Expression of MAO gene in both starved and non-starved glioblastoma cancer cell line

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Abstract

Background: The relationship between cancer cell aggression induced by serum starvation and cell metastasis has been studied. Serum starvation is a typical way to induce tumor cell apoptosis, and stress may affect cell growth. Monoamine oxidase A (MAOA) was discovered as a mitochondrial enzyme that plays a role both as tumor suppressor and as an oncogenic factor in promoting tumor cell growth. Glioblastoma is a deadly cancer in the 4th rank reported in the Iraqi Cancer Registry 2020.

Aim of the study: The deprivation of serum from cultured media for glioblastoma cancer cell line (AMGM5) was tested after three different durations 24, 48, and 72h.

Methods: Comparison of MAO gene expression between starved and non-starved glioblastoma cell lines was measured using real-time PCR.

Results: The results indicated an increase in MAO gene expression in serum deprivation of cultured cells compared significantly (p<0.03) to non-starved cancer cell lines.

Conclusion: the increase in MAO expression in well-established cell populations of the AMGM5 cancer cell line in a serum-containing microenvironment compared to a lower MAO level in starved cells suggests the importance of cancer therapy using deprived microenvironments.

Keywords: serum starvation, glioblastoma cancer cell lines, MAO gene expression, Iraqi cancer registry.

Introduction

Brain tumors were the leading cause of cancer-related deaths worldwide, according to data on cancer incidence and cancer mortality extracted from the World Bank for Cancer in 2018 (GLOBOCAN 2020) was estimated that the incidence rate for both sexes with brain cancer was higher in Asia 54.2% compared to Europe incidence rate 21.8% while the mortality rate in Asia was 54.8% compared to Europe 21.4%. The new cases recorded the number for brain cancer in the 19th rank while the number for death in the 12th rank (1).

In Iraqi statistics, brain tumor incidence rates were in the top 5 of cancer distribution after breast, lung and colorectal with incidence rates of 19.7%, 7.8%, and 6.8% respectively, and for brain 6.2% according to the Iraqi Cancer Registry for the year 2020 for both genders. While the incidence rate of brain tumors in men was recorded at 7.2% after lung, colorectal, urinary bladder and prostate; in women it was recorded at 5.4% after breast, thyroid gland and colorectal (2).

Brain tumors are malignant neoplasms that grow and can invade and metastasize to distant sites in the body and undergo many genetic and molecular events that occur within nascent cancer cells and microenvironments. Cancer cells can grow in medium, the ability to grow as a three-dimensional mass, and an ability to resist various forms of physical stress such as less oxygen, fewer substrate levels, deviation from normal pH, and high- or low-temperature extremes. Cancer cells adapt better than normal diploid cells to hostile environments, either in vitro or in vivo. In response to environmental stress, cancer cells produce cytokines to create new blood vessels in a mechanism known as angiogenesis. These observed cell behaviors serve as potential opportunities for the treatment of cancer in that they suggest the possibility of directing therapies to weak-

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nesses in these adaptive mechanisms (3).

Cancer cells need glucose, amino acids, and lipids at an accelerated rate to support growth and energy production. In cell cultures, cancer cells need media that contains serum, vitamins, and growth factors that promote cell growth and multiply, and all environments that help cells divide. When serum is deprived of media, cells will undergo different mechanisms to grow or cells will undergo apoptosis (4).

Monoamine oxidases (MAO) have been determined as an enzyme present in the outer mitochondrial membrane, play a role in the production of H2O2 and apoptosis. ROS produced by MAOA inhibits apoptosis by reducing p53 transactivation function (5).

Two isoenzymes, MAO-A and MAO-B, have been discovered in mammals, present on the outer mitochondrial membrane, but each isoenzyme has different substrate and inhibitor sensitivities (6).

MAOA was previously identified as a neurotransmitter regulator, in recent studies revealed its unanticipated roles in tumorigenesis. However, both the tumor suppression and promotion characteristics of MAOA have been identified. Down-regulation of MAOA was associated with cancers including cholangiocarcinoma, squamous cell carcinoma of the esophagus, and hepatocellular carcinoma (7, 8). In general, MAOA functions as a tumor suppressor by reducing biogenic amines that stimulate tumor progression by increasing their degradation. In contrast, up-regulation of MAOA was identified in high-grade carcinomas, such as renal cell carcinoma (9) and PCa and lung (10).

