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Research Article

Investigation of the Impact of Foretinib on *AURKA* and *AURKB* Expression in T98 Glioblastoma Cell Line

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Abstract

Background: Gliomas are a common type of the primary brain tumors and account for more than 40% of all central nervous system (CNS) tumors. Glioblastoma (GBM) remains one of the most fatal human malignancies as it is highly angiogenic. Foretinib is an oral multikinase inhibitor that has been shown to exhibit antitumor activity in previous clinical studies. *AURKA* and *AURKB* have been shown to be overexpressed in various cancers.

The purpose of this study was to investigate the effect of foretinib on the expression of *AURKA* and *AURKB* in the T98 cell line.

Materials and Methods: In this study, the T98 cell line was selected as an experimental model of glioblastoma. The cultured cells were exposed to different concentrations of foretinib (5 μM, 10 μM, 20 μM, and 0 μM as control). Following that, we examined the changes in the expression of *AURKA* and *AURKB* under the influence of foretinib compared to control using quantitative real-time polymerase chain reaction (qRT-PCR).

Results: The expression of *AURKA* and *AURKB* were found to be significantly reduced in the foretinib treated group compared to the control. The results demonstrated that increasing the concentration of foretinib led to reduction in the expression of both the genes.

Conclusion: These findings indicate that the foretinib can decrease the mRNA levels of *AURKA* and *AURKB*. Thus, we suggest that foretinib may be an effective drug for GBM treatment and can be considered for future studies.

Keywords: *AURKA*, *AURKB*, Foretinib, Glioblastoma.

1. Introduction

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Malignant glioma is a frequently occurring form of cancer originating in the central nervous system (CNS) and glioblastoma multiforme (GBM) is the most prevalent as well as

lethal type [1]. GBM is a World Health Organization grade IV astrocytoma, which indicates that it is a highly aggressive form of primary brain tumor. In the recent years, significant advances have been made in understanding the molecular biology of GBM [2]. However, despite the advances in cancer investigation and treatment over several decades, there has been only 2% improvement in the 5-year survival of GBM patients. It has been demonstrated that this disease can be resistant to radiotherapy and chemotherapy [3]. The median survival of GBM patients is approximately 12 months. However, only 3 to 5% of the patients survive for more than 3 years and they are referred to as long-term survivors. The molecular factors that help to long-term survival remain unknown [4]. Foretinib is an oral multikinase inhibitor that primarily targets the HGF/MET and the VEGF receptor-2 (VEGFR-2) signaling pathways by binding to the adenosine triphosphate pocket of both MET and VEGFR-2. In addition, clinical investigations have shown that foretinib can exert antitumor activity [5]. Recently, there have been a number of phase II clinical trials in progress using foretinib to treat breast, liver and gastric and neck cancers. Therefore, foretinib can be considered as a novel treatment option for GBM. Based on a previous study, foretinib has been found to inhibit the migration and invasion of GBM in vitro [6]. Aurora kinases (AURKA, AURKB, and AURKC) regulate mitosis and meiosis in all eukaryotes, and there has been increasing evidence linking these kinases to oncogenesis. They are key regulators of chromosome segregation during mitosis. *AURKA* and *AURKB* encoding serine/threonine kinases are involved in different phases of mitoses [7]. *AURKA*, located on chromosome 20q13, is highly amplified in human malignancies such as GBM, gastric and breast cancers. AURKA overrides the mitotic assembly checkpoint and induces chemoresistance. It has also been linked with the development of chemoresistance in breast and esophageal cancers [8]. *AURKB* is located on chromosome 17p13 and it has been found to be overexpressed in various human cancer cell lines as well as in primary tumors such as colorectal and lung cancers [9-10]. An important step in the rational identification of new drug targets for cancer therapy is to understand the gene expression pattern of the tumor cells [10]. There are limited studies evaluating the effect of foretinib on overexpressed aurora kinase genes in human malignancies. Therefore, in this study, we attempted to examine whether foretinib has an effect on the deregulated aurora kinase genes (*AURKA* and *AURKB*) using T98 glioblastoma cell line. In other words, the purpose of this study was to investigate the impact of different concentrations of foretinib, which is an oral multikinase, on the expression of *AURKA* and *AURKB* in a GBM cell line.

2. Materials and Methods

2.1. Cell culture

The T98 cell line used in this study was purchased from the National Cell Bank of Iran (NCBI, Tehran, Iran) and the cell culture was maintained at 37°C and 5% CO_2 in Dulbecco's modified Eagles medium (DMEM) (Gibco, USA) that was supplemented according to NCBI's recommendations. Foretinib was obtained from ChemieTek (ChemieTek, IN, USA). To investigate the impacts of foretinib on the T98 cell line, the cultured cells

were exposed to various concentrations of foretinib (5 μM, 10 μM, and 20 μM) and the cells that were not treated with the drug were used as control. The total treatment duration was 24 h, based on a previous study [11].

