HOXC10

To study the effects of Homeobox C10 (HOXC10) on biological characteristics such as migration, invasion and proliferation of glioma cancer cells and to explore the role of HOXC10 gene in glioma microenvironment.

The expression level of HOXC10 in high grade glioma (glioblastoma) and low-grade glioma and its effect on patient survival were analyzed by using The Cancer Genome Atlas (TCGA) and Chinese Glioma Genome Atlas (CGGA) database. Hoxc10-siRNA-1, HOXC10-siRNA-2 and siRNA negative control (NC) were transfected into U251 cells according to the operation instructions of HOXC10-siRNA transfection. 100 ng/ mL recombinant protein chemokine ligand 2 (reCCL2) was added into the transfection group, and was labeled as HOXC10-siRNA-1+ reCCL2 and HOXC10-siRNA-2+ reCCL2 groups. The expressions of HOXC10 mRNA and target protein in each group was detected by real-time fluorescence quantitative polymerase chain reaction (qRT-PCR) and western blot. The proliferation ability of cells in each group was detected by cell counting kit 8 (CCK8) method. The migration ability of cells was detected by Transwell assay and Nick assay, and cell apoptosis was detected by flow cytometry. The expression of chemokines in each group was detected by multiple factors. Co-incubation assays were performed to determine the role of HOXC10 and chemokine ligand 2 (CCL2) in recruiting and polarizing tumor-associated macrophages (M2-type macrophages). Results: The median expression level of HOXC10 in high grade gliomas was 8.51, higher than 1.00 in low-grade glioma s ($P<0.001$) in TCGA database. The median expression level of HOXC10 in high grade gliomas was 0.83, higher than 0.00 in low-grade glioma s ($P=0.002$) in CGGA database. The 5-year survival rate of patients with high HOXC10 expression in TCGA database was 28.2%, lower than 78.7% of those with low HOXC10 expression ($P<0.001$), and the 5-year survival rate of patients with high HOXC10 expression in CGGA database was 20.3%, lower than 58.0% of those with low HOXC10 expression ($P<0.001$). The numbers of cell migration in HOXC10-siRNA-1 group and HOXC10-siRNA-2 group were (45±3) and (69±4) respectively, lower than (159±3) in NC group ($P<0.05$). The cell mobility of HOXC10-siRNA-1 group and HOXC10-siRNA-2 group at 48 hours were (15±2)% and (28±4)% respectively, lower than (80±5)% of NC group ($P<0.05$). The expressions of vimentin in HOXC10-siRNA-1 group and HOXC10-siRNA-2 group were (141 740.00±34 024.56) and (94 655.00±5 687.97), N-cadherin were (76 810.00±14.14) and (94 254.00±701.45), β-catenin were (75 786.50±789.84) and (107 296.50±9 614.53), lower than (233 768.50±34 114.37), (237 154.50±24 715.50) and (192 449.50±24 178.10) of NC group ($P<0.05$). The A value of HOXC10-siRNA-1 group and HOXC10-siRNA-2 group were (0.44±0.05) and (0.32±0.02) at 96 hours, lower than 0.92±0.12 of NC group ($P<0.05$). The apoptosis rates of HOXC10-siRNA-1 group and HOXC10 siRNA-2 group were (10.23±1.24)% and (13.81±2.16)%, higher than (4.60±0.07)% of NC group ($P<0.05$). The expression levels of CCL2 in U251 cells in HOXC10-siRNA-1 and HOXC10-siRNA-2 groups were (271.63±44.27) and (371.66±50.21), lower than (933.93±29.84) in NC group ($P<0.05$). The expression levels of CCL5 (234.81±5.95 and 232.62±5.72), CXCL10 (544.13±48.14 and 500.87±15.65) and CXCL11 (215.75±15.30 and 176.18±16.49) in HOXC10-siRNA-1 and HOXC10-siRNA-2 groups were higher than those in NC group (9.98±0.71, 470.54±18.84 and 13.55±0.73, respectively, $P<0.05$). The recruited numbers of CD14(+) THP1 in HOXC10-siRNA-1 and HOXC10-siRNA-2 groups were (159.33±1.15) and (170.67±1.15), respectively, lower than (360.00±7.81) in NC group ($P<0.05$), while addition of reCCL2 promoted the recruitment of CD14(+) THP1 cells (287.00±3.61 and 280.67±2.31 in HOXC10-siRNA-1+ reCCL2 group and HOXC10-siRNA-2+ reCCL2 group, respectively, $P<0.05$). The expressions level of M2-type macrophage-related gene TGF-β in HOXC10-siRNA-1 group and HOXC10-siRNA-2 group were (0.30±0.02) and (0.28±0.02), respectively, lower than (1.06±0.10) in NC group ($P<0.05$). The expressions level of M1-related gene NOS2 in HOXC10-siRNA-1 and HOXC10-siRNA-2 were (11 413.95±1 911.85) and (5 894.00±945.21), respectively, higher than (13.39±4.32) in NC group

(P<0.05). Conclusions: The expression of HOXC10 in glioma is high and positively correlated with the poor prognosis of glioma patients. Knockdown of HOXC10 can inhibit the proliferation, migration and metastasis of human glioma U251 cells. HOXC10 may play an immunosuppressive role in glioma microenvironment by promoting the expression of CCL2 and recruiting and polarizing tumor-associated macrophages (M2 macrophages) [1].

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