Cdk inhibitor flavopiridol enhances temozolomide-induced cytotoxicity in human glioma cells.

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Introduction

Recent progress in chemotherapy for malignant gliomas has been made by introduction of the DNA–methylating agent temozolomide (TMZ); however, drug resistance remains a major problem. Previous studies have shown that TMZ induces prolonged arrest of human glioma cells in the G2/M phase of the cell cycle followed by a senescence–like phenomenon (p53–wild type cells) or mitotic catastrophe (p53–non–functional cells). These findings suggest that the G2 checkpoint system is linked to DNA repair mechanisms.

Materials & Methods

We investigated the effect of a cdk inhibitor (flavopiridol; FP) that inhibits the action of a key protein in the G2 checkpoint pathway, i.e., cdc2 (Cdk1), on TMZ–treated human glioma cells.

Cell culture

U87MG-, U87MG-E6-, -AktERM+ cell; UCSF

DMEH-21, 10% FCS, 37 C in 5% CO2. Cells (5000)were plated for 2d, exposed toTMZ for 3H, replaced in TMZ-free media or in fresh media with FP for 3d.

Anti-tumor agents

TMZ, FP; National Cancer Institute

CDDP, 4-hydroxytamoxifen; Sigma

Immuno-blot analyses & fluorescence

phosphorylated cdc2, Polo-like kinase 1(Plk1), Aurora B, Survivin, gH2AX; Cell Signaling Technology

Alexa 488, 568; Molecular Probes

Cell cycle Becton-Dickinson FACscan.

Colony formation efficiency

5000 cells/ well, at 37 C O/N,treated with ATA for 15d, staining with methylene blue (Sigma). Colonies of over 50 cells were counted.

Glioma xenografts

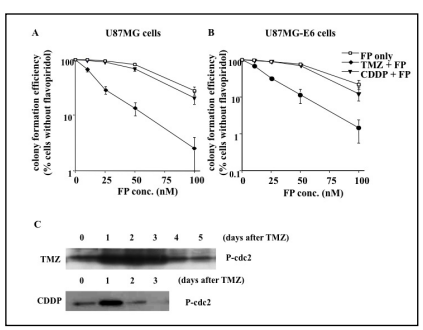
The cells were transplanted *sc* into the frank of 4 week-old female BALB/ c nude mice (Charles River).

TMZ (25mg/ kg) for 3d, and FP (50mg/ kg) for 5d were injected *ip*.

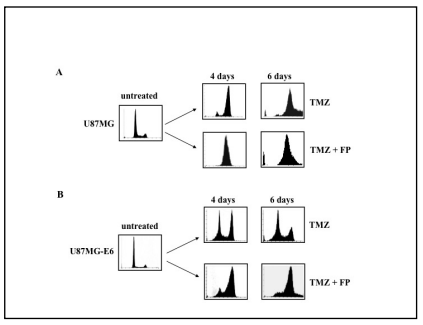
Results

Drug toxicity and the activation of cell–signaling proteins were evaluated by colony formation efficiency(Fig.1), cell cycle study(2), and immunoblotting(3,4). FP potentiated the cytotoxicity of TMZ in human glioma cells in a p53–independent manner. This effect was clearly associated with suppression of key proteins at the G2–M transition (Plk 1, Aurora B kinase, and Survivin) (3), accumulation of the cells exclusively at the G2 phase, and increased expression of g–H2AX, which is a double strand DNA break marker (4). To analyze the action of cdk inhibitor in TMZ–resistant cells, we treated U87MG cells repeatedly with dose escalation to establish TMZ–resistant clones and used U87MG cells transfected with myristoylated Akt and murine estrogen receptor (U87MG–AktER cells) fusion protein, whose Akt activity could be enhanced by exogenous treatment with estrogen analogue. TMZ–resistant clones showed activation of the G2 checkpoint in response to TMZ, while FP treatment re–sensitized these clones to TMZ(5). In addition, FP also enhanced cytotoxicity of TMZ in U87MG–AktER cells with overactivated Akt(6). Moreover, administration of TMZ and/or FP to nude mice with xenografted U87MG cells revealed that FP sensitized xenografted U87MG cells to TMZ in these mice(7).

