Silencing of MEG3 gene promoted anti-cancer activity and drug sensitivity in glioma

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Research Article

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Abstract

Aberrant expression of MEG3 has been shown in various cancers. The purpose of this study is to evaluate the effect of MEG3 on glioma cells including U87MG and patient-derived primary tumors cells and compare with nonglial HUVEC cells. Cell viability, migration and chemo sensitivity was assayed. Apoptosis was evaluated in MEG3 overexpressing and MEG3 suppressed cells. MEG3 expression was compared in patient-derived tumor cells with respect to IDH1 mutation status and WHO-grades. Silencing of MEG3 inhibited cell proliferation and reduced cell migration while overexpression of MEG3 promoted cell proliferation in glioma cells. MEG3 inhibition improved chemo sensitivity of glioma cells to 5-fluorouracil(5FU) but not to navitoclax. Suppression of MEG3 gene in patient-derived oligodendrogliaoma cells also showed the same effect whereas glioblastoma cell proliferation and chemo sensitivity were not affected by MEG3 inhibition. Further, cell death mechanism of action was found as apoptosis. Although MEG3 is a widely known tumor suppressor gene and its loss is associated with several cancer types, here we reported that MEG3 inhibition can be used in drug sensitivity for improving efficiency of known chemotherapeutics. We propose that the level of MEG3 should be evaluated in treatment of different glioma subtypes that are resistant to effective drugs.

Introduction

Treatment modalities for glioblastoma are limited due to the complex nature and malignant progression of the tumor. In recent years, efforts to develop novel therapeutic strategies for efficient treatments in glioma cells focused on inhibiting or activating essential regulatory genes (Costa et al. 2015; Gangemi et al. 2009). Understanding the role of non-coding genes in glioma may lead us to discover novel therapeutic targets. Non-coding genes make up the vast majority of the human genome. MicroRNAs (miRNA) and long non-coding RNAs (lncRNA) are expressed differently in cancer cells than healthy cells. Noncoding RNAs regulate the basic characteristics of cancer cells such as cell proliferation, survival, invasion and therapy resistance. The functions of lncRNAs emerge through epigenetic, transcriptional and post-transcriptional mechanisms which have not yet been fully understood. Understanding the role of lncRNAs in these mechanisms will help us in the treatment of glial tumors as well as in many types of cancer. (Zhang et al. 2017b; Mercer et al. 2009; Ponting et al. 2009)

Maternally Expressed Gene 3 (MEG3) is an imprinted gene residing in the chromosome 14 which also hosts long non-coding RNAs, microRNAs and nucleolar RNAs. Hence, it plays several vital roles in growth and development. A defect in the expression of MEG3 can lead to various diseases including cancer as a result of the disruption caused in the regulation of cellular proliferation and its putative role as tumor suppressor. Several studies showed that MEG3 played various roles in different cancer types. Overexpression of MEG3 was found to inhibit EMT and result in a reduction of the migration and invasion of cervical cancer cells. Whereas in hepatocellular carcinoma, MEG3 was found to contribute to the EMT phenotype and therefore increased the migration and invasion of the cancer cells. Its role may vary depending on the type of cancer. (Yang et al. 2020) The most prominent role of MEG3 in glioma is cell cycle regulation. MEG3 overexpression causes the cell cycle to stop in the G2/M phase, thus reducing the
proliferation of glioma cells. MEG3 can also upregulate tumor suppressors by interacting with regulatory miRNAs. This interaction is also required for the activation of p53. (Momtazmanesh and Rezaei 2021)

The precise role of MEG3 in gliomas is still not well understood due to confusing results in previous studies. The aim of this study is to discover the role of MEG3 in glioma cells and to investigate how MEG3 affects cell proliferation, migration and chemo sensitivity in glial tumors when overexpressed by the transfection of the MEG3 gene and suppressed by small interference RNA (siRNA). Furthermore, the effects of MEG3 gene expression were compared between non-cancerous human umbilical vein endothelial cord (HUVEC) cells and U87-MG glioblastoma cells either derived from ATCC or from patients with different pathological grades and histopathological subtypes of glioma tumors.

