行政院國家科學委員會專題研究計畫　期中進度報告

MKP-1 的細胞保護作用(1/3)

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一、中文摘要

人類原生性顱內腫瘤多數為膠質母細胞癌。由於膠質癌細胞生長快速且具高度侵襲性，侵襲大腦週邊組織而造成臨床治療的問題。在成年人膠質母細胞瘤中而最惡性的為多型性膠質母細胞瘤(Glioblastoma multiforme ，簡稱GBM)。GBM的治療與術後普遍不佳。膠質母細胞癌的高侵襲性和過度表現間質金屬蛋白腖(MMPs)有關。因此抑制腦瘤間質金屬蛋白腖的活性可望降低其侵襲性並有效的控制膠質細胞癌轉移能力。 MMPs的基因表現已知可循p38 MAPK、ERK等訊息傳遞路徑。因此若能降低MAPK的酵素活性，可以有效降低腦瘤間質金屬蛋白腖的活性。MKP-1 (MAP kinase phosphatase 1) 屬於 dual specificity MAPK phosphatases (DS-MKPs)其中一員，其功能可以抑制MAPK (MAP kinase)的活性。目前我們發現，糖尿病用藥peroxisome proliferator activated receptor-γ (PPAR-γ)的活化物 rosiglitazone與抗發炎藥物dexamethasone可以活化MKP-1蛋白的產生且有效抑制腦瘤間質金屬蛋白腖的活性。因此我們認為常用來抗發炎藥物dexamethasone或糖尿病用藥Rosiglitazone也許可用來做為膠質母細胞癌的治療或術後的輔助療法(adjuvant therapy)。 此外，iNOS普遍表現於惡性度較高的腦瘤細胞中，在本實驗中，利用NO合成腖抑制劑（*l*-NAME）與 NO donor（SNP）證實NO的存在可促進MMP-2蛋白活化過程。Dexamethasone與rosiglitazone透過MKP-1可以有效抑制iNOS的表現，降低NO的產生，影響MMP-2活性。綜合上述結果，可知增加 MKP-1蛋白生成可以抑制MAPK的活性 ，減低MMP-2蛋白的產生與活化達到抑制腫瘤侵襲性的效果。

關鍵詞： 間質金屬蛋白腖-2 (MMP-2)、腦瘤、MKP-1、 dexamethasone 、 Rosiglutazones

Abstract

The majority of primary intracranial tumors in human are gliomas. Tumor cell hyperproliferation and invasiveness are key features of glioma. Glioblastoma multiforme (GM) is the most common form of astrocytomas in adults. Despite radical surgery, radiation therapy and conventional chemotherapy prognosis remains poor and is associated with low survival rate. Matrix metalloproteinases (MMPs) have been implicated as important factors in the control of the invasive capability of glioma cells. Induction of MMPs is known to mediate through many signaling pathways including MAPK (MAP kinase) dependent pathways. MKP-1 (MAP kinase phosphatase 1), which is a member of the dual specificity MAPK phosphatases (DS-MKPs), inactive MAPK activity. We found that Dexamethasone, an anti-inflammatory agent and Rosilitazone, an agonist of peroxisome proliferator activated receptor-γ (PPAR-γ) have been shown to inhibit MMP-2 activity via induction of MAPK phosphatase -1 (MKP-1). Thus, we propose to explore the possibility of whether dexamethasone and Rosiglitazones can be used as a therapeutic agent to treat malignant glioma invasiveness and cell growth. In addition, iNOS only expressed in high grade of malignant glioma cells. Treatment of glioma cells with *l*-NAME (NOS inhibitor) or Sodium nitroprusside (Nitric Oxide donor), We found that Nitric Oxide regulate MMP-2 activity. By using siRNA to knockdown MKP-1 also reversed Dexamethasone- and Rosiglitazones-reduced MMP-2 activity. These data suggesting NO is the positive regulator of MMP-2 in malignant glioma cells and dexamethasone and Rosiglitazone-induced MKP-1 which regulates MMP-2 activity and invasiveness in human malignant glioma cells via iNOS.