2.2. RNA isolation

The total RNA from the treated samples was extracted using TRizol reagent (Invitrogen, USA) according to the manufacturer's instructions. The quality of the extracted RNA was determined based on its 260/280 absorbance ratio, which was measured by the NanoDrop spectrometer (Thermo Scientific, Waltham, MA, USA).

2.3. Complementary DNA synthesis

Complementary DNA (cDNA) synthesis was performed using a standard kit (TaKaRa, Kusatsu, Shiga Prefecture, Japan) based on the manufacturer's instructions.

2.4. Quantitative real time polymerase chain reaction

The primers were designed using the National Center for Biotechnology Information (NCBI) website and Gene Runner software (Table 1). The uniqueness of the primers was confirmed using the BLAST program. Real-time quantitative PCR (RT-qPCR) reactions were performed in triplicate using an ABI PRISM 7500 instrument (Applied Biosystems, USA). Briefly, in a total reaction volume of 10 μl, the cDNA was added to a master mix comprising AURKA and AURKB primers; 0.5 μl of forward primer, 0.5 μl of reverse primer, 3 μl of DEPC-treated water, and 5 μl of SYBR premix ExTaq II (TaKaRa, Kusatsu, Shiga Prefecture, Japan). The run program was set at 95°C for 10 min followed by 40 cycles of 95°C for 15 s, 60°C for 10 s and 72°C for 20 s. All reactions were performed in triplicate. GAPDH was quantified as the reference in order to normalize the differences in the total RNA levels. The calculation was performed using $2^{-\Delta\Delta CT}$ method [12].

Table 1: Sequences of the primers used for real-time PCR **(5'-3')**.

Gene	Forward Primer	Reverse Primer	Size (bp)
AURKA	GGATATCTCAGTGGCGGACG	GCAATGGAGTGAGACCCTCT	211
AURKB	GCTCTCCTCCCCCTTTCTCT	TGTGAAGTGCCGCGTTAAGA	245
GAPDH	CCACTCCTCCACCTTTGACG	CCACCACCCTGTTGCTGTAG	107

2.5. Statistical analysis

For statistical analysis, Graph Pad Prism statistical software, version 8.0.2 (Graph Pad, Inc., San Diego, CA, USA) was used. Normality was evaluated using the Kolmogorov–Smirnov test. One-way ANOVA was used to compare the data. The data was expressed as the mean ± SD. For all tests, $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. *AURKA* expression

The impact of foretinib on *AURKA* expression was evaluated by quantitative real-time PCR in T98 cell line. The C_t values of real-time PCR were used for the quantification of the relative *AURKA* expression using the $2^{-\Delta\Delta Ct}$ method. A significant reduction was observed in the expression of *AURKA* compared to the control, the increase was in a dose-dependent manner of foretinib (Figure 1). The highest reduction of gene expression was seen when cells were treated with 20 µM of foretinib. At this concentration, the reduction in *AURKA* expression observed was higher compared to that seen in the cells treated at foretinib concentrations of 5 µM and 10 µM ($p < 0.004$ and $p < 0.0005$, respectively).

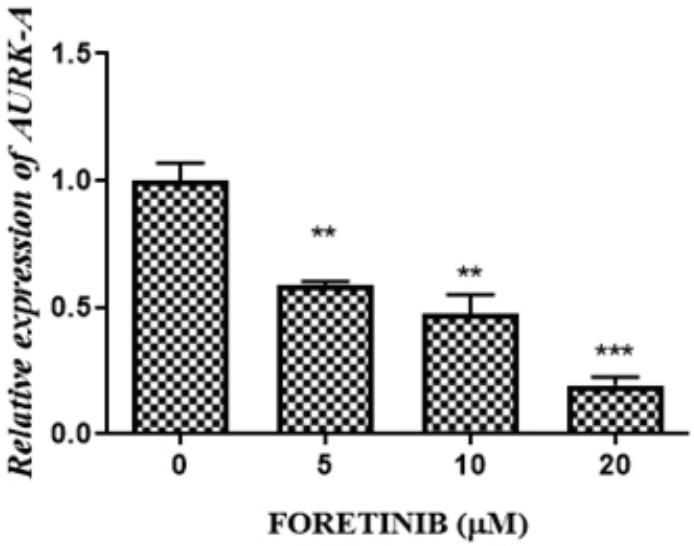

Figure 1: Decrease in the expression of *AURKA* in cells treated with increasing concentrations of foretinib. A reduction in the expression of *AURKA* was observed in T98 cells treated with various concentrations of the foretinib (5, 10, and 20 µM) compared to the control (0 µM) (** : $p<0.004$ and *** : $p< 0.0005$, respectively). The results are presented as mean ± SD.

3.2. *AURKB* expression

To explore the effect of foretinib on *AURKB* expression in T98 cell line, we examined the expression level of *AURKB* under the influence of various concentrations of foretinib by qRT-PCR. Our findings showed that the expression level of *AURKB* was significantly decreased under the influence of foretinib. The expression of *AURKB* was reduced by increasing the concentration of foretinib compared to control (Figure 2). We observed the highest decrease at 20 µM of foretinib compared to the levels seen in cells treated with 10 µM and 5 µM concentrations of foretinib ($p < 0.0005$ and $p < 0.0001$ respectively).

