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Li et al. from Suzhou investigated the roles and anti-cancer mechanism of artificially synthesized EGF-containing fibulin-like extracellular matrix protein (EFEMP1) derived tumor suppressor ZR30 protein in glioma (GBM).

ZR30 protein were in vitro expressed using a wheat germ cell-free system. GBM cell lines (U251, U251NS, and U87) were cultured for 2-3 days in the presence or absence of ZR30 treatment. MMP-2 level was detected by gelatin zymography assay, moreover, the expression of EGFR, Notch-1 and p-Akt/Akt levels were determined by western blot. Additionally, MTT assay was used to measure ZR30's effect on the cell proliferation of U251 and U251NS cells. Furthermore, pre-mixed U251-GFP and U251NS-RFP cells (1:9) were injected into the brain of nude mice, and then ZR30 or PBS was injected into the intra-tumor after 10 and 21 days, respectively. Then DNA was extracted from the right brain of nude mice in each group. Comparative quantitative polymerase chain reaction (CQ-PCR) was used to examine the copy numbers of human gene hSPAG16, mouse gene mSpag16, GFP and RFP. The survival status of each group of nude mice was also observed. Results: The levels of activated MMP-2 in U87 and U251 cells were lower after 10, 50 and 100 ng/ml ZR30 treatment for 2-3 days. Western blot analysis showed that ZR30 treatment reduced the expression of EGFR, Notch-1 and p-Akt/Akt in U251 cells, and inhibited Notch-1 and p-Akt/Akt expression in U251NS cells, and then decreased the response of U251 cells to EGF stimulation. Moreover, ZR30 inhibited the cell proliferation of U251 and U251NS two days after exposure. The in vivo orthotopic GBM xenografts were successfully constructed. CQ-PCR results indicated that the hSPAG16/mSpag16 ratios of mice in PBS group and ZR30 treatment groups at 180, 700, and 1 800 ng dosages were 3.67±2.82, 1.18±0.97, 1.75±1.55 and 1.38±1.17, respectively, and ZR30 treatment groups showed significantly lower ratios than the PBS group (P<0.05 for all). Correspondingly, the ratios of GFP/RFP in each group were 1.97 $\pm$ 0.80,  $1.97\pm0.85$ ,  $1.48\pm0.71$  and  $1.73\pm0.77$ , respectively, showing no statistical significance (P>0.05 for all). When treatment was performed 10 d after cell implantation, and the median survival time of mice in PBS group and ZR30 group was 40.5 days and 59.0 days, respectively. When treatment was performed 21 d after cell implantation, the median survival time of mice in PBS group and ZR30 group was extended to 57.0 days and 74.5 days, respectively. The median survival time of ZR30 treatment groups significantly prolonged (P<0.05 for all). Conclusions: ZR30 inhibits in vitro cell growth, invasion, angiogenesis and stemness maintenance in glioma via suppressing activated MMP-2, EGFR, p-Akt/Akt and Notch-1 proteins. In vivo, ZR30 markedly increased survival of mice harboring glioma xenografts, even for only one intra-tumoral injection at the time of early tumor formation. Overall, the in vivo and in vitro experiments supported the therapeutic potential of ZR30 for GBM 1).

Li YY, Chen XH, Sun T, Hu Y, Zhou YH, Zhou YX. [The anti-cancer effect of ZR30 protein via targeting extracellular signal proteins of different cell subpopulations of glioma]. Zhonghua Zhong Liu Za Zhi. 2018 Nov 23;40(11):812-817. doi: 10.3760/cma.j.issn.0253-3766.2018.11.003. Chinese. PubMed PMID: 30481930.

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