CCL5 and endoglin as a Therapeutic Target in ER positive Breast Cancer

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Abstract

Estrogen receptor (ER) drives approximately 75% of all breast cancers and serves as the primary target for endocrine therapies. However, resistance to these therapies remains a major clinical hurdle, especially in metastatic ER-positive breast cancers harboring activating ESR1 mutations (e.g., Y537S, D538G) within the ligand-binding domain. These mutations are commonly detected in metastatic lesions and circulating tumor DNA (ctDNA) from patients undergoing endocrine treatment. While combination therapies with CDK4/6 inhibitors have shown promise, the mechanisms driving resistance in ESR1-mutant tumors and their associated therapeutic vulnerabilities remain poorly defined. To address this gap, we investigated the interaction between endocrine-resistant breast cancer (ERBC) cells and the tumor microenvironment. Using co-culture systems involving seven ERBC cell lines—including genome-edited ESR1 mutants—and four stromal cell types, we profiled 28 tumor-stroma secretome combinations via cytokine antibody arrays. This approach identified CCL5 and endoglin as significantly upregulated in resistant tumor-stroma interactions. We hypothesized that CCL5 promotes endocrine resistance and metastatic progression through paracrine signaling with stromal components. To test this, we generated CCL5 knockout EO771 cells using CRISPR-Cas9 and confirmed CCL5 loss through qRT-PCR and ELISA. CCL5-deficient cells exhibited markedly reduced proliferation and migration in vitro. In vivo, orthotopic mouse models demonstrated that CCL5 knockout tumors had significantly decreased growth and metastasis. Moreover, treatment with maraviroc, a CCR5 antagonist, selectively impaired the viability of wild-type but not CCL5-deficient cells. Together, these findings identify CCL5 as a key driver of endocrine resistance and metastatic potential in ERBC, highlighting it as a promising target for therapeutic intervention. This study lays the groundwork for future clinical strategies aimed at overcoming resistance in ESR1-mutant breast cancers by disrupting CCL5 signaling.

Objective

To investigate if CCL5 plays a critical role in EO771 cells.

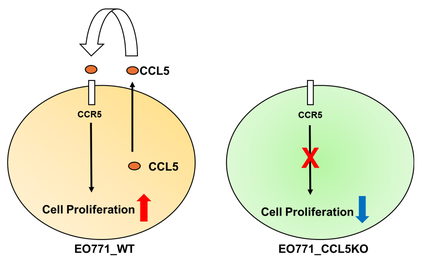


Fig.1. Schematic representation of the research strategy for investigating tumor growth in CCL5 depletion.

Results

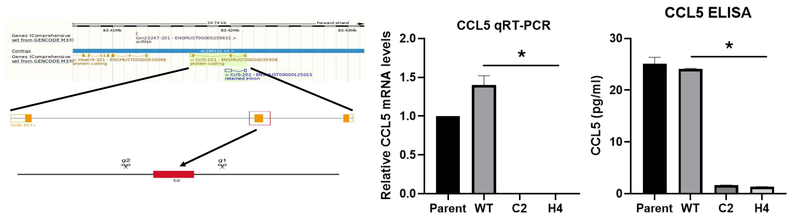


Fig.2. CRISPR-Cas9 mCCL5 gene knockout in EO771 cell line. 3 exons are identified with the ATG start codon in Exon1 and the stop codon in Exon 3. Exon2 is the target region. (Left) Real time qRT-PCR and (Right) ELISA analysis of mCCL5 in EO771 wild type cells and two clones of EO771_mCCL5 knockout cells (C2 and H4). *P<0.001.

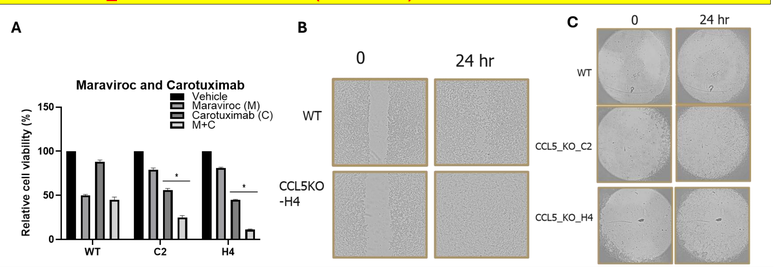


Fig.3. (A) Cell viability assay of EO771 WT and CCL5 KO cells with 1 uM maraviroc (CCR5 inhibitor), 10 uM carotuximab (endoglin mAb), and the combination treatment for 48 hours. Cell migration assay of EO771 WT and CCL5 KO cells using the wound healing assay (B) and (C) Oris migration assay with collagen coated plate. *P<0.05.

