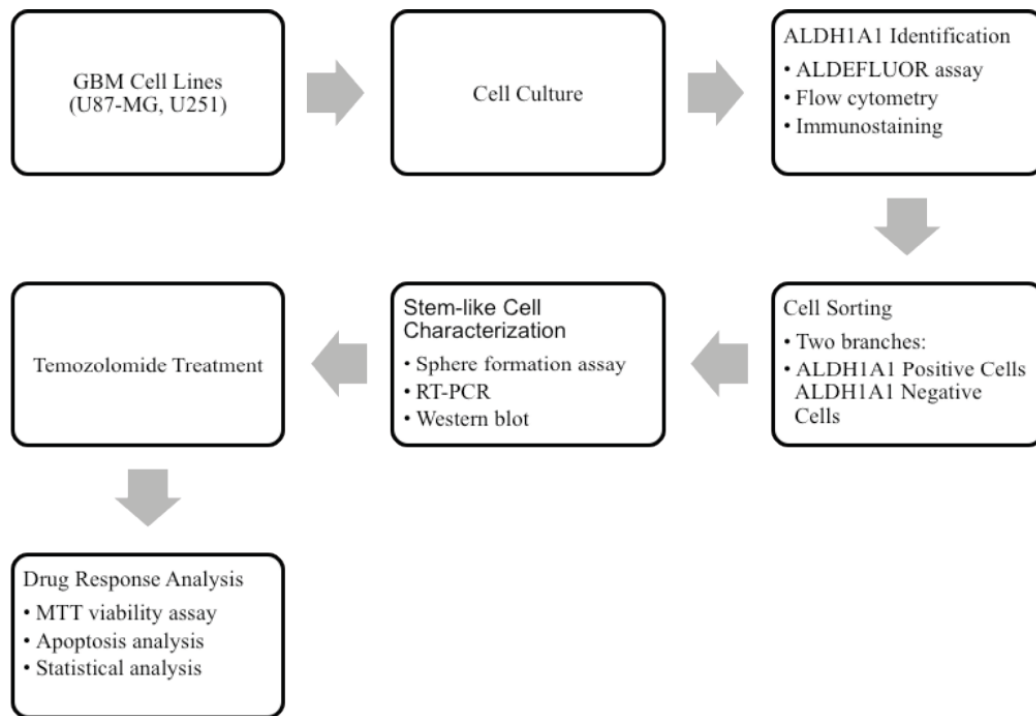


Figure 2: Experimental Workflow for Identification and Drug Response Analysis of ALDH1A1-Positive Glioblastoma Cells.

Using the fluorescence intensities, the cells were classified into ALDH1A1 positive and ALDH1A1 negative fractions. The negative controls were obtained through DEAB and employed for the determination of the activity of ALDH.

Figure 2 illustrates the experimental process that was used to test the nature and drug reaction of ALDH1A1-positive glioblastoma cells. GBM cell line cultures (U87-MG and U251) were the first step of the investigation and further the ALDH1A1-positive cells were detected using ALDEFLUOR test, flow cytometry, and immunostaining procedures. After the detection stage, the sorting procedure was undertaken to isolate ALDH1A1 positive and negative cell populations. Further, the populations of cells underwent cancer stem cell analysis by performing spheres' formation, RT-PCR, and Western blot analyses. Then, the cells were exposed to temozolomide treatment and tested for differences in chemotherapy sensitivity through the MTT cell viability test and apoptosis test.

Table 1 is a presentation of the culture and experiment conditions involved in growing U87-MG and U251 glioblastoma cell lines in order to establish the cell populations that are positive for ALDH. It gives an account of the culture medium, the antibiotic, the incubation condition, the ALDEFLUOR test, flow cytometry cell sorting, and immunofluorescence validation.

Table 1: Experimental Parameters and Conditions of Cell Culture

Parameter	Description
Cell Lines	U87-MG, U251
Culture Medium	DMEM supplemented with 10% FBS
Antibiotics	1% Penicillin–Streptomycin
Incubation Conditions	37°C, 5% CO ₂
Subculture Confluence	~80%
ALDH Detection Method	ALDEFLUOR assay
Cell Sorting Technique	Flow Cytometry
Confirmation Method	Immunofluorescence staining

3.2. Characterization of the Cancer Stem Cell Properties

A sphere formation assay was done to assess the stem-like properties of ALDH1A1-positive cells in serum-free culture conditions. The sorted cells were seeded in ultra-low attachment plates using a stem cell medium that was supplemented with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). Formation of the neurospheres was followed over a period of 7-10 days, and the efficiency of sphere formation was determined to assess the self-renewal capacity of the cells.