Deletion of MAO-A reduced prostate cancer stem cells and suppressed invasive adenocarcinoma. MAO-A was also overexpressed in classical Hodgkin lymphoma and glioma brain tumors. MAO B was overexpressed in glioma and nonsmall cell lung cancer. MAO-A inhibitors reduce prostate cancer growth, drug-sensitive and resistant gliomas, and classical Hodgkin lymphoma, and enhance standard chemotherapy (11, 8).

Serum starvation is a common feature of solid tumors during anti-angiogenesis, irradiation, and chemotherapy. Under low-nutrient conditions, antibiotics are effective due to the dependence of cells on mitochondria (12).

The current study evaluated MAOA gene expression in glioblastoma cancer cell line and cell proliferation in different duration times (24, 48, and 72) hours of serum deprivation condition: starvation compared to nonstarved glioblastoma cells.

Materials and Methods

The scheme chart of the current study is briefly shown in figure (1).
Expression of MAO gene in glioblastoma

Cell maintenance

The human brain carcinoma cell line (AMGM, Ahmed Majeed Glioblastoma Multiform) cancer cell line was established and obtained from the experimental therapy department / Iraqi center for cancer and medical genetic research / Mustansiriyah University / Baghdad/ Iraq. Glioblastoma cells were maintained in RPMI1640 supplemented with 10% fetal bovine serum (FBS) at 37 °C under a humidified atmosphere saturated with 5% CO2 (13).

Withdrawal of serum (Serum-deprivation) starvation

In a six-well plate, AMGM5 cells per well were plated in a medium supplemented with 10% FCS. After 24 h, cells were cultured either in RPMI-1640 medium without serum or medium containing serum. During a 24-48 and 72-hour duration, serum-free medium and medium-containing serum cells were collected to measure MAO gene expression. Glioblastoma cells were cultured in EMDM medium containing 10% FBS and digested with 0.25% trypsin to make a cell suspension of 1×105 cells/mL, about 200 μL of cell suspension per well was inoculated into 96-well plates, and cultured in incubator at 37 °C, saturation humidity of 5% CO2 for 24 h.

Cell viability assay

After the exposure experiment, the duration was 24, 48, and 72 h. Cell viability was measured by adding crystal violet stain to the attached 96-well monolayer plate for 20 min. at 37 °C in an incubator. The absorbance was measured spectrophotometrically at 490 nm with a microplate reader. The cell culture non-starved was used as a blank control. Cell viability was calculated using the method of (Feoktistova, M 2016) (14).

% viability = ((Mean O.D sample)/ (Mean O.D blank)) × 100 %.

Real-time reverse transcription (RT) quantitative PCR (qPCR) analysis

In a 5-cm dish, 105 cells were grown for 24 h, then cultured in the medium without serum for 24, 48, or 72 hours. Then total RNA was isolated from each group using an RNA isolation reagent (Invitrogen). Total RNAs were isolated using TRIzol (Invitrogen) following a standard protocol using the one-step kit (KAPA Roch, South Africa). The amount of mRNA for each group was analyzed by real-time RT-PCR using an (Agilent, stratagem mx3005p) system. Real-time PCR was performed with an SYBR kit, and the β-actin CTAB (was used as a normalizer) mRNA primer was included in each plate to avoid sample variations. Relative gene expression using ΔΔCT value was then calculated to determine the fold expression as described (15). The conditions used include 95°C for 10 min, followed by 40 cycles of 95°C for 5 s and 55°C for 31 s.

The primer MAO sequence used was R: 5’-GAACG-GACGCTCCATTCGGA-3’, F:5’-ACAGCCTGACCGTG-GAGAAAG-3’; and β-actin primer sequence used was R: 5’-TGTTTTCTTGCGCAAGTTAGGT-3, F: 5’-CCGCAAAT-GCTTCTAGGCCG-3.

Statistical analysis:

All data were presented as mean ± standard deviation. Each experiment was repeated at least three times in triplicate. Statistical evaluation was performed using the Student’s t test with GraphPad Prism (version 8.0.1; Institute Inc., Cary, NC, USA). P≤0.05 was considered to indicate a statistically significant difference.

