

## The Anti-Glioma Effect of Tanshinone IIA in U-118MG Glioblastoma Cells via Decreasing the Mitochondrial Membrane Potential and Induction of Apoptosis

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**SUMMARY.** Glioma is the most common intracranial malignancy, finding a suitable therapeutic drug is of great significance. Tanshinone IIA (Tan IIA) is a biologically active ingredient isolated from the traditional Chinese medicine *Salvia miltiorrhiza*, which has an anti-glioma effect. This study is to observe the effect of Tan IIA on human brain glioblastoma U-118MG. To find the suitable concentration of Tan IIA to promote apoptosis of glioma cells, and further explore possible mechanisms. MTT assay was used to detect the effect of Tan IIA on the cell viability of U-118MG cells, flow cytometry was used to detect apoptosis, fluorescence spectrophotometer was used to detect intracellular calcium and mitochondrial membrane potential (MMP), and RT-PCR and western-blot were used to detect the expression of metallothionein 1E (MT1E), cytochrome C (Cyt C), and caspase 3. The cell viability of U-118MG cells decreased with the increase of Tan IIA concentration, and the percentage of apoptotic U-118MG cells is significantly increased. With the increase of intracellular calcium concentration, the MMP also decreased. And we detected that the expression of Cyt C and Caspase-3 mRNA in U-118MG cells increased with the increase of Tan IIA concentration. And the protein level of Cyt C and active-caspase 3 show the same results. Interestingly, in this study that the MT1E protein of 10  $\mu$ M Tan IIA group increased while that of 100  $\mu$ M group decreased. The anti-glioma mechanisms of Tan IIA are likely due to modulating  $Ca^{2+}$  homeostasis by up-regulating MT1E expression, to promote the Cyt C release and MMP decreases, thereby activate the caspase-3, leading to apoptosis.

**RESUMEN.** El glioma es la neoplasia maligna intracranial más común, por lo que encontrar un medicamento adecuado es de gran importancia. Tanshinone IIA (Tan IIA) es un ingrediente biológicamente activo aislado de la medicina tradicional china *Salvia miltiorrhiza*, que tiene un efecto anti-glioma. Este estudio es para observar el efecto de Tan IIA en el glioblastoma de cerebro humano U-118MG. Para encontrar la concentración adecuada de Tan IIA para promover la apoptosis de las células de glioma y explorar aún más los posibles mecanismos. Se usó el ensayo MTT para detectar el efecto de Tan IIA en la viabilidad celular de las células U-118MG, se usó citometría de flujo para detectar apoptosis, un espectrofotómetro de fluorescencia para detectar calcio intracelular y potencial de membrana mitocondrial (MMP) y RT-PCR y Western-blot se utilizaron para detectar la expresión de metalotioneína 1E (MT1E), citocromo C (Cyt C) y caspasa 3. La viabilidad celular de las células U-118MG disminuyó con el aumento de la concentración de Tan IIA y el porcentaje de células U-118MG apoptóticas se incrementa significativamente. Con el aumento de la concentración de calcio intracelular, la MMP también disminuyó. Detectamos que la expresión de ARNm de Cyt C y Caspasa-3 en células U-118MG aumentó con el aumento de la concentración de Tan IIA y que el nivel de proteína de Cyt C y caspasa-activa 3 muestran los mismos resultados. Curiosamente, en este estudio, la proteína MT1E del grupo Tan IIA 10  $\mu$ M aumentó mientras que la del grupo 100  $\mu$ M disminuyó. Los mecanismos anti-glioma de Tan IIA probablemente se deben a la modulación de la homeostasis de  $Ca^{2+}$  mediante la regulación positiva de la expresión de MT1E, para promover la liberación de Cyt C y la disminución de MMP, activando así la caspasa-3, lo que lleva a la apoptosis.

**KEY WORDS:** apoptosis, glioma, intracellular calcium, metallothionein 1E, mitochondrial membrane potential, tanshinone IIA.

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