**Methods**

**Cell Culture**

U87MG and HUVEC cells were acquired from American-Type Culture Collection (ATCC, USA). Patient derived tumor tissues were obtained through surgical operation upon the consent of the patients and with approval from the institutional ethics board (BAU-2020/02). Primary tumor cells were incubated under the appropriate conditions and regularly passaged. Cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 1% antibiotic, antimycotic and 10% fetal bovine serum. Whereas primary cells were grown in DMEM mixed with nutrient mixture F-12 (DMEM/F-12) supplemented with 1% antibiotic, antimycotic and 10% fetal bovine serum. All cells were incubated in a humidified air incubator (5% CO$_2$) at 37°C.

**Cell Transfection**

The overexpression plasmid pCI-MEG3 (Addgene, catalog number 44727) which was generously gifted to Addgene by Yunli Zhou and colleagues was obtained (Zhou et al. 2007). BLOCK-iT™ RNAi Designer web tool (Thermo Fisher) was used to design siRNA against the mRNA of the MEG3 gene. The design was based on the transcript variant 1 of MEG3 and the MEG3 gene sequence was obtained from the NCBI Gene database. One of the three different siRNA sequences suggested by the BLOCK-iT™ RNAi Designer web tool was selected and ordered from Thermo Fisher. The MEG3 siRNA sense sequence was: CAUCAUCGUCCACCUCCUUGCUUU and the MEG3 siRNA antisense sequence was: AAGACAAGGAGGACGGAUGAUG. Cells were transfected with the MEG3 overexpression plasmid, which was introduced to the cells with the PEI chemical transfection method. Polyethylenimine (PEI) (1mg/ml), 500 µL of DMEM medium and 7,5µg of plasmid DNA were used for transfection. PEI was given the day after the cells were seeded and the cells were harvested for RNA and protein isolation 48 hours after transfection. siRNA transfection was done using the Neon™ Transfection System 100µL kit protocol with 30 nM oligonucleotide. 1300 volts were applied to the samples for 30 ms and for 1 pulse. siRNA was given while the cells were seeded, and the cells were collected for RNA and protein isolation after 72 hours of incubation.
Quantitative Real-time PCR (qRT-PCR)

RNA was isolated with the High Pure RNA isolation kit (Roche, 11828665001) and the extracted RNA (100ng) was reverse transcribed into cDNA with the A.B.T.™ cDNA Synthesis Kit (dT20, Cat# C01-01-25) The relative expression levels of MEG3 were calculated using the 2−ΔΔCT method. GAPDH was used as an internal control in the qPCR assays. The primers used are as follows: MEG3 Forward: GCCTGCTGCCCATCTACAC, MEG3 Reverse: CCTCTTCATCCTTTGCCATC, GAPDH Forward: TGCACCACCAACTGTTAGC and GAPDH Reverse: GGCATGGACTGTGGTCATGAG

Cell proliferation Assay

Cell proliferation and viability was measured with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. 10,000 cells were seeded into each well, next day 10µl of MTT solution (5mg/ml in PBS) was added to each well and incubated for 2.5 hours in the incubator. 0.1M HCl and 10% SDS solubilization buffer was added to dissolve the formazan crystals and incubated for 15 minutes at 37°C. The absorbance was measured at 570nm.

Chemo sensitivity assay was performed by administration of two different drugs Fluorouracil (5FU) and Navitoclax (Nvtx). Cell viability upon the administration of these drugs was measured via the MTT assay. 5FU is a common cytotoxic drug that acts as a pyrimidine analog. It has several uses in different cancer types (Wei et al. 2018). Nvtx is another anti-cancer agent that acts as a Bcl-2 inhibitor especially in myeloid chronic lymphocytic leukemia (Kipps et al. 2015). Although both drugs are previously approved for other cancers none of them are used in the standard treatment of glioma tumors. The purpose of using these drugs was to increase glioma cell sensitivity to other drugs. Cells were treated with 100µM 5FU, and 100µM Navitoclax. The drugs were administered to the cells after the transfection of the siRNA and overexpression plasmids for 96 hours.

Wound-healing Assay

Cells were seeded into 12-well plates with each well containing 30,000 cells. After 90% cell confluence was achieved, a scratch was created in the middle of the wells with the help of a pipette tip. Images of the healing process were taken until the wound in the control group cells were closed.

