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Rho-GDIγ inhibits the proliferation of human glioma cells

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Abstract Rho-GDIγ, one of GDP-dissociation inhibitors (GDIs) in the GTPases Rho-subfamily, plays a key role in modulating the activation of GTPases, and contributes to reorganization of the actin cytoskeleton, cell aggregation, cell-to-cell adhesion, membrane ruffling, cell motility, and so on. This study primarily shows that Rho-GDIγ inhibits the proliferation of human glioma cells (HGC) U251. The result indicates that overexpression of Rho-GDIγ in U251 significantly inhibits cell proliferation and migration. Bioinformatics analyses indicate that Rho-GDIγ in mouse and human have the similar motifs, domains and structures. The results suggest that Rho-GDIγ in human may be a regulation target in controlling the proliferation of human glioma cells.

Keywords Rho-GDIγ ; Glioma cells

1 Introduction

Central nervous system (CNS) tumors are the most prevalent solid neoplasm of childhood, and are responsible for approximately 2% of all cancer deaths and around 50% of these cases are gliomas, in particular those derived from astrocytes [1] [2]. The current therapies for glioblastoma multiforme (GBM) are ineffective [3]. Therefore, it is urgently important to identify novel therapies that target specific differences between normal and malignant cells. Several genetic alterations have been found that associated with GBM including regulating cell cycle, growth factor receptors (EGF and PDGF), the signal transducer and activator of transcription (Stat) family [4] [5] [6] [7]. However, there is few research focuses on the effect of Rho-GDIγ in Human Glioma Cell at present.

Rho family members regulate various cell functions through reorganization of the actin cytoskeleton, such as cell shape change, cell aggregation, cell-to-cell adhesion, membrane ruffling, formation of stress fibers and focal adhesions, cell motility, cytokinesis, and smooth muscle contraction [8] [9] [10]. Rho-GDIγ (Guanine nucleotide dissociation inhibitors) is a member of the Rho family of small GTPase. Our previous studies showed that neural stem cells (NSC) migration ability declined when Rho-GDIγ was over expressed [not published]. In this research, we show that when Rho-GDIγ is overexpressed in U251, cell proliferation is inhibited. Bioinformatics techniques are also utilized to identify the motifs, domains and structures in both mouse and human.

2 Results

2.1 Rho-GDIγ inhibits the proliferation of U251 cells

Expression construct vector pEGFP-C1- Rho-GDIγ was transfected into U251 cells as mediated by Lipofectamine. Green fluorescence was used to assess the transfection efficiency. The results was observed at 36 hours post transfection through a fluorescence microscope (Fig1 A.). In addition analysis of cellular morphology showed that no matter U251 cells were transfected with the pEGFP-C1- Rho-GDIγ or not, they all appeared similar phenotype (data not shown). MTT assay was applied to detect the proliferation every 12 hours after U251 cells were transfected with the over-expression vector (Fig1B.). It showed that U251 proliferation was inhibited in the U251- Rho-GDIγ group as compared to U251 control group, but there was no significant difference between U251-mock group and U251- Rho-GDIγ group, so it is need further verification. Besides, DAPI was used to dye cell nucleus. U251 nucleus in control cells were plump and clear, which present homogeneous blue fluorescence under ultraviolet light, while those of transfecetd cells presented half-moon-like formation or chipped fluorescence (Figure 2C,D).