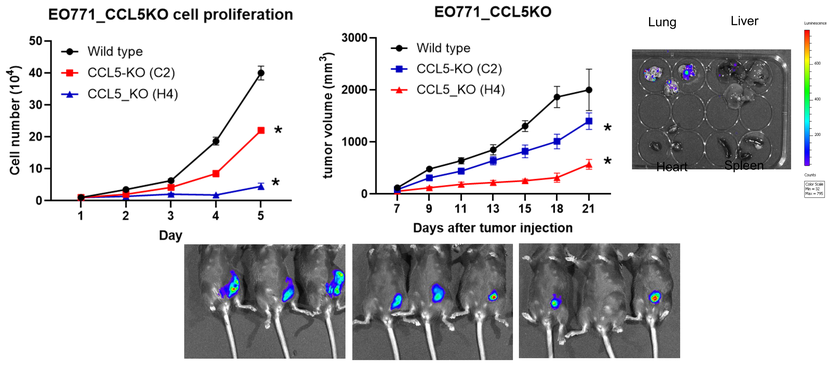


Fig.4. Cell counts was conducted for 5 days with a hemocytometer. Tumour volumes were measured after implantation of EO771_WT and CCL5KO cells into the 4th mammary gland of C57BL/6 mice [mean tumour volume (mm3) ± s.d.]. Representative mammary tumors and organs observed with the IVIS imager. *P<0.05.

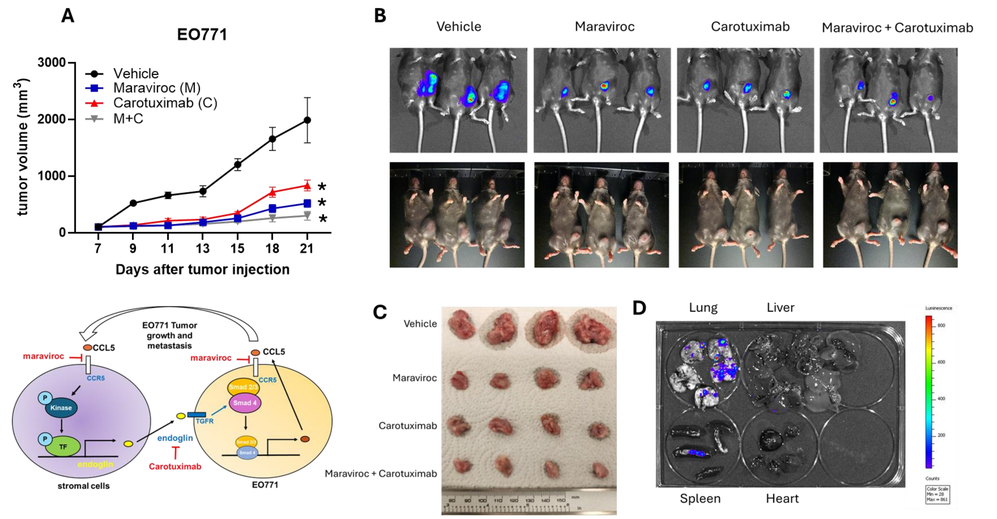


Fig.5. (A) Tumour volumes were measured following the treatment of maraviroc, carotoximab, and the combination after implantation of EO771_CCL5KO cells into the mammary gland of C57BL/6 mice [mean tumour volume (mm3) ± s.d.]. (B) Representative mammary tumors observed with the IVIS imager. (C) Representative tumors and (D) organ images of WT tumor under the IVIS imager. *P<0.05.

Conclusions

1. We successfully generated EO771_mCCL5 knockout (KO) cell lines.
2. The WT cells exhibited a faster growth rate than the CCL5 KO cells, suggesting that CCL5 plays a crucial role in EO771 cell proliferation.
3. Using an EO771 tumor-bearing C57BL/6 mouse model, we observed that the growth rate of WT tumors significantly increased over 21 days compared to the CCL5 KO tumors. These findings indicate that the autocrine loop of CCL5 is a key factor in EO771 tumor growth and metastasis.
4. Inhibiting CCR5 using maraviroc, a CCR5 inhibitor, reduced the proliferation of EO771_WT cells while carotuximab inhibited CCL5 KO cell proliferation.
5. The combination of maraviroc and carotuximab enhanced the suppression of CCL5 KO cell proliferation more effectively than either treatment alone.
6. These findings suggest that the autocrine loop of CCL5 and endoglin plays a critical role in EO771 cell growth.
7. Utilizing an EO771 tumor-bearing C57BL/6 mouse model, we confirmed that both maraviroc and carotuximab significantly suppressed tumor growth and metastasis, with the combination treatment showing a synergistic enhancement of the inhibitory effect.

Acknowledgements

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