MicroRNA Targetting MGMT Enhances Sensitivity to Themozolomimde Therapy in Glioblastoma

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Introduction

Epigenetic silencing of the MGMT gene by promoter methylation has been associated with longer overall survival in patients with glioblastoma who, in addition to radiotherapy, received alkylating chemotherapy with TMZ. It has remained unclear whether other factors affect MGMT silencing and TMZ resistance. MicroRNAs (miRs) are about 22-nucleotide small, non-coding RNAs that negatively regulate their target mRNAs by inhibiting translation. These miRs have often been reported as associated to TMZ resistance.

To understand the mechanism of TMZ-resistance and MGMT gene regulation by microRNA, we searched for candidates of MGMT-targeting microRNAs.

Methods

We picked up 6 microRNAs out of tens of candidates from several binding site prediction softwares, which have complementary seed region with 3'-UTR of MGMT messenger RNA (mRNA).

Results

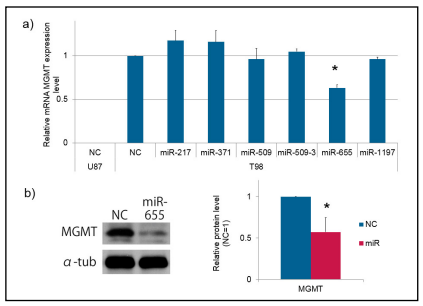

Fig.1 MGMT was suppressed by microRNA-655.

As a result of our in silico analysis, we selected 6 microRNAs, which putatively target MGMT mRNA. T98G cells were used for MGMT expressing positive control, whereas U87MG were used as a negative control. MGMT mRNA level was measured by qPCR after cell lines were transfected with selected miRs and negative control (NC, Scramble miRs). Out of 6 selected microRNAs only miR-655 significantly down-regulated MGMT mRNA level (0.59 ± 0.03 SD fold reduction, $p<0.01$, Fig.1a), as well as MGMT protein level (0.53 ± 0.13 SD fold reduction , $p<0.05$, Fig.1b) compared to negative control in the MGMT-positive T98G glioma line.

Learning Objectives

By the conclusion of this session, participants should be able to:

1) Describe the importance of how to manage drug resistance gene,

2) Discuss, in small groups,drug sensitivity,

3) Identify an effective treatment for glioblastoma.

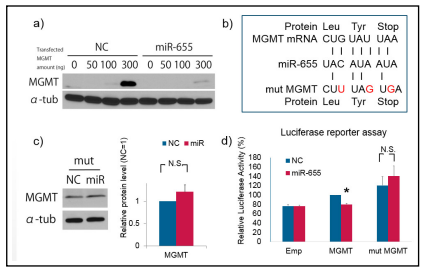

Fig.2 Dose-dependent reduction by miR-655 and mutation-mediated inhibition of downregulation.

In order to confirm specific targeting of miR-655 for MGMT mRNA, MGMT-negative U87MG cells were transfected with a construct carrying MGMT cDNA along with miR-655 or negative control miR, followed by western blot (Figure 2a). To prove specific targeting, we overexpressed MGMT from the vector with mutated miR binding site (Figure 2b). U87MG were co-transfected with MGMT construct that has mutation (mut) at predictive microRNA binding site, along with either miR-655 or negative control (Figure 2c). The result of western blot analysis showed that mutation inhibited suppression of MGMT expression by miR-655 (1.21 ± 0.13 SD fold compared to negative control, N.S.=non-significant, Figure 2c). Additionally, in order to exclude internal mechanisms of regulating MGMT expression level, reporter assay using luciferase activity showed no significant difference between negative control and miR-655 respectively co-transfected with mutated 3'-UTR of MGMT (mut MGMT) inserted after luciferase expression vector, while significant difference was seen in vector with 3'-UTR of MGMT (MGMT), using internal control as luciferase vector without 3' -UTR (Emp) (Fig2d, 0.80 fold ± 0.02 SD, *;$p<0.01$, N.S. = non significant).

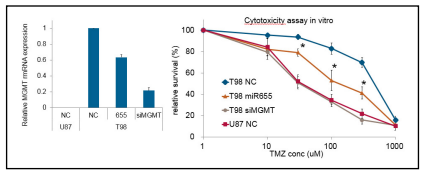

Fig.3 MiR-655 transfected cells showed enhanced chemo -sensitivity to TMZ.

Ectopic miR-655 expression enhanced cytotoxicity in TMZ-resistant T98G cells, showing similar cytotoxicity as the TMZ-sensitive U87MG cells (*; $p<0.05$). DMSO concentration was adjusted. SiRNA of MGMT (siMGMT) was transfected for positive control. Cytotoxicity was measured by Lactate dehydrogenase (LDH) assay.

Conclusions

Specific suppression of MGMT by microRNA-655 can be achieved at both mRNA and protein level.

MGMT suppression increased chemo-sensitivity to TMZ both in vitro and in vivo.

This study will provide a new target to enhance efficacy of chemotherapy.

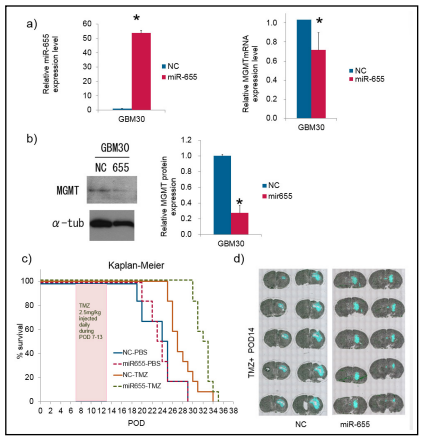

Fig.4 Athymic mice injected with primary glioma cells stably expressing miR-655 are characterized by better survival than control cells.

Primary glioma cells (GBM30) were stably infected with either control empty vector or miR-655 expressing lentiviral constructs. MiR-655 expression level and reduction of MGMT level both at mRNA and protein levels in GBM30 cells stably expressing miR-655 are shown on Figure 4a,b, (*=$p<0.01$). The cells were injected into right striatum of athymic mice. During POD7-13 (post-operation day) , mice were daily injected intra-peritoneally with either PBS or TMZ (2.5mg/kg). Survival analyses were performed using Kaplan-Meier method. GBM30 stably expressing miR-655 injected with PBS did not differ from control, while within TMZ injected group, there was significant extension of survival with miR-655 expression compared to control group (Figure 4c, $p<0.01$). From brain slice sections injected with tumor cells labeled by GFP, cells stably expressing miR-655 were obviously suppressed from growing than control, showing miR-655 is effective in vivo as well (Figure 4d) .