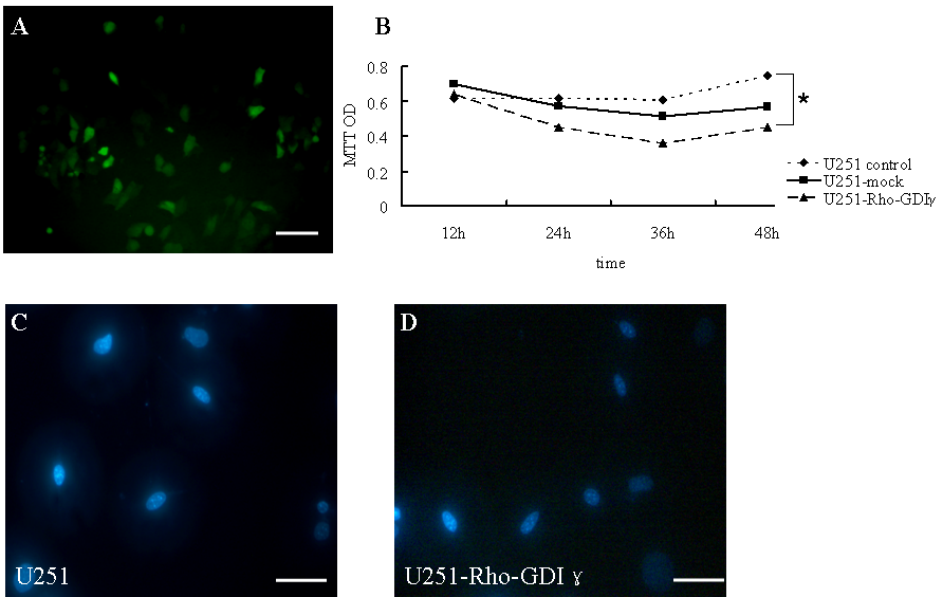

Figure 1: Effects of Rho-GDIγ on the proliferation, transfection effciency of U251 cells. (A)The cells were viewed under a Nikon fluorescent microscope 36 hours after U251 cells were transfected with pEGFP-C1-Rho-GDIγ. (B) Effects of pEGFP-C1-Rho-GDIγ on the proliferation of U251. (C, D) Cell nuclei were counterstained blue with 4,6-diaminodino-2- phenylindole (DAPI).

2.2 Rho-GDIγ declined the migration of U251 Cells

Cell migration was confirmed by wound-healing (scratch) assay in vitro [11]. As shown in Fig.2. I-L, U251 cell migration ability was declined when treated with pEGFP-

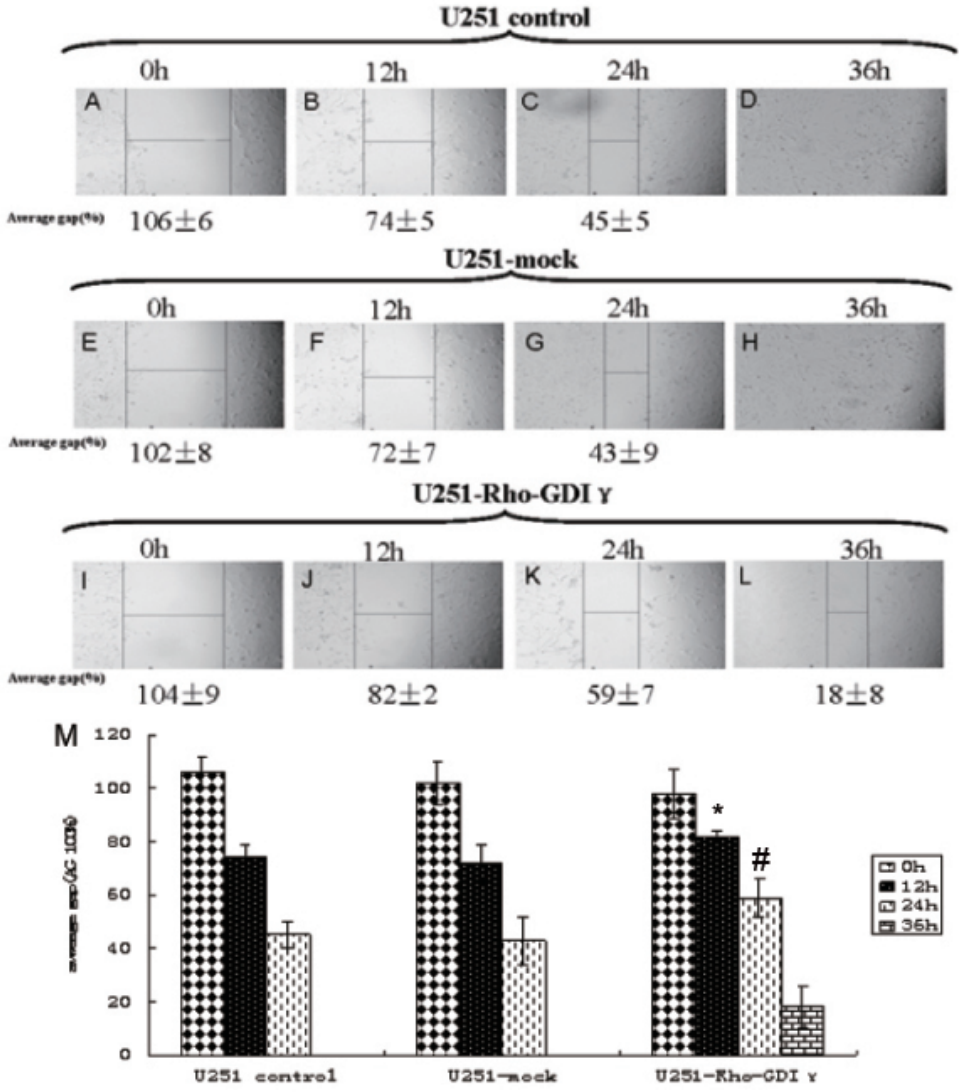

Figure 2: Effects of Rho-GDIγ on U251 cells migration are shown by in vitro wound-healing assay. Cells were plated densely in 6cm culture dishes in each group. A standardized scratch was made with a p-20 pipette tip[11]. After washed with PBS by three times, representative images of the scratched areas under each condition at different time points were photographed and the quantitation (average gap) was carried out as described [11]. *, -$p<0.05$ as compared to no treatment group afters 12 hours. #, -$p<0.05$ as compared to no treatment group after 24 hours.

