Coexpression of Leukocyte Cytosolic Protein 1 and Matrix Metalloproteinases are Integral for Metastatic Progression of Chondrosarcoma

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INTRODUCTION: Chondrosarcoma (CSA) lacks effective systemic therapies for metastatic disease. Thus, novel targets are necessary. Our previous work identified overexpression of Leukocyte Cytosolic Protein 1 (LCP1) in metastasizing CSAs and expression levels further correlated with patient outcomes. We hypothesize that LCP1 directly effects cell motility, which may be accomplished via cytoskeletal effects that promote formation of invadopodia, and increase production of matrix metalloproteinases (MMPs).

METHODS: Four patient derived CSA cell lines (2 LCP1 high metastasizing; and 2 LCP1 low non-metastasizing) and their conditional knockout/overexpression conditions were used. Cell viability, wound healing, and 3D cell invasion assays were performed to assess proliferation, migration, and invasion. Bulk RNA sequencing data and protein quantification via Luminex assay were performed to measure expression of MMP-1 and MMP-9. Hydrogel matrices were generated to recapitulate a 3D environment. Protein localization was demonstrated with immunocytochemistry.

RESULTS: While cell viability remained unchanged, LCP1 knockout decreased, and overexpression increased cell migration and invasion. MMP-1 and MMP-9 were significantly increased at the RNA and protein level in the LCP1 high cell lines with immunocytochemistry demonstrating localization of MMPs at the invadopodia edge.

DISCUSSION AND CONCLUSION: Our findings suggest that LCP1 directly contributes to cell motility and invasiveness in CSA. This effect may be further mediated by increased coexpression of MMP-1 and MMP-9 activity. Our findings suggest common regulatory machinery for the expression of LCP1 and MMP1/9, as well as potential for targeted agents with multiple therapeutic effects that we continue to investigate.

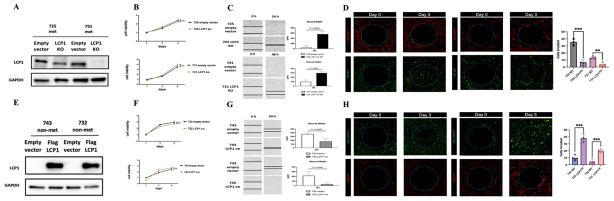

Figure 1: Impacts of LCP1 expression on CSA behavior *in vitro* and invasion potential. **A)** LCP1 KO cell lines for the 725 and 731 primary tumor was confirmed via western blotting. **(B)** Five-day cell viability with perturbations in LCP1 expression in the 725 and 731 cell lines. **(C)** Scratch wound assays with perturbations in LCP1 expression in the 725 and 731 cell lines. **(D)** 3D cell invasion with comparison of day 0 to day 3 in wild type and KO, 725 and 731 cell lines. **(E)** LCP1 OE cell lines for the 743 and 732 primary tumor was confirmed via western blotting. **(F)** Five-day cell viability with perturbations in LCP1 expression in the 743 and 732 cell lines. **(G)** Scratch wound assays with perturbations in LCP1 expression in the 743 and 732 cell lines. **(H)** 3D cell invasion with comparison of day 0 to day 3 in wild type and OE, 743 and 732 cell lines.

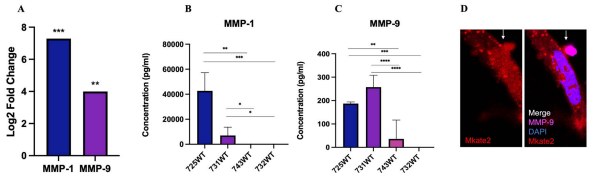

Figure 2: MMP-1 and MM-9 expression in CSA. **(A)** Differential expression of MMP-1 and MMP-9 in CSA metastatic vs. non-metastatic primary tumors via RNA sequencing **(B)** MMP-1 and **(C)** MMP-9 excreted protein expression in metastatic lines (725, 731) and non-metastatic lines (732, 743) via Luminex assay. **(D)** Fluorescent Immunocytochemistry of the 3D hydrogel matrix. White arrows localizing invadopodia and co-staining of MMP-9 [Red; Mkate2 (endogenous fluorophores), Blue; DAPI, Magenta; MMP-9].