Apoptosis Assay

Control and transfected cells were removed with Accutase and then collected. Cells were washed twice with a solution containing PBS and sodium azide. Cells were then resuspended in the binding buffer included in the APC Annexin V Apoptosis Detection Kit with PI (Biolegend #640932). 5µL APC Annexin V was added into the test tube containing the suspended cells. Then 10µL Propidium Iodide Solution was added. This mixture was gently vortexed, then incubated for 15 minutes at room temperature and in the dark. Finally, 400µL binding buffer was added to each tube. Ratio of early apoptotic, late apoptotic and dead cells were measured with flow cytometry (Acea NovoCyte 3005, Thermo).

Statistical Analysis
Gene expression analysis was done for target genes with One-way Anova, Pearson Correlation Test and Simple Linear Regression Analysis in GraphPad Prism 9. Cell viability, cell migration and apoptosis assays were also compared with a simple T Test.

Results

Silencing of the MEG3 gene inhibits the proliferation of glioma cells whereas MEG3’s overexpression promotes cell proliferation

MEG3 silenced and overexpressed non-cancerous HUVEC cells and U87MG glioma cells were compared to untreated control cells. U87MG glioma cells showed reduced cell proliferation when MEG3 was silenced. Whereas, cell proliferation was enhanced in these cells when the MEG3 gene was overexpressed for 96 hours (Fig. 1a). On the other hand, amongst the three groups of MEG3 silenced and overexpressed HUVEC cells, which were incubated for either 24 hours, 48 hours or for 72 hours, only the 72 hour samples showed significant change in cell proliferation (Fig. 1b). The cell proliferation and viability analysis of HUVEC cells were done until the 72nd hour after seeding whereas this was done until the 96th hour in U87MG cells. This difference in the time frame for analysis was due to HUVEC cells showing more proliferation potential which limits the amount of time needed for cell proliferation as a result of the contact inhibition of cells.

MEG3 gene silencing reduces the migration of both U87MG glioma and HUVEC endothelial cells

In order to understand the effects of the MEG3 gene on cell migration, a wound healing assay was performed. Silencing of the MEG3 gene significantly reduced cell migration in both U87MG glioma (Fig. 2a) and HUVEC cells. (Fig. 2b) As for the MEG3 overexpression samples, their cell migration was not significantly different from that of the nontransfected cells. Cell migration was observed for up to 130 hours following wound formation. MEG3 gene overexpressing cells re-covered the wounded area within 48 hours in both cells types whereas MEG3 silenced cells could not fully re-cover the wounded area even after 130 hours upon scratch formation (Fig. 2c). Moreover, MEG3 silencing caused slower cell migration in HUVEC cells compared to U87-MG cells.

MEG3 gene silencing improves chemo-sensitivity for 5-Fluorouracil in glioma cells

The role of the MEG3 gene in chemo sensitivity of cells to two different drugs with different mechanism of actions were evaluated by the administration of these drugs and the assessment of cell viability. Although the 5FU treatment had a minor negative effect on U87MG glioma cell viability in nontransfected control cells expressing normal levels of MEG3, it did not significantly inhibit cell proliferation. However, when MEG3 was suppressed, cells immediately died after 24 hours of treatment and there was no resistant cell proliferation after 72 hours of incubation. On the other hand, MEG3 overexpression
significantly increased the proliferation of U87MG cells despite the cells being treated with 100 µM 5FU (Fig. 3a). The effects of MEG3 silencing on HUVEC cells were almost the same as it was on glioma cells. However, the overexpression of MEG3 did not significantly improve cell viability in the transfected cells compared to the untreated HUVEC cells (Fig. 3b).

The effects of navitoclax were also tested. The navitoclax treatment did not significantly inhibit the viability of glioma cells expressing MEG3 at normal levels. As for the transfected cells neither suppression nor the overexpression of the MEG3 gene showed significant inhibition to cell proliferation. However, MEG3 overexpression significantly promoted cell proliferation upon 24 and 48 hours of treatment in U87MG glioma cells (Fig. 3a). HUVEC cells were also not affected by MEG3 activity when treated with navitoclax. Neither an increase nor a decrease in cell viability was observed in the MEG3 gene silenced and MEG3 overexpressed HUVEC cells treated with navitoclax (Fig. 3b). Hence, we can propose that MEG3 activity is important in the chemo sensitivity of glioma cells to 5 fluorouracil but not to navitoclax. The reason for this selective activity may be due to the MEG3 gene's interactions with the selected drug's mechanism of action.