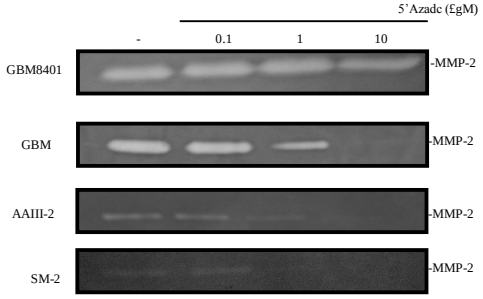
Keywords: MMP-2、Glioma 、 MKP-1、dexamethasone、Rosiglitazone

二、緣由與目的

Human malignant glioma cells are characterized by uncontrolled growth and rapid invasion of adjacent tissues. Despite radical surgery, conventional chemotherapy prognosis and radiation therapy remains poor and is associated with only 5% survival after 5 years of indication. As a consequence, available therapeutic strategies are still largely unsuccessful, showing only a minimal impact on the survival rate of patients [Zalutsky, 2005; Ohgaki, 2005]. Matrix metalloproteinases (MMPs) have been implicated as important factors in the control of the invasive capability of glioma cells. MMPs constitute a family of enzymes with more than 20 members identified, which are extracellular endopeptidases requiring metal ions for their enzymatic activity. [Price et al., 2001]. MMP-2 (also known as collagenase A) has been shown to be a prognostic factor in various malignancies such as ovarian cancer, gastric carcinoma and stage I non-small cell lung carcinoma [Davidson et al., 1999; Monig et al., 2001; Herbst et al., 2000]. The invasiveness of human gliomas has been attributed to high level of MMP-2 Indeed, inhibition of MAPK pathways resulted in suppression of MMP-2 expression [Galli et al., 2005; Choi et al., 2004]. Dexamethasone (dex) is one of glucocorticoids broadly used in anti-inflammation. Glucocorticoids has been shown to inhibit angiogenesis and suppress MMP-2 expression and reported to act as an angiogenesis inhibitor. These studies have shown that dexamethasone may regulate cell invasiveness through inhibitions of MAPK activity. Thiazolidinediones (TZDs) are PPAR-γ agonists, which have been shown to improve insulin sensitivity in vivo and have been used as new class of antidiabetic drugs [Mooradian et al.,2002]. Recently, TZDs have been shown to suppress MMP-2 activity by PPARγ independent pathway in the pancreatic cancer cells [Galli et al., 2004; Abe et al., 2002]. We previously found that rosiglitazone inhibited advanced glycosylation end products induced iNOS via p38MAPK signal pathway [Chang et al., 2004]. Thus, TZDs may regulate cell cycle progression through regulating MAPK activity. MAP kinase phosphatase 1 (MKP-1) (also known as CL100, 3CH134, Erp and hVH-1) belongs to dual-specificity phosphatase family, which inactivate MAPKs by dephosphorylation of both threonine and tyrosine residues within the activation motif. [Keyse and Emslie, 1992; Liu et al., 1994 and 1995; Gupta et al., 1996; Lai et al., 1996; Duff et al., 1995; Engelbrecht et al., 2003; Wadgaonkar et al., 2004]. We demonstrated that Dexamethasone and Rosiglitazone inhibited matrix invasiveness in cell lines derived from human gliomas via a mechanism dependent upon MKP-1 induction.