C1-Rho-GDIγ. In contrast, cell migration in both of no treatment control group or mock group was significant increased. Quantitation of the data (Fig.2. M), defined as the "Average Gap", was performed as described previously [11].

2.3 Bioinformatics analysis indicate similar function between Rho-GDIγ in mouse and human

The biological experiments described above illustrate that mouse Rho-GDIγ gene plays a key role in Human Glioma Cells U251. Furthermore, the sequences of Rho-GDIγ in mouse and human were respectively queried against the database in GeneBank, and the results indicate that the two sequences shared 82% identity (data not shown). Generally, proteins have distinct functions because they vary in structures. In this work, bioinformatics techniques were utilized to identify their motifs, domains and corresponding 3D structures on the InterPro database (Fig.3) [14] [15]. The results indicate that Rho-GDIγ in mouse and human have the similar motifs and domains. Therefore, the results imply that human Rho-GDIγ gene in HGC may have the same function as the one of mouse in the HGC.

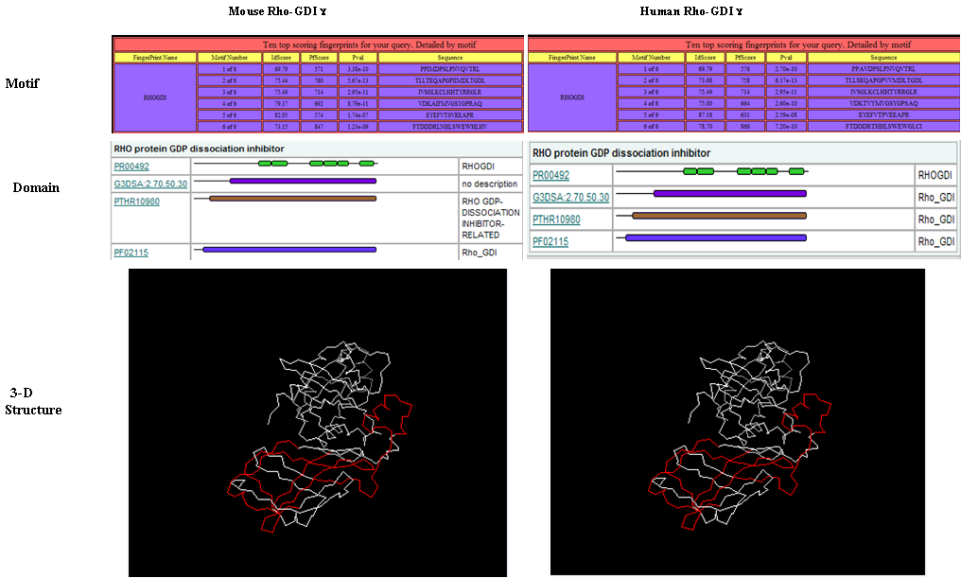

Figure 3: The identified motifs, domains and corresponding structures of Rho-GDIγ gene in mouse and human on the InterPro database.

3 Materials and methods

3.1 Cell culture

Human Glioma cells were grown in high-glucose DMEM (Invitrogen) supplemented with 10% fetal calf serum (Hyclone, USA) on 6cm Corning culture dishes in standard

humidified 5% CO_2at 37°C(Snyder).

3.2 Gene transfection

Lipofectamine 2000 reagent (Invitrogen) was used for gene transfection according to the protocol provided. The cells were seeded in 96-well plates at the density of 1×10^4 cells/100μl each well. Tweenty-four hours later, the vectors 1μg in the Lipofectamine 2000/DMEM (W/V, 1:1) 50μl was added. Five hours later, the cells were changed with fresh medium, and then cultured for use.

3.3 MTT assay

The cells were seeded in 96-well plates at the density of 1×10^4 cells/100μl each well and then transfected, incubated for 12 to 48 h, added 10μl MTT reagent (Sigma) and incubated for another 12h until purple precipitate is visible, added 100μl DMSO and gently shocked 10min and record absorbance at 490nm.

3.4 In vitro scratch assay

The effect of pEGFP-C1- Rho-GDIγ on cell's migration was also assessed in an in vitro scratch assay [11][12][13]. Cells were plated densely in 6cm culture dishes and after transfection for 12h, a standardized scratch was made through the Cell Culture dish with a P20 pipette tip[12]. The cells were then washed extensively with PBS. Representative images of the scratched areas under each condition at different time points were photographed. To estimate the relative migration of the cells, we used average gap (AG,%) to quantify the data [11][13].The condition at 0 hour was considered 100% AG.

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