**MEG3 has different roles in different glioma cells and pathological subtypes of glioma tumors**

Patient derived glioma tumor samples were used to assess the effects of the MEG3 gene on the cell viability, chemo sensitivity and cell migration of glioma cells. Oligodendroglial (PD-OG) and glioblastoma (PD-GBM) cells were obtained from oligodendroglioma and glioblastoma patient’s tumors respectively, and the cells were subjected to the same viability, migration and chemo sensitivity assays as the other cell lines. Suppression of the MEG3 gene significantly decreased the viability of PD-OG cells at the 48th and 72nd hours whereas PD-GBM cell viability was promoted upon MEG3 silencing. On the other hand, overexpression of MEG3 did not significantly affect cell viability in either of these cell types (Fig. 4a, 4c). The drug responses of patient derived cells also differed between different glioma cells. MEG3 silenced PD-OG cells treated with 5-FU showed significant reduction in cell viability whereas PD-GBM cells did not show less cell viability upon treatment. The MEG3 silenced and MEG3 overexpressed cells which were treated with Navitoclax did not show any significant change in cell viability (Fig. 4b, 4d). The migration potential of PD-OG cells were not significantly affected by t MEG3 activity. Neither silenced nor overexpressed MEG3 oligodendroglial cells showed any change in cell migration (Fig. 4e, 4f). PD-GBM cells were also not affected by MEG3 activity (Data not shown). Although both cell lines originated from glioma tumors they were affected differently by MEG3 activity with respect to cell viability and 5FU response, indicating the different roles of MEG3 in different types of glioma cells.

MEG3 gene expression levels were analyzed in patient derived glioma tumors including different histopathological subtypes, grades and IDH status. RNAs were derived from 27 patients. The level of MEG3 gene expression was significantly higher in IDH mutant glioma subtypes compared to IDH wild type glioma (Fig. 5a). Moreover, the level of MEG3 had a partial correlation with the tumor grade. Grade III showed the maximum level of MEG3 gene expression whereas grade I tumors showed the least amount
of expression (Fig. 5b). It might be speculated that the reason why the MEG3 overexpression and siRNA transfections were less impactful on GBM cells is due to the already low levels of MEG3 expression in grade 4 tumor cells. Similarly, the fact that MEG3 expression is higher in grade 3 patients might be causing a greater difference between MEG3 overexpressed or siRNA treated oligodendroglioma cells and their controls. Additionally, the finding that MEG3’s expression is higher in IDH mutant patients than wild-type patients is consistent with the tumor suppressor role of the MEG3 gene demonstrated in the previous studies.

**Suppression of MEG3 induces apoptotic cell death**

The MEG3 associated cell death mechanism of action was investigated by evaluating the apoptosis in U87-MG glioma cells. MEG3 silencing in glioma cells switched the cell populations into early apoptotic and late apoptotic cell phenotypes whereas the control and MEG3 overexpressed cells showed no significant apoptotic induction (Fig. 6a). In the MEG3 silenced glioma group 25% of the cells were engaged in an apoptotic process while only 0.5% and 2% of control and MEG3 overexpressing cells were respectively undergoing apoptosis (Fig. 6b).

**Discussion**

Glial tumors are a heterogeneous group of tumor categories which include various molecular signatures that form distinct types of tumors. Recent evidence confirmed that long noncoding RNAs (LncRNAs) are modulating tumorigenesis for various cancer types. Therefore, exploring the role of LncRNAs in glioma can facilitate our understanding of its’ development. Maternally expressed gene 3 (MEG3) is an imprinted gene located at the chromosome 14q and expressed in various tissue types. MEG3 has a tumor suppressor role in normal tissues and its loss of function has been associated with various cancer types including bladder, breast, bone marrow, cervix, colon, liver lung and meninges.(Zhou et al. 2012) Loss of MEG3 activity in these different cancer types is attributed to aberrant DNA methylation.(Gao et al. 2017; Zhang et al. 2017a)