三、結果與討論

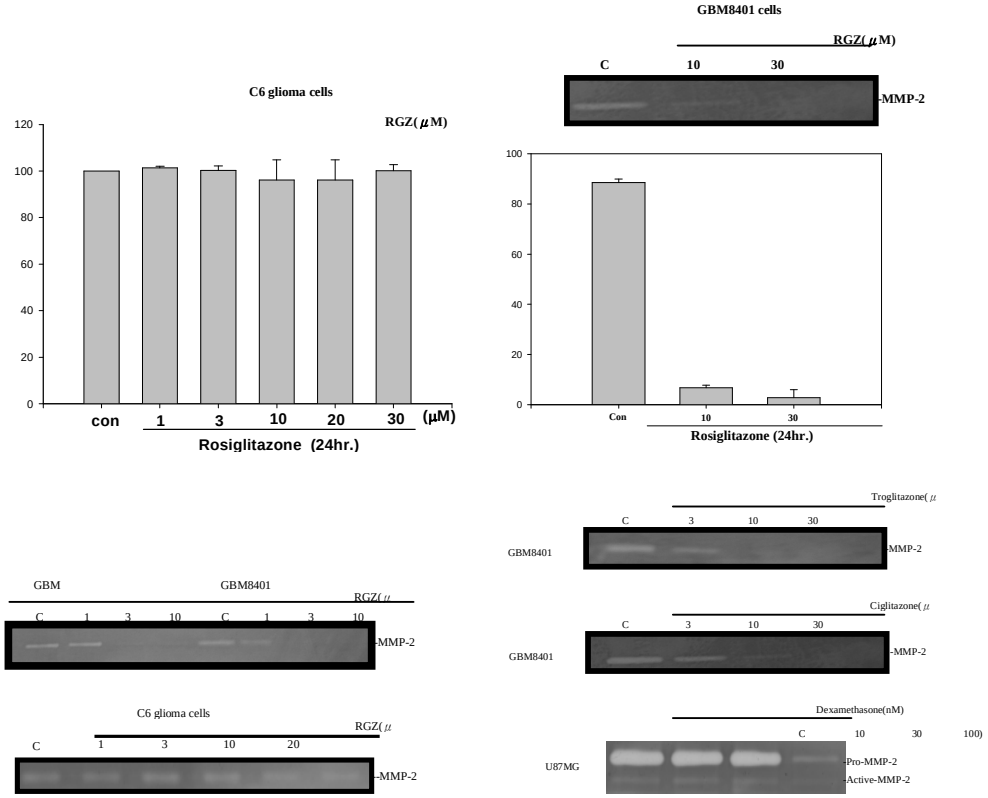
MMP-2 activity is correlated with maliganancy：



The endogenous MMP-2 activity in WHO grade IV gliomas, GBM8401 and GBM, were much higher than that in grande III glioma (AAIII-2) and in non-glial menigioma (SM-2). Inhibition of MMP-2 in grade IV gliomas cells required more than10μM of 5'Aza-deoxycytidine (5'-aza dC).

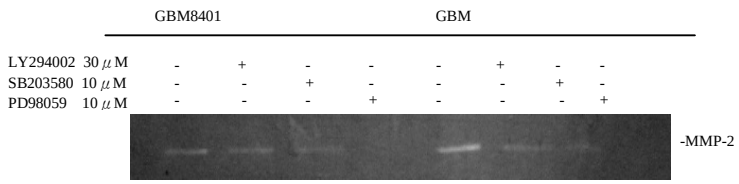
Gitazones and Dexamethasone inhibits MMP-2 activityin human glioma cells.

The effects of thazolidinediones and Dexamethasone on MMP-2 activity were analyzed using gelatinolytic zymography. Treatment of cells with glitazones on GBM8401 cells and Dexamethasone on U87 cells significantly inhibited MMP-2 activity in a dose dependent manner. glitazones have no cytotoxic effects on human glioma cells using MTT assay.



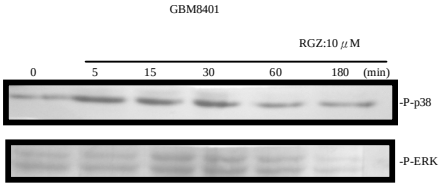
MMP-2 activity is regulated by MAPK signal pathway

Treatment of cells with pharmacological inhibitors specific for p38 MAPK (SB203580) or p42/44 ERK (PD98059) significantly inhibited MMP-2 acivity. We found that p38MAPK and ERK regulate MMP-2 activity in GBM8401 and GBM cells