RT-PCR and Western blotting were used to study stemness markers based on expression. Both

Table 2: Markers and Analysis Techniques of Stemness

Marker	Biological Role	Detection Method
SOX2	Maintains stem cell self-renewal	RT-PCR, Western Blot
NANOG	Regulates pluripotency and stemness	RT-PCR
OCT4	Controls stem cell differentiation potential	RT-PCR, Western Blot
ALDH1A1	Functional marker of cancer stem cells	ALDEFLUOR Assay, Immunostaining

ALDH1A1-positive and ALDH1A1-negative populations were extracted for RNA and proteins. The relative expression levels of the significant stemness genes such as SOX2, NANOG and OCT4 were examined to identify the molecular phenotypes of the cancer stem cells. Further experiments were done to determine the proliferation and differentiation capability. Growth rates were measured using cell proliferation assays, and differentiation assays were done by cultivating cells in serum-containing medium to examine morphological and marker alterations.

Table 2 shows the essential markers of stemness that were investigated in this study and how they were analyzed to determine their levels in glioblastoma cells. The SOX2, NANOG, and OCT4 markers were chosen because they have been previously shown to preserve the self-renewal and pluripotency of cancer stem cells, and the primary functional marker used to determine the stem-like tumor population was ALDH1A1 [20]. Methods such as RT-PCR, Western blotting, and immunostaining were used to test the level of expression of these markers and to determine the features of the molecular structure of cancer stem cells' behavior.

Experiments were conducted using three biological replicates. Biological replicates were measured using technical replicates wherever required. In flow cytometry experiment, roughly 10,000-12,000 cells were measured per biological replicate.

3.3. Analysis of Temozolomide Treatment and Drug Response

Temozolomide (TMZ) was used to assess chemotherapy response by treating the ALDH1A1-positive cell population and the ALDH1A1-negative cell population. Cells were grown in 96-well plates, and the cells were exposed to TMZ doses varying between 25 μ M and 200 μ M for 48 hours. The major point of interest was the percentage of viable cells following TMZ treatment, whereas the rate of apoptosis served as a secondary endpoint. As controls, untreated cells were used. Viability of the cells after exposure to drugs was determined by the MTT assay, which measures

mitochondrial metabolic activity and hence is a measure of cell survival. The percentages of cell viability were determined against untreated controls, and the absorbance values were read with the help of a microplate reader. The analysis of apoptosis was conducted through the staining of Annexin V with FITC and propidium iodide and flow cytometry. In this way, it was possible to determine early apoptotic, late apoptotic, and viable cell populations. The findings gave an overview of the chemoresistance profiles of ALDH1A1-positive stem-like cells and their contribution to glioblastoma response to therapy.

3.4. Dataset Details

The data in this research were composed of experimental findings in the U87-MG and U251 glioblastoma cell lines in a culture medium. During the flow cytometry, the number of cells analyzed was about 10,000- 12,000 per sample, and the experiment was repeated in three biological replicates. The parameters used in the dataset were the values of fluorescence intensity, the number of spheres, cell viability measurement, and the percentage of apoptosis of both treated and untreated groups.

3.5. Software Details

The flow cytometric data were analyzed using FlowJo software (version 10.8.1; BD Biosciences, Franklin Lakes, NJ, USA). The statistical analysis and data visualization were performed using GraphPad Prism (version 9.5.1; GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics (version 28.0; IBM Corp., Armonk, NY, USA). Image analysis of sphere formation and immunofluorescence was conducted using ImageJ/Fiji software (version 1.53c; National Institutes of Health, Bethesda, MD, USA).

4. RESULTS

4.1. Identification of ALDH1A1 Positive Cell Population

From the analysis of glioblastoma cell culture, it was revealed that there is a subpopulation of cells with high

Table 3: Efficiency of Sphere Formation and Stemness Marker Expression in Populations of ALDH1A1 Cells

Parameter	ALDH1A1 Positive Cells	ALDH1A1 Negative Cells
Sphere Formation Efficiency	46%	18%
SOX2 Expression Level	High	Moderate
NANOG Expression Level	High	Low
OCT4 Expression Level	Elevated	Low
Relative Growth Rate	1.6×	Baseline

ALDH1A1 activity. The separation of the ALDH1A1 positive cells and tumor cell population in general became possible because of the use of flow cytometry in the ALDEFLUOR assay. It was revealed that there is a fraction of about 31-35% of all GBM cells with high activity of the enzyme ALDH. This proves the presence of the cells which are enriched in ALDH1A1 and can be considered as stem cells. The specificity of the test was clearly proven by the diagram of the intensity of fluorescence of ALDH1A1 positive cells and control cells treated with the ALDH inhibitor. In the course of phase contrast microscopy and morphological analysis, it was found that there are significant structural differences between the two groups of cells. ALDH1A1 positive cells have the morphology of small rounded cells which are able to aggregate in neurospheres. Conversely, the ALDH1A1-negative cells had a more flattened and differentiated morphology, as seen with adherent glioblastoma cells. These observations were further confirmed by immunofluorescence staining that revealed positive cytoplasmic expression of ALDH1A1 in the stem-like population of cells.