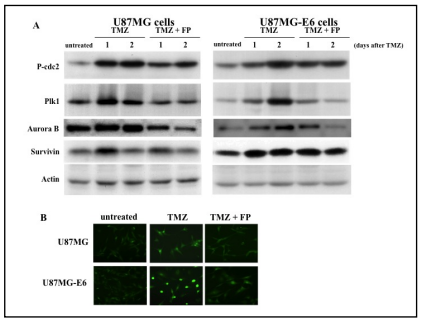
1. Colony–formation efficiency of glioma cells treated with FP and CDDP



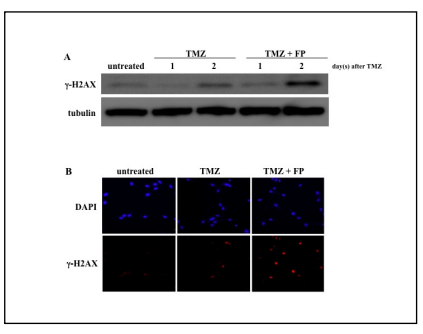
2. Analysis of cell cycle distribution of U87MG (A) and U87MG–E6 (B) cells induced by TMZ w/ or w/o FP (25 nM).

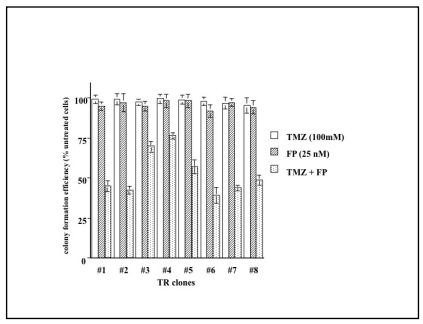


3. Effects of FP on G2/M phase proteins.
(A) Immunoblot analyses and (B) immunostaining showing the effects of FP on the expression of key proteins at the G2–M transition in glioma cells treated w/ TMZ (50 uM) w/ or w/o FP (25 nM).



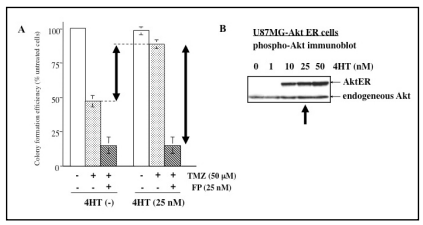
4. Protein level analyses showing the effects of FP on the expression of g–H2AX in glioma cells treated w/ TMZ (50 uM) w/ or w/o FP (25 nM).
(A)Western blot analysis.
(B) Immunocytochemical analysis.



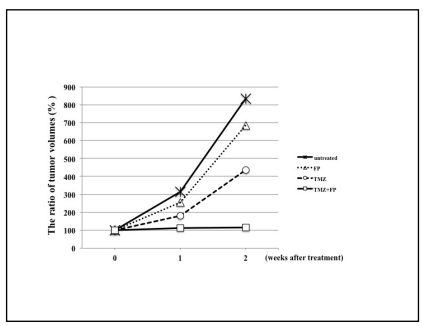


5. Flavopiridol re–sensitized U87MG–derived TMZ–resistant Clones to TMZ

6. Effect of Flavopiridol on TMZ–treated Glioma Cells with Overactivated Akt
Cells were pretreated with 4–HT (50 uM) for 24h and/or incubated with TMZ (50 uM) and/or FP (25 nM) for 3d.
(A) Colony–formation efficiency of U87MG cells transfected with a fusion protein of U87MG–AktER treated with TMZ and/or FP.
(B) Immunoblot analysis of Akt in U87MG–Akt ER cells.



7. Effects of combined temozolomide (TMZ) and flavopiridol (FP) treatment on U87MG xenografts in vivo.



Hypothetical illustration of the association between a G2 checkpoint protein and DNA damage repair in TMZ–treated glioma cells. Akt may be involved in a DNA repair mechanism after cell cycle arrest of the cells, suggesting that the Akt pathway may link the DNA checkpoint to cell survival.

Conclusions

Both in vitro and in vivo findings presented here suggest that TMZ resistance could be promoted by enhanced DNA repair activity in the G2–M transition and that a cdk inhibitor could suppress this activity, leading to potentiation of TMZ action on glioma cells. Moreover, the cdk inhibitor re–sensitized TMZ–treated cells, including those with Akt hyperactivity to TMZ. Further investigation into this resensitization mechanism would aid in the development of a novel chemotherapeutic regime.

Discussion

FP could enhance G2/M arrest through cdc2 inhibition, and an investigation of the damaged–DNA repair mechanism in glioma cells might lead to some pivotal findings regarding the acquisition of chemotherapeutic agent resistance in glioma cells. Here, we hypothesized that Akt might be involved in the DNA repair mechanism in the glioma cells after cell cycle arrest. If the Akt pathway does link the DNA checkpoint to cell survival, this pathway would provide a promising therapeutic target against TMZ–resistant glioblastoma.