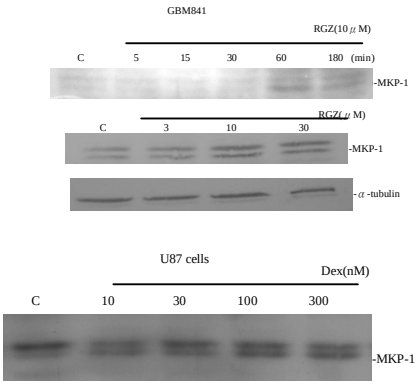
Effect of Rosiglitazone on p38MAPK and p42/44 ERK

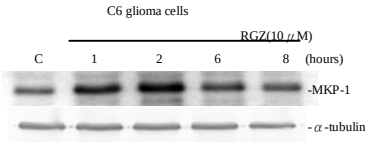
Incubation of GBM8401 cells with rosiglitazone transiently increase p38 MAPK and ERK phosphorylation, and the phosphorylation was declined at 60 minutes.



Glitazones and dexamethasone induce MKP-1 expression in glioma cells.

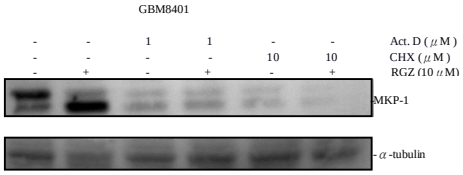
MKP-1 has been suggested to play a role in the negative regulation of cellular responses mediated by MAPK. We then examined whether Dexamethasone and Rosiglitazone would induce MKP-1 expression. Rosiglitazone increased MKP-1 protein levels at the time course when 38MAPK and p42/44 ERK was dephosphorylated.

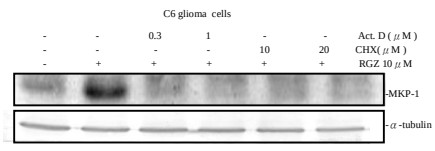


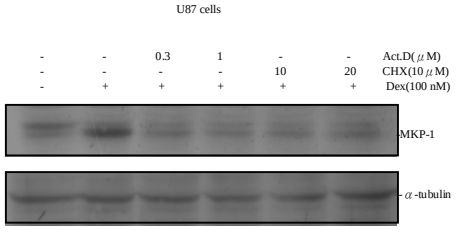


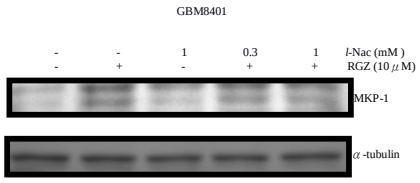
Mechanisms by which Dexamethasone and Rosiglitazone induce MKP-1 expression in glioma cells.

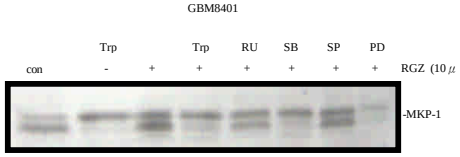
Treatment of glioma cells with actinomycin D (inhibition of transcription) or cycloheximide (inhibition of translation) for 30 min before addition of Dexamethasone and Rosiglitazone decreased MKP-1 protein levels, suggesting Dexamethasone- and Rosiglitazone- induced MKP-1 expression required de novo protein synthesis. We found that Rosiglitazone induce MKP-1 via reactive oxygen species (ROS). Rosiglitazone induced MKP-1 via p38MAPK and p42/44 ERK activation.





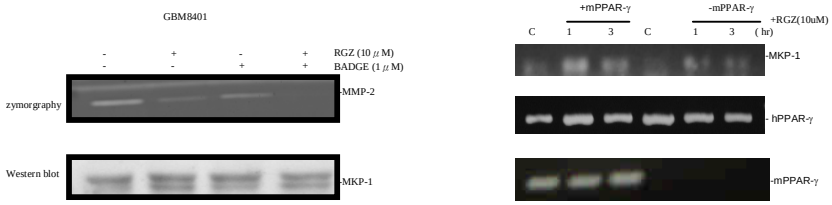


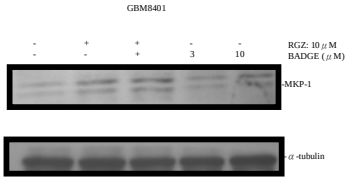




TZDs inhibit MMP-2 activity and MKP-1 expression through a PPAR-γ independent mechanism