4.2. Stem-Like Characteristics of ALDH1A1-Positive Cells

Sphere formation assays were conducted under serum-free conditions in order to identify whether the ALDH1A1-positive cells had cancer stem cell properties or not. The findings showed that neurospheres formed by the ALDH1A1-positive cells were much more efficient than those formed by the ALDH1A1-negative cells. The efficiency of modulation of sphere formation in the positive population ranged to about 46%, and the efficiency of modulation in the negative population was about 18%, which implies improved self-renewal ability. Moreover, the stem-like characteristics of the ALDH1A1-positive cells were further demonstrated through molecular studies. For instance, markers of stemness like SOX2, NANOG, and OCT4 were present in relatively higher concentrations based on RT-PCR and Western blot analysis [7]. These have been widely recognized as

pluripotency and tumorigenicity controllers. The elevated expression of these factors showed that the ALDH1A1-positive cells were still maintaining certain transcriptional programs associated with stem cell functions and tumor growth.

Similarly, in the case of cell proliferation, it can be seen that the ALDH1A1-positive cells exhibited a higher rate of growth as compared to the negative group. These cells showed a 1.6 fold increase in the proliferation rate within 72 hours of observation.

The ability of the spheres to form, stemness-related marker expression, and growth features of the populations of ALDH1A1-positive and ALDH1A1-negative glioblastoma cells were compared in Table 3. The findings show that ALDH1A1-positive cells have a significantly higher sphere formation ability and show a high expression of major stemness markers like SOX2, NANOG, and OCT4. Moreover, the relative growth rate of the ALDH1A1-positive population was higher than that of the negative cells, which indicates a higher ability to proliferate and self-renew, which is also common to cancer stem cells.

4.3. Response of ALDH1A1 Positive and Negative Cells to Temozolomide

ALDH1A1-positive and ALDH1A1-negative populations were assessed in terms of their viability as an evaluation of the response of glioblastoma cells to temozolomide treatment. Cells were subjected to different concentrations of TMZ, and viability was assessed using an MTT assay after 48 hours of treatment. The findings showed a considerable decline in viability of the total tumor cell population; the decline was not as much in the case of the ALDH1A1-positive cells. Whereas a reduction in viability of the negative population by 52% was observed, the number of ALDH1A1-positive cells decreased by just 26%, implying that ALDH1A1-positive cells were less sensitive to TMZ treatment than ALDH1A1-negative cells. The analysis of apoptosis by the staining

Table 4: Cell Viability of ALDH1A1 Cell Populations after Treatment with Temozolomide

TMZ Concentration	ALDH1A1 Positive Viability	ALDH1A1 Negative Viability
25 µM	86%	72%
50 µM	78%	61%
100 µM	74%	48%
200 µM	64%	36%

Table 5: Rates of Apoptosis in Cell Populations Exposed to Chemotherapy of ALDH1A1

Cell Population	Early Apoptosis	Late Apoptosis	Total Apoptosis
ALDH1A1 Positive	9%	6%	15%
ALDH1A1 Negative	18%	14%	32%

procedure with Annexin V showed the same tendency. The percentage of the cell population that was undergoing apoptosis was significantly lower in the ALDH1A1-positive group in comparison to the negative group after treatment with TMZ. This finding indicates that the stem-like cells have some protective systems that prevent cell death under the influence of drugs.

Table 4 is a summary of the percentage of cell viability of ALDH1A1-positive and ALDH1A1-negative glioblastoma cells under the influence of rising levels of temozolomide. These data show that, despite the decrease in the viability of both populations under the influence of temozolomide treatment, ALDH1A1-positive cells show higher survival rates under all concentrations tested. This trend shows that there is increased chemotherapy toleration and indicates that the ALDH1A1- positive population is one that leads to drug resistance in glioblastoma.

Table 5 indicates the percentages of early apoptosis, late apoptosis, and total apoptotic cells in the ALDH1A1-positive and ALDH1A1-negative groups after treatment with temozolomide. These findings demonstrate that the overall apoptosis rate is greater in the ALDH1A1-negative cells than in the ALDH1A1-positive cells. This difference implies that the stem-like ALDH1A1-positive cell population is not as susceptible to cell death following treatment with chemotherapeutic agents as previously believed, thereby explaining the resistance to TMZ demonstrated by the cells.