The level of MEG3 expression in glial cells has been investigated in different studies with patient derived tissues and various cell lines. Studies including glioma tumors and controls reported that MEG3 expression level was decreased in glioma tissues compared to the non-cancerous glial cells of the patients.(Qin et al. 2017; Zhang and Guo 2019; Gong and Huang 2017) In vitro studies including the U251, U87 an A172 cell lines have also reported that MEG3 gene expression was relatively down regulated in these cell lines compared to the control cells.(Wang et al. 2012; Li et al. 2016) Furthermore, Tong et al. showed that MEG3 suppressed the proliferation, migration and invasion of glioma cells(Gong and Huang 2017) whereas, Gong et al. reported that MEG3 suppressed glioma cell proliferation and induced cell cycle progression, overexpression of MEG3 which weakened the Wnt/β catenin pathway. (Gong and Huang 2017) Considering the results of these previous studies, we conducted a comparative analysis of MEG3 activity in glioma and non-cancerous HUVEC cells by suppressing and overexpressing this gene. Interestingly, we found out that the suppression of MEG3 limited the proliferation of U87 cells
while it promoted it this process in the HUVEC cells. As for the overexpression of MEG3, it promoted cell proliferation in both U87 and HUVEC cells. Consistent with cell proliferation, cell migration was also suppressed when MEG3 was downregulated in U87 and HUVEC cells and it did not significantly change when MEG3 was overexpressed in both cell types. Although experiments were repeated at least 3 times the results were completely inconsistent with previously reported data on MEG3. Therefore, we suspected the U87 cells that we were experimenting on and decided to conduct the same experiments on patient derived glioma cells. Primary glioma cells were obtained from oligodendroglioma (PD-OG) and glioblastoma patients’ (PD-GBM) cells. Upon the suppression of the MEG3 gene, PD-OG cells showed reduced cell proliferation and cell migration similar to the U87-MG cells, whereas the PD-GBM cells demonstrated increased cell proliferation and migration. In other words, the PD-OG results did not fit the previously acquired data on MEG3 activity but the PD-GBM results were consistent with them. Therefore, we proposed that MEG3 activity is dependent on the context of the cell and the cell type the gene is found in. This hypothesis was also partially formed considering the epigenetic differences between cells which have previously been drawn attention to by several studies. (Li et al. 2016; Modali et al. 2015; Iyer et al. 2017)

Based on our hypothesis that cells can be affected in different ways by MEG3 activity, we evaluated how MEG3 gene expression impacts drug sensitivity in glioma cells. Ma et al, previously showed that the overexpression of MEG3 enhanced the chemo sensitivity of U87 cells to cisplatin whereas the suppression of MEG3 increased the cells’ resistance(Ma et al. 2017) to the same drug. Here, we wanted to evaluate the effects of two different drugs with different mechanisms of action on glioma cells. 5-Fluorouracil (5FU) and navitoclax were chosen for increasing the range of drugs available for glioma treatment, since none of these drugs are currently used for this purpose. However, there are many experimental studies indicating their partial efficacy in suppressing tumor growth and cell proliferation. (Menei et al. 2005; Takahashi et al. 2014; Karpel-Massler et al. 2017; Levesley et al. 2013) Suppression of MEG3 in glial and HUVEC cells that have been treated with 5FU, caused cell proliferation in these cells to become inhibited. Whereas, the MEG3 downregulated cells that have been treated with navitoclax showed no significant change in their proliferation. In patient derived oligodendroglioma cells 5FU significantly decreased cell viability when MEG3 was suppressed. However, navitoclax did not induce any change in cell viability when MEG3 was suppressed. Neither 5FU nor navitoclax was found to be effective in reducing the cell viability of MEG3 suppressed and overexpressed patient derived glioblastoma cells. The suppression of MEG3 enhanced the sensitivity of oligodendrogliaoma cells to the 5FU drug. A previous study by Li L. et al, reported that the overexpression of MEG3 promoted chemo sensitivity to oxalipaplatin. (Li et al. 2017) This result was consistent with the activity of cisplatin demonstrated in a study done by Ma et al. (Ma et al. 2017) Our results are opposing with the conclusions made by these studies in that higher levels of chemo sensitivity were achieved when MEG3 was suppressed rather than overexpressed. However, this may be due to the abundant expression of MEG3 in the cells we worked on and tested for chemo sensitivity. Therefore, we can once again propose that the activity of MEG3 is specific to the cell type and the cellular context which together dictate chemo sensitivity.
MEG3 activity differences between different cell lines pushed us to pose this question: Are there any significant differences between patient, disease and tumor associated characteristics of various glioma types? It has been reported that MEG3 gene expression levels were lower in glioma samples compared to normal and para-carcinogenic samples taken from patients. High grade (Grade III-IV) glioma tumors showed decreased expression of MEG3 compared to low grade glioma (Grade I-II) tumors.(Li et al. 2016; Zhang and Guo 2019; Gong and Huang 2017) There is a significant correlation between MEG3 expression and overall survival.(Gong and Huang 2017) Furthermore, studies have shown that lower levels of MEG3 expression is associated with higher WHO grade, older age at the time of diagnosis, low Karnofsky performance score (KPS), the presence of the wild-type isocitrate dehydrogenase (IDH), tumor recurrence, and poor overall survival.(Momtazmanesh and Rezaei 2021) Although there is a significant reverse correlation between tumor malignancy and MEG3 expression in previous reports, we found that MEG3 activity differs between different cell lines and is mostly correlated with the IDH1 mutation status. IDH1 mutant tumors showed significant overexpression of MEG3. The maximum MEG3 expression was observed in Grade III tumors indicating the contribution of IDH1 mutations since IDH1 mutant gliomas are the most frequently observed amongst all the grades of glioma tumors.(Avsar et al. 2020)