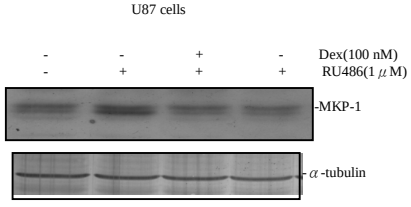
Inhibition of PPAR-γ by pharmacological antagonist (BADGE) or Overexpression of mouse PPAR-γ in human glioma cells. We found that Rosiglitazone inhibits MMP-2 activity and induces MKP-1 expression via PPAR-γ independent mechanism.

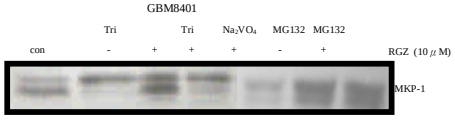


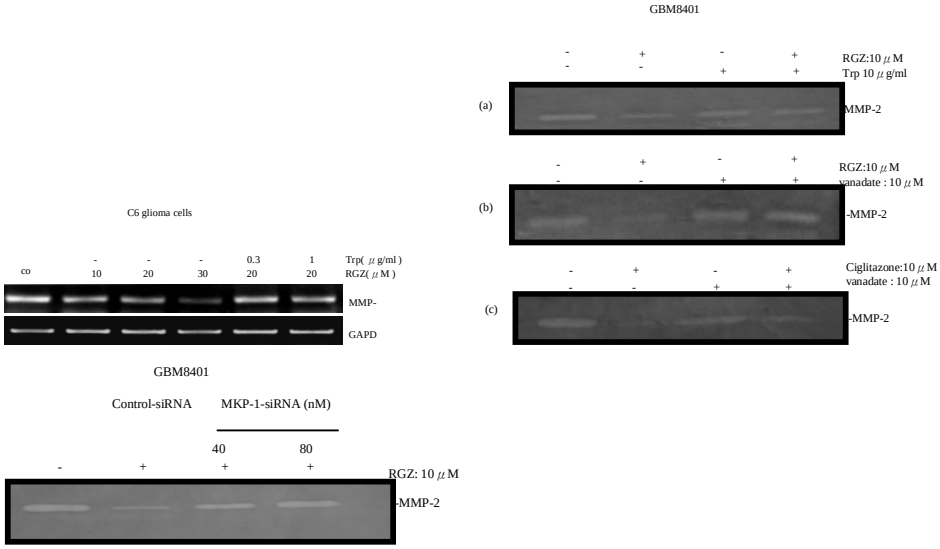


Roles of MKP-1 in MMP-2 expression

Treatment of cells with MKP-1 pharmacological inhibitors reduced Rosiglitazone-induced MKP-1 expression in GBM cells. Treatment of U87 cells with RU486, an antagonist glucocorticords receptor before treatment with dexamethasone. RU486 blocked dexamethasone-induced MKP-1 expression. MKP-1 siRNA were introduced to GBM8401 cells, we found that Rosiglitazone-inhibited MMP-2 activity is reversed. We found that induction of MKP-1 is linked to inhibition of MMP-2, and rosiglitazone inhibition of MMP-2 was examined