4.4. Comparative Analysis of ALDH1A1-Positive and Negative Populations

According to the results of the comparative study, the ALDH1A1-positive cells exhibited better sphere formation capability, higher stemness marker

expression, greater proliferation capacity, and decreased sensitivity to TMZ than ALDH1A1-negative cells. Therefore, ALDH1A1 enrichment implies that the cancer cells possess stem cell-like properties and change their drug response profile. These cells show an augmented capacity for forming spheres, an amplified expression of stemness markers, and augmented proliferative ability. More to the point, the experiments conducted with TMZ treatment have shown that the ALDH1A1-positive group has a better survival rate and a reduced rate of apoptosis than the negative group. Such results suggest that ALDH1A1-positive cells play a great role in the cases of therapeutic resistance and tumor persistence in glioblastoma.

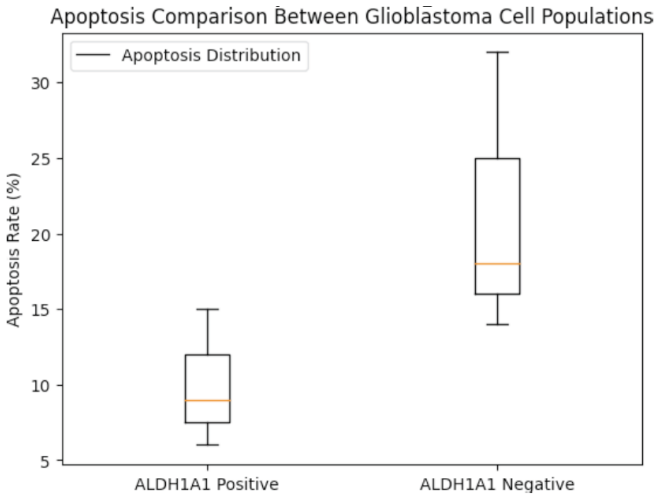


Figure 3: Comparison of the Apoptosis Rates of the Glioblastoma Cell Populations.

Figure 3 is a comparison of the rate of apoptosis in ALDH1A1-positive and ALDH1A1-negative glioblastoma cells following exposure to chemotherapy. The box plot reveals that the negative cell population has a

more extensive distribution and a higher median rate of apoptosis, and the cells of the ALDH1A1-positive group reveal lower levels of programmed cell death. This disparity implies that the ALDH1A1-positive group has superior survival systems that diminish chemotherapy-caused apoptosis.

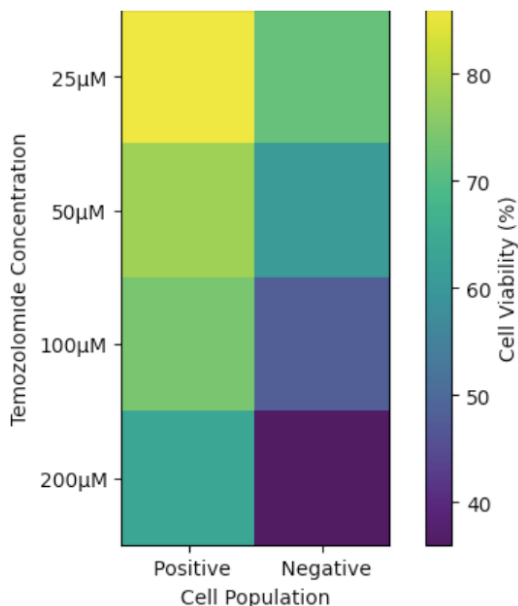


Figure 4: Response of Temozolomide Drug on Glioblastoma Cells.

Figure 4 demonstrates the change in cell viability of ALDH1A1-positive and ALDH1A1-negative glioblastoma cell with various concentrations of temozolomide. The heatmap shows that the level of drug concentration gradually decreases viability in both populations, but cells with ALDH1A1 are always characterized by a higher level of survival. This figure substantiates the finding that the stem-like cells associated with ALDH1A1 play a role in the development of chemoresistance to the treatment of glioblastoma.

4.5. Comparison of Sorted and Unsorted Cell Populations

Comparing TMZ responses in different cell states was achieved by conducting experiments in unsorted GBM cells, and also in the isolated positive and negative ALDH1A1 GBM cells. In a single setup, TMZ was administered on the entire population of tumor cells without cell sorting. In yet another setup, ALDH1A1-positive and negative populations had been pre-isolated and then treated. In comparison, the isolation of the cell populations gave a better understanding of the variation in drug sensitivity. The findings indicated that the overall outcomes of

treatment were strongly influenced by the presence of ALDH1A1-positive cells, and such cells play a role in chemoresistance, which is why it became important to consider targeting stem-like tumor cells in the future.

