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#681 Knockdown of connective tissue growth factor and treatment with temozolomide inhibited invasion, angiogenesis, and tumorigenesis of human glioblastomas.

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Connective tissue growth factor (CTGF) is a putative proto-oncogene and plays a crucial role in endothelial cell migration and tumor angiogenesis. CTGF is highly upregulated in proliferating endothelial cells and glioblastoma cells. In this investigation, we examined whether knockdown of CTGF at the mRNA level and treatment with temozolomide (TMZ) could inhibit cell invasion, angiogenesis, and growth of human glioblastoma SNB-19 and T98G cells in vivo. The cells were stably transfected with a plasmid vector carrying the human CTGF siRNA cDNA and then treated with 10 μM TMZ for 48 h. Semiquantitative PCR, Western blotting, and immunohistochemical staining demonstrated 80% downregulation of CTGF both at mRNA and protein levels after stable transfection. Matrigel invasion, cell migration from spheroids, and cell proliferation studies demonstrated significant inhibition of cell invasion, migration, and proliferation, respectively, of both cell lines after downregulation of CTGF and treatment with TMZ. In vitro and in vivo angiogenesis assays demonstrated inhibition of network formation of endothelial cells and neovascularization under the dorsal skin of nude mice, respectively, after knockdown of CTGF and treatment with TMZ. Both subcutaneous and intracerebral tumorigenesis in nude mice was markedly reduced in CTGF downregulated cells after TMZ treatment. Mechanistic studies demonstrated significant reduction of PCNA, VEGF, c-Myc, CDK2, CDK4, and cyclin D1 and upregulation of the cell cycle inhibitors p21Waf1 and p27Kip1. Flow cytometric analysis showed cell cycle arrest at G2/M phase in both cell lines after CTGF knockdown and TMZ treatment. Taken together, our study indicates that the CTGF knockdown and TMZ treatment effectively prevents cell invasion, migration, angiogenesis, and growth of glioblastomas in vivo. Therefore, CTGF knockdown during TMZ treatment offers a novel and potential therapeutic strategy for controlling the growth of glioblastomas. This investigation was supported in part by the R01 grants (CA-91460 and NS-57811) from the National Institutes of Health (Bethesda, MD).

Citation Format

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