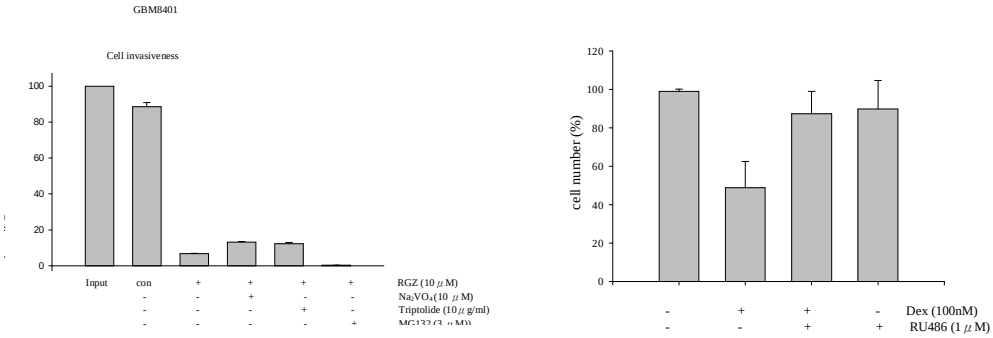






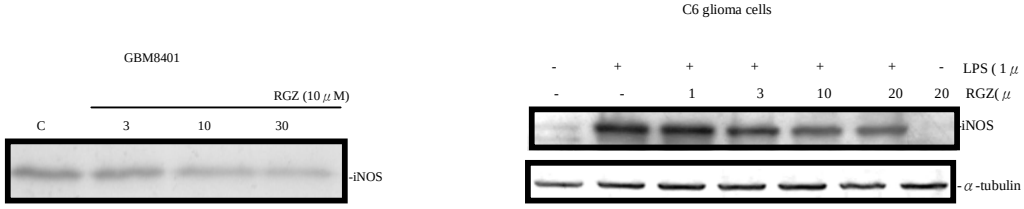
Rosiglitazone and Dexamethasone inhibit glioma cell invasiveness via MKP-1

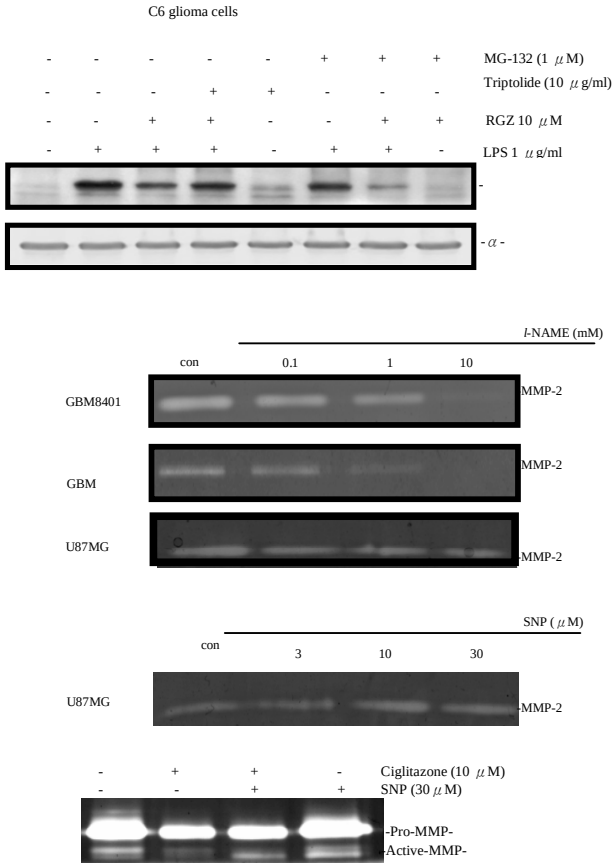
We use the Matrigel coated chamber to investigate the invasiveness of these glioma cells. Glioma cells exhibits high matrix invasiveness. Rosiglitazone and Dexamethasone inhibit cell ivasiveness. By using pharmacological inhibitor, we found that Rosiglitazone- and Dexamethasone-inhibited effect via MKP-1.



Roles of Nitric Oxide in regulating MMP-2 activity

We investigate whether iNOS expression is linked to MMP-2 expression in glioma cells. Treatment of glioma cells with *l*-NAME, a NOS inhibitor, reduced MMP-2 expression. And SNP, Sodium nitroprusside (Nitric Oxide donor) increased MMP-2 activity, suggesting SNP reverses Rosiglitazone inhibited MMP-2 activity. These data suggest that Nitric Oxide is the positive regulator of MMP-2.





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