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The role of intratumoral CXCR3 in Glioblastoma (GBM)

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BACKGROUND

CXCR3 is a chemokine receptor which plays the role of a "double-edged sword" in cancer progression and resistance through the **autocrine** and **paracrine** pathways. The autocrine pathway increases tumor formation and growth, whereas the paracrine pathway recruits immune cells including Th1, CD8+ and NK cells which all help to suppress tumor growth (1).

This study investigates the phenotypic functions, potential mechanisms and clinical translations of intratumoral CXCR3 in GBM.

METHODS

In vitro, U87 & U251 GBM cells w/wo CXCR3-siRNA are cultured investigated using western blot assay, qPCR, MTT, Colony formation, IHC.

In vivo, U87 & GL261 cells w/wo CXCR3-shRNA are injected intracranially into nude and C56 WT mice. If CXCR3-shRNA tumors demonstrate decreased growth, CXCR3 inhibitor AMG487 is given at different dosages. Growth is quantified using IVIS luminescence system.

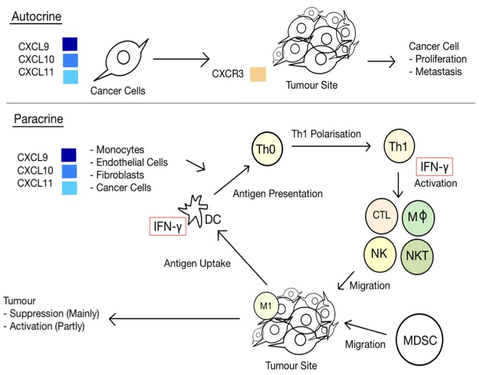

Figure 1) Autocrine and paracrine pathways of CXCR3.

IN VITRO

Compared to our control, both siRNA-CXCR3 U87 and U251 cells lines demonstrated **significantly reduced proliferation** from day 1 up till day 3.

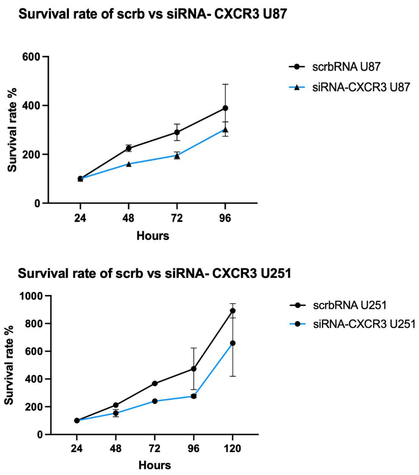

Figure 2a) MTT assay, siRNA-CXCR3 U87 & U251 compared to control. Growth was recorded for 4 days from day 0 to day 3.

Mechanistically, we briefly investigated a range of primary antibodies from DNA damage, ER stress, Phospho-MAPK family, death receptor antibody and mesenchymal transition, and discovered that IRE1a, E-Cadherin, Phospho-AKT decreased when silencing CXCR3, whilst Phospho-P46 SAPK/ JNK levels increased. CXCR3 also reduces PD-L1 levels also drastically, although it is only visible in U251.

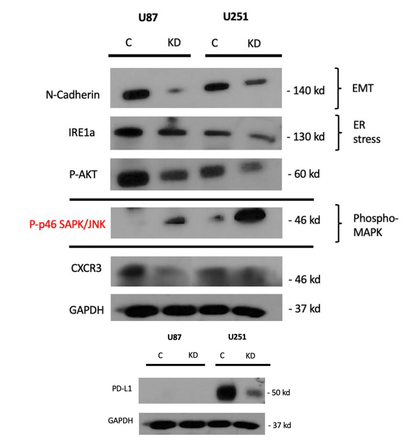

Figure 2b) Western blot, different mechanistic markers and PD-L1 in siRNA-CXCR3 U87 and U251 compared to control.

IN VIVO

a) Compared to negative control, Sh-RNA CXCR3-homo453 U87 tumor was **significantly smaller** at day 7. This draws parallels to previous studies which demonstrated CXCR3 contributes to invasion in GBM (2).

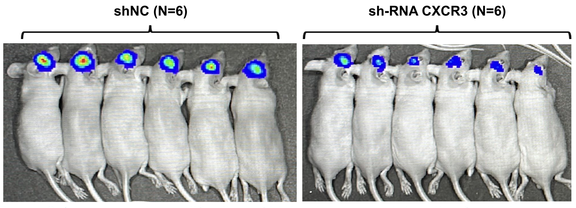

Figure 3a) Image and quantification of bioluminescence intensity of orthotopic xenograft in nude mice bearing U87 cells at day 7 after injection. N = 3 per group.

b) CXCR3 inhibitor (AMG487) is given subcutaneously at a dosage of 5mg/kg and 10 mg/kg every day after the 2nd week. Compared to vehicle, CXCR3 inhibitor is shown to **clearly reduce tumor size** at a dose-dependent response.

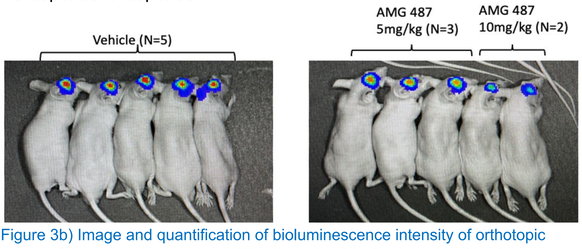

Figure 3b) Image and quantification of bioluminescence intensity of orthotopic xenograft in nude mice bearing U87 cells at day 21 after injection. N = 3 per group.

INTERACTIONS WITH TEMOZOLOMIDE

Contradicting to what we predicted, post treatment siRNA-CXCR3 cell lines with TMZ at different dosages actually had relatively lower levels of cell death, with the effect particularly prominent in U251. Thus, we propose that TMZ addition could increase CXCR3 levels, which had previously been shown to be the case in cutaneous melanoma (3). Perhaps specificity and efficacy of **CXCR3 inhibitor** can be increased with more specific drug delivery to achieve maximal effect.

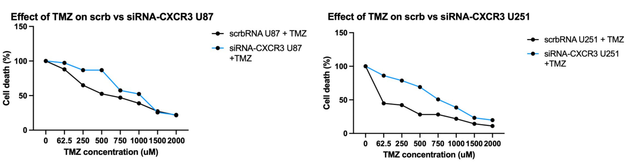

Figure 4a) MTT assay, different concentrations of TMZ in siRNA-CXCR3 U87 and 251 vs control. Cell death was recorded 72 hours post treatment.

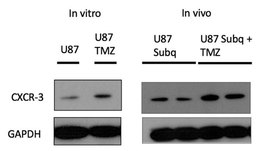

Figure 4b) Western blot, changes in CXCR3 after TMZ induction in vitro and in vivo.

CONCLUSION

CXCR3 reduces growth both in vitro and in vivo in GBM. The autocrine pathway can be targeted mechanistically as shown with initial success with CXCR3 inhibitor therapy, although it is unclear what it's exact paracrine function is and how it may interact with current conventional therapy.

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