

OMTO, Volume 22

Supplemental information

Exosome-mediated transfer of MIF confers temozolomide resistance by regulating TIMP3/PI3K/AKT axis in gliomas

Q.T. Wei, B.Y. Liu, H.Y. Ji, Y.F. Lan, W.H. Tang, J. Zhou, X.Y. Zhong, C.L. Lian, Q.Z. Huang, C.Y. Wang, Y.M. Xu, and H.B. Guo

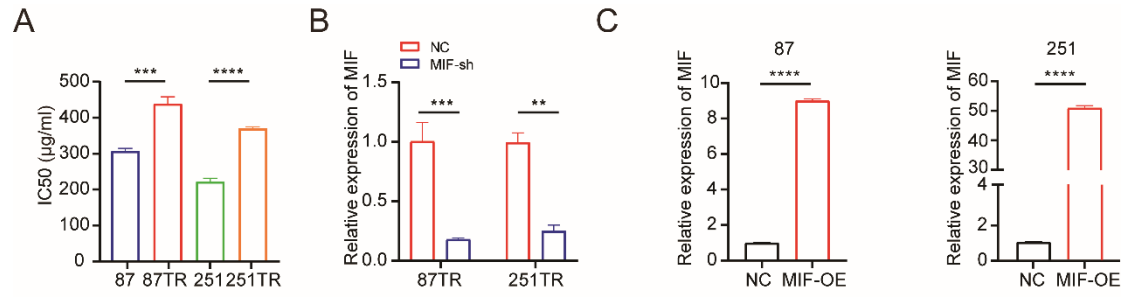


Figure S1. TR cells were verified and the efficiency of MIF knockout or overexpress was confirmed. (A) The IC50 value of TR cells verified by CCK8 was significantly higher than that of TS cells. (B) RT-qPCR confirmed the low efficiency of MIF knockout in TR cells. (C) RT-qPCR confirmed the high efficiency of MIF overexpression in TS cells.

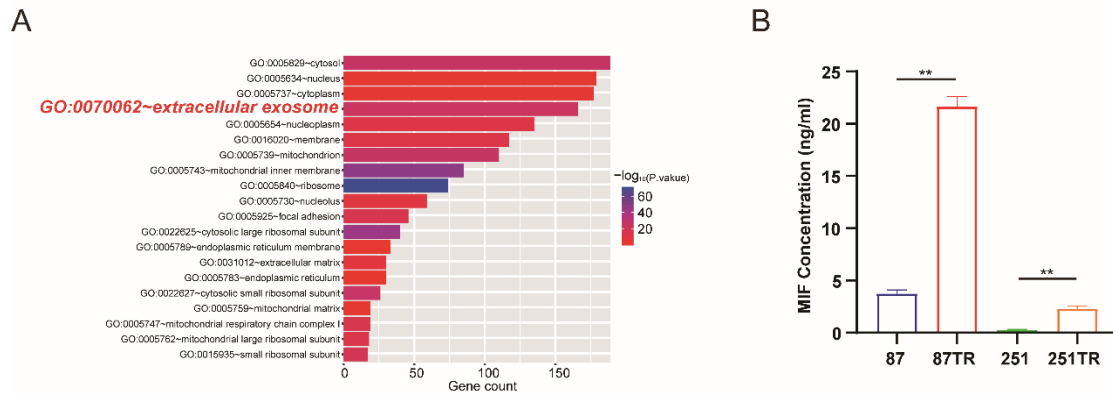


Figure S2. MIF was validated to be related to exosomes. (A) Differential expressed genes (DEGs) analysis of MIF in TCGA database showed the DEGs were enriched in extracellular exosome. (B) ELISA results for MIF expression in cell culture supernatant. Cells, 2×10^5 each, were cultured in 2ml medium for 48 hours. The culture supernatants were assessed by ELISA for MIF.

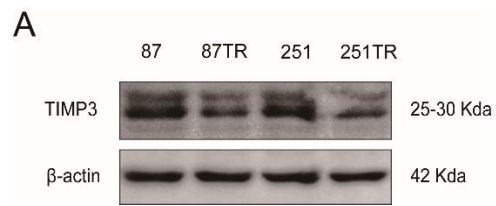


Figure S3. TIMP3 was significantly down-regulated in TR cells. (A) Western blot analysis showed that TIMP3 was significantly down-regulated in TR cells compared to homologous TS cells.

Supplementary Table S1: The primers used in RT-qPCR analysis in glioma cells.

mRNA	Forward primer	Reverse primer
MIF	CGGACAGGGTCTACATCAA	CTTAGGCGAAGGTGGAGTT
TIMP3	CATGTGCAGTACATCCATACGG	CATCATAGACGCGACCTGTCA
GAPDH	CAATGACCCCTTCATTGACC	GACAAGCTTCCCGTTCTCAG

Supplementary Table S2: Information on antibodies used in glioma cells.

Reagent	Source	Identifier
Anti-MIF	Novus Biologicals	NBP1-81832
Anti-TIMP3	Abcam	ab39184
Anti-PI3K	Abcam	ab151549
Anti-CD81	Abcam	ab219209
Anti-TSG101	Abcam	ab125011
Anti-ALIX	Abcam	ab186429
Anti-Caspase-3	Cell Signaling Technology	Cat#9662
Anti-Caspase-9	Cell Signaling Technology	Cat#9508
Anti-P-c-Raf	Cell Signaling Technology	Cat#9421
Anti-AKT	Cell Signaling Technology	Cat#4691
Anti-P-Akt (Ser473)	Cell Signaling Technology	Cat #4060
Anti-P-Akt (Thr308)	Cell Signaling Technology	Cat #13038
Anti- β -actin	Cell Signaling Technology	Cat#4970
Anti-BAX	Bioss ANTIBODIES	bsm-33279M
Anti-P21	Proteintech	10355-1-AP
Anti-CDK2	ImmunoWay	YT0832
Anti-Cyclin H	ZenBio Science	200542-1A7