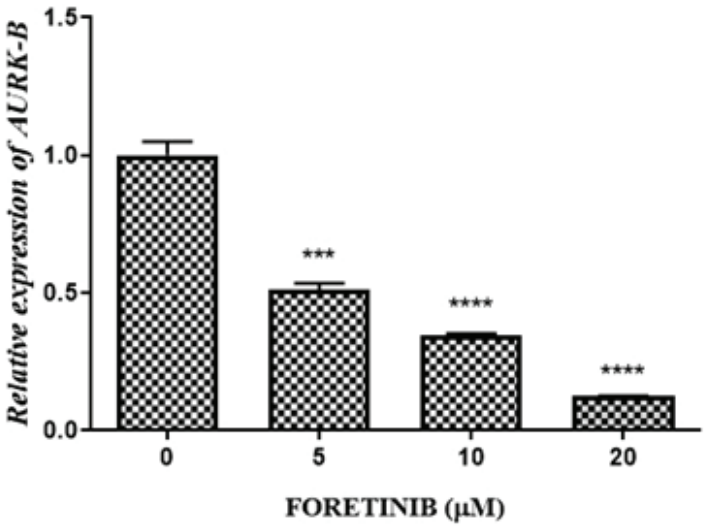

Figure 2: Decrease in the expression of *AURKB* in cells treated with increasing concentration of foretinib. The reduction in *AURKB* expression is observed the T98 cells were treated with various concentrations of the foretinib (5, 10, and 20 µM) compared to the control (0 µM) (***; $p<0.0005$ and ****; $p< 0.0001$, respectively). The results are presented as mean ± SD.

4. Discussion

In order to have more insight into the possible effects of foretinib on the genes belonging to the Aurora family (A and B), we studied the expression profile of *AURKA* and *AURKB*. One of the major questions that need to be answered while examining gene expression data is whether a certain kind of drug, such as foretinib, can affect the expression of that particular gene. Based on a similar question, in this study, we attempted to investigate whether foretinib leads to deregulation of aurora kinase genes (*AURKA* and *AURKB*) in GBM cell line. Previous studies have suggested that tumors display higher levels of *AURKA* and *AURKB* expression than their corresponding normal tissues [10]. We observed a reduction in the expression of both genes (*AURKA* and *AURKB*) by increasing the concentration of foretinib. According to these results, it can be assumed that foretinib can lead to decreased expression of *AURKA* and *AURKB* and may be an effective drug for the treatment of GBM that can be considered for future investigations. GBM has a poor prognosis along with very low relevant survival rate [13]. Despite the therapeutic advances, GBM is still one of the most lethal human malignancies and, there are no drugs that can significantly amend the survival rate of the patients. Investigation of genetic aberrations and expression patterns may provide insight into the molecular pathogenesis of GBM and hence, facilitate the identification of the prognostic markers for treatment. Changes in the expression levels of the Aurora kinases were found to be linked with various malignancies, and hence, their inhibition has been explored in the field of cancer therapy [14-15]. Moreover, it has proposed that foretinib exhibits significant antitumor activities in patient-derived hepatocellular carcinoma [16]. Although it has been shown that there is a correlation between the overexpression of the *AURKA* and clinical aggressiveness in breast, bladder, colon, ovarian and pancreatic cancers, and GBM [10], the role of *AURKB* in tumorigenesis and cancer progression remains to be

clarified. It has been shown that certain tumors, such as anaplastic or undifferentiated thyroid carcinomas and seminomas, overexpress *AURKB* [9]. In a previous study, *AURKB* was found to be overexpressed in GBM cell lines [9]. Hence, three members of the Auroras family (A, B, and C) have been shown to be overexpressed in different kinds of cancer [17]. This hypothesis, which might explain the gene expression patterns in malignant cells, clearly requires extensive examination in the future. Based on previous studies that have shown high levels of expression of *AURKA* and *AURKB* in different types of cancers, there were two intriguing questions: could foretinib bring about low levels of expression of *AURKA* and *AURKB* in GBM cell line and can it act as a potential chemotherapeutic agent for the treatment of GBM? We have attempted to answer these questions in this present study. Our data showed that foretinib could lower the mRNA levels of both the genes studied. We believe that our work validates that foretinib has an impact on the expression of *AURKA* and *AURKB* in GBM cell line and it might have potential anti-tumor effects in glioma. In conclusion, the results of the current study demonstrated that foretinib leads to down-regulation of *AURKA* and *AURKB* in the T98 cell line. Based on these results, it can be assumed that foretinib has potential anti-tumor effects and could be used in combination with chemotherapy for GBM therapy in the future. However, further in-vitro and in-vivo experiments are required to confirm this hypothesis.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Author Contributions

M.S and M.M; Conceived and designed the study. H.S and F.N; Analyzed and interpreted the data and performed all of the experiments. H.S and F.N; Wrote the manuscript. M.S; Financial support. M.M; Discussed the results and approved the manuscript. All authors read and approved the final manuscript.

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