Finally, MEG3 suppression was found to induce apoptotic cell death. Previous studies showed that MEG3 overexpression would induce apoptosis in lung carcinoma and glioma cells.(Zhao et al. 2018; Wang et al. 2012) Contrary to these findings, our experiments revealed that the induction of apoptosis was observed when MEG3 was suppressed. Other studies showed that when MEG3 was overexpressed in U251 and U87 MG cells the frequency of apoptotic cells was at somewhere between 12–15%. This meant that only a minor group of cells were affected from MEG3 overexpression.(Wang et al. 2012) In our study we reported that 25% of MEG3 downregulated and 2,5% of MEG3 overexpressed cells underwent apoptotic induction. In both our study and previous studies, only a minor fraction of the cell total showed apoptosis indicating a more complicated role of MEG3 in cell death due to its heterogeneous expression in different cells.

Conclusions

Despite various studies indicating that the loss of MEG3 expression was detected in various cancer types including glioma, here we report that different glioma cells may be affected differently by MEG3 expression and MEG3 manipulation. U87-MG glioma cells and patient derived OLG cells showed that when MEG3 is post transcriptionally suppressed cell proliferation, and migration in glioma cells are reduced and chemo sensitivity to the 5FU drug is achieved. This sensitivity to the 5FU drug upon MEG3 suppression was not observed in glioblastoma and endothelial HUVEC cells. Here, we propose that the MEG3 IncRNA plays a complicated role that changes for different cancer types. The exact part this IncRNA plays in cancer cells requires further attention to be fully understood.

Declarations

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Conflict of interest:
Authors declare no conflict of interest.

Ethical Approval and consent:
Study was approved by Bahcesehir University institutional ethics board (BAU-2020/02). Patients were provided informed consent form before they were included in study.

Author Contribution:
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Zehra Degirmenci. The first draft of the manuscript was written by Timucin Avsar. Clinical materials were provided by Turker Kilic and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References


**Figures**

**Figure 1**

![Figure 1](image_url)
Cell proliferation assay of MEG3 gene suppressed and overexpressed a) glioma b) HUVEC cells. Assay conducted for 96 hours in glioma and 72 hours in HUVEC cells.

Figure 2

Cell migration assay of MEG3 gene suppressed and overexpressed a) glioma b) HUVEC cells. Migration of cells to wounded area has been photographed at 0, 8, 24, 48, 72 and 130 hours in both cells.
Microscope photographs were taken at 40X magnification. Bars indicate 100mm length.

Figure 3

Chemo sensitivity to 5 fluorouracil and navitoclax in a) glioma b) HUVEC cells. Assay conducted for 96 hours in glioma and 72 hours in HUVEC cells.
Figure 4

Cell proliferation (a, c), chemo sensitivity (b, d) and cell migration e) assay of MEG3 gene suppressed and overexpressed patient derived oligodendroglioma and patient derived glioblastoma cells. Migration of cells to wounded area has been photographed at 0, 8, 24, 48, 72 and 130 hours in both cells. Microscope photographs were taken at 40X magnification. Bars indicate 100mm length. *** p <0.001
Figure 5

qPCR results of MEG3 gene in a) IDH mutant and IDH wild type glioma cells. b) MEG3 expression in glioma tumors with different WHO grades. **** p < 0.0001

Figure 6

Evaluation of apoptosis. a) Flow cytometry dot plot b) Comparison of groups. *** p < 0.001