5. DISCUSSION

The current findings provide evidence for the link between ALDH1A1 enrichment and stem-like properties in glioblastoma cells and can be used to understand possible correlations of this protein with changes in therapy resistance. The detected proportion of ALDH1A1-positive cells [20] and their elevated capacity to form spheres indicate that this population has typical properties of stem-like tumor cells, such as high proliferative capacity and extended self-renewal potential. These characteristics are in line with the morphological variations and the varied high expression of the stemness markers (SOX2, NANOG, OCT4) [7, 20] that are observed in the course of the experimental analysis.

The decreased sensitivity towards TMZ exhibited by cells positive for ALDH1A1 could be connected to some of the roles of the ALDH enzymes described before. Namely, these enzymes are involved in the detoxification process and oxidative stress response. However, in the current research, the molecular pathways of this phenomenon have not been studied. The enzymes of the ALDH1A1 system are also known to be involved in aldehyde detoxification and redox regulation within cells [12], and it is believed that they counteract the action of cytotoxic agents and alleviate the effects of oxidation caused by drugs [1, 4]. This survival metabolic response may allow stem-like cells to survive exposure to DNA-damaging reagents like temozolomide [1, 11, 13]. Such resistant populations may be maintained as a result, and hence this may be one of the factors contributing to tumor recurrence after conventional treatment [1, 2, 11].

The above-noted data indicate that ALDH1A1-enriched cell populations might be one possible area to explore further in the future. Functional studies using ALDH1A1 pharmacological blockade or gene knockout are necessary to elucidate whether targeting this pathway can overcome TMZ resistance and sensitize resistant cells to chemotherapy [1, 4]. Furthermore, combining ALDH inhibitors with standard chemotherapy offers a promising strategy to reduce the survival benefit of cancer stem cells and inhibit tumor regeneration [1, 2, 11]. Further studies of the signaling pathways and metabolic networks that accompany the activity of ALDH can also contribute to the creation of more efficient targeted treatments [1, 9, 10].

6. LIMITATIONS

There are multiple shortcomings of this research. First of all, the experiments have been conducted on known GBM cell lines that may not reflect the variability found in patient-derived tumors. Second, the ALDH1A1 protein was used as an enrichment marker associated with stem-like properties, but no knockdown or inhibition of ALDH1A1 was done to functionally validate its role. Third, the mechanisms that can underlie the change in sensitivity to TMZ treatment have not been verified through experimental work.

7. CONCLUSION

This study aimed to assess the relationship between enrichment with ALDH1A1, stem-like features, and TMZ sensitivity in glioblastoma cell lines. It was found that the ALDH1A1-enriched subpopulations of the U87-MG and U251 cells displayed greater potential for sphere formation, higher levels of stem cell-specific markers such as SOX2, NANOG, and OCT4, and a stronger proliferation rate than the ALDH1A1-negative subpopulations. Additionally, the subpopulation enriched with ALDH1A1 was found to have greater resistance to TMZ, which was manifested in their higher cell viability and less apoptosis caused by the drug. The results of the study show that enrichment with ALDH1A1 is related to stem-like characteristics and sensitivity to TMZ. Nevertheless, ALDH1A1 can be regarded only as an associated marker. This particular pattern of response may rely on several biological processes, including adaptation and survival responses in the cells, and more research will have to be done to validate this finding. The results of this study contribute to our understanding of how populations enriched for ALDH1A1 expression influence heterogeneity and therapy response in glioblastomas. In order to establish the medical significance of the observed ALDH1A1-driven processes and their possible contribution to improving TMZ treatment, further research will have to be performed.

ETHICAL APPROVAL STATEMENT

The present study involved commercially available human glioblastoma cell lines (U87-MG and U251) and did not involve human participants, animal subjects, or primary clinical tissue samples. Therefore, formal institutional ethical approval was not required.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

All authors contributed to the conceptualization, experimental execution, data collection, and writing of this manuscript. BP, RNA, and PG conceived and designed the study. LM, BSK, TS, and RJ performed the experiments, data collection, and statistical analyses. All authors drafted, reviewed, and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Received on 08-08-2026

Accepted on 05-09-2026

Published on 30-09-2026

<https://doi.org/10.30683/1929-2279.2026.15.29>© 2026 Patel *et al.*; Licensee Neoplasia Research.

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