Results

To investigate the role of serum deprivation on cell viability, the first glioblastoma cancer cell line was cultured in 96 well plates with RPMI 1640 media and calculated cell growth in three different duration times 24-, 48-, and 72-hours using crystal violet stain. Next, cells were cultured in serum-free media also for three duration time and cells were stained and read optical density using an ELISA reader. Cell viability was correlated with control cells, the results represent the mean of three replicates as shown in Figure 2. Cells grow well in the presence of cultured medium with all its components, especially serum, which represents the basic requirement that supplements cell growth and differentiation. While cells that are cultured in serum-free medium (starved / serum-deprived) lose their growth when compared to cells grown in media with serum (non-starved).

![Figure 2: glioblastoma cancer cell line (AMGM5) cells growth rate before/after serum deprivation 24, 48, and 72 hrs.](image)
Glioblastoma tumors exhibit invasive behavior, growth, and poor patient survival, and their subtypes have aggressive invasion into surrounding normal tissue. A recent review mentioned that the large interpatient and intratumor heterogeneity of gliomas, and the role of the tumor microenvironment in general makes a deep understanding of glioma mechanisms, so that research on the complex interaction between tumor cells of different subtypes and the surrounding brain parenchyma is still at its beginning (16).

The current study described the level of MAOA expression in the glioblastoma cancer cell line under different environmental conditions: serum starvation/non-serum starvation. The monoamine oxidase (MAO) represents a mitochondrial outer membrane monoamine oxidase enzyme that produced H2O2, this ROS will inhibit apoptosis by reducing the p53 gene transactivation function and activates the autophagy process through induction of mitophagy functions, for that Lin and his colleague 2017 called the MAO gene as a novel “decision maker” between autophagy and apoptosis (17).

The growth of the serum starved and nonstarved growing glioblastoma cancer cell line was affected during the duration of incubation (24, 48, and 72 hrs) respectively. The growth of cancer cells needs a complete RPMI media to grow well when these media reduce some important components such as (here in the current study) the serum (as the precursor growth of cells) cells will undergo shrinkage in shape and start to die during the duration of starvation. The importance of the microenvironment for the growth of cancer cells was previously described. The expression of the MAO gene as an aggressiveness gene in the starved and nonstarved cancer cell line was affected by the presence of serum in the micro-environment of grown cells. Significant differences between the starved and non-starved glioblastoma cancer cell line in MAO gene expression were observed with (p<0.03) higher expression in nonstarved cells.

In a recent study by Rutvi Vaja. (2021) found that the MAOA and MAOB genes contributed to cell migration in glioblastoma cells, and also found that gene expression changes in amyloid β-binding protein between glioma neural stem cells and normal neural stem cells that plays an important role in the formation of tumor microenvironments (18). And Kushal and his team workers (2016) found that MAO-A protein increased levels in glioma tissue and was cytotoxic to the glioma cancer cell line, and it reduced proliferation and invasion of glioma tissue in an in vivo study (19).

Several studies highlighted that the expression of the MAO-A gene was highly associated with advanced oral and pharyngeal cancer (20), colon cancer, gastric cancer (21) prostate cancer (22), liver (23), lung (24), and brain (23, 25). In the study of 106 protein and 12 cancer cell line, they found that the expression of proteins and phosphoproteins 24 h after serum starvation increased more often in glioma lines than in adenocarcinoma lines, in adenocarcinomas, the expression of proteins and phosphoproteins generally increased in apoptosis pathways, while there were minor fluctuations in the other pathways. While gliomas become resistant to apoptosis after 24 h of serum starvation (26).

This study presented that MAO-A gene expression decreased in the absence of serum content of media in glioblastoma cancer cell line after 24, 48, and 72 h respectively. The increase in MAO-A activity plays a role in increasing oxidative stress and increasing susceptibility to stress-induced impairment in cell viability. Although oxidative stress is only one contributing factor to cell death given its role in oxidative metabolism (27). Elevation of MAO expression was not confined to a cellular model as a similar trend was observed in Huntington disease patient iPSC-derived neural cells (28).

In conclusion, the effect of increased MAO expression in well-established cell populations of AMGM5 cancer cell line in a microenvironment containing serum compared to a decreased level of MAO in starved cells suggest the importance of cancer therapy using deprived microenvironments.

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**Conflict of interest**

None

**Author contribution**

Concept and design: zaynab S Abdulghany, Noah A Mahmood

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Data analysis: zaynab S Abdulghany

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![Figure 3: MAO gene expression analyzed by qPCR in glioblastoma cells before and after serum deprivation for 24, 48, and 72 hours compared to control cells. Blots were normalized to β-actin.](image-url)
References:


