Development of a Novel Combination Therapy targeting MET and LGR5 to overcome Colorectal Cancer Resistance

Shraddha Subramanian[1,2], Tressie Posey[1,2], Joan Jacob[1,2], and Kendra S. Carmon[1,2]

[1] Center for Translational Cancer Research, Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center, Houston, TX, USA

[2] University of Texas MD Anderson Cancer Center UTHealth Houston Graduate School of Biomedical Sciences, Houston, TX, USA

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center® | UTHealth® Houston

Graduate School of Biomedical Sciences

INTRODUCTION

Therapy-induced resistance and recurrence contribute to a majority of the challenges encountered in the clinical management of colorectal cancer (CRC). The chief culprits behind colorectal tumor relapse are cancer stem cells (CSCs). CSCs promote tumor progression and clonal heterogeneity owing to their self-renewability, plasticity, and differentiation capacity. Upon therapy termination, CSCs exit dormancy and circulate to secondary sites where they spawn metastases leading to disease-induced mortality. Leucine rich repeat-containing G protein-coupled receptor 5 (LGR5) is highly expressed in CRC and is a bona fide marker of functional CSCs. LGR5$^+$ CSCs are often responsible for tumor initiation and metastatic outgrowth, however their conversion into a chemo-resistant LGR5$^-$ state is vital for metastatic dissemination. LGR5-targeted therapy results in tumor regression, yet LGR5$^-$ tumors eventually relapse due to plasticity. Successful elimination of CRC tumors can be achieved by targeting both LGR5$^+$ CSCs and LGR5$^-$ CRC cell populations. LGR5$^-$ CRC cells, at least in part, rely on the MET/STAT3 pathway to evade therapy. MET is a well-characterized oncogene upregulated and associated with poor prognosis in many solid tumor types, including CRC. For this project, we are generating MET-targeted antibody-drug conjugates (ADCs), which will act as guided missiles to deliver cytotoxic agents to CRC cells expressing high levels of MET, including therapy-resistant LGR5$^-$ CRC cells.

WORKING MODEL

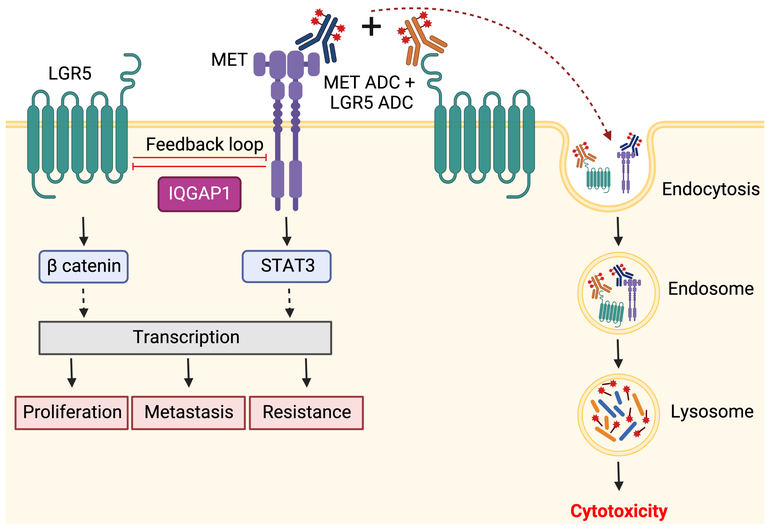

Figure 1. ADCs can serve as guided missiles that deliver potent therapeutic agents to drug resistant colorectal cancer cells. The cytotoxic drug is released upon ADC internalization and lysosomal trafficking in tumor cells.The combination of MET and LGR5-targeted ADCs should effectively destroy both LGR5$^+$ CSCs and LGR5$^-$ CRC cell populations.

RESULTS

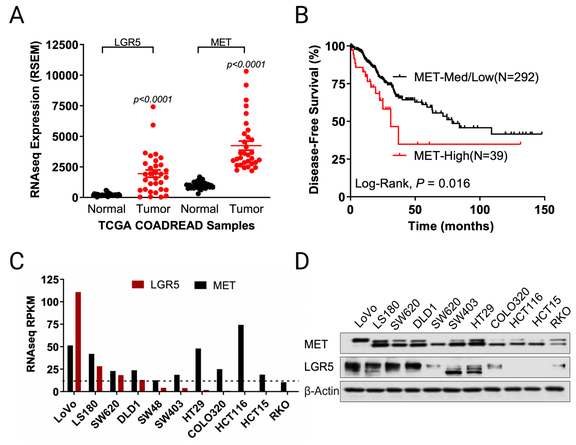

Figure 2. (A) LGR5 and MET RNA-Seq RSEM values for matched tumor and adjacent normal from the colorectal adenocarcinoma (COADREAD) cohort of the Cancer Genome Atlas (TCGA). (B) Kaplan Meier curves comparing Disease-Free Survival with MET expression status. (C) Comparison of MET and LGR5 RNA-Seq RSEM values in CRC cell lines. (D) Immunoblotting of endogenous MET and LGR5 expression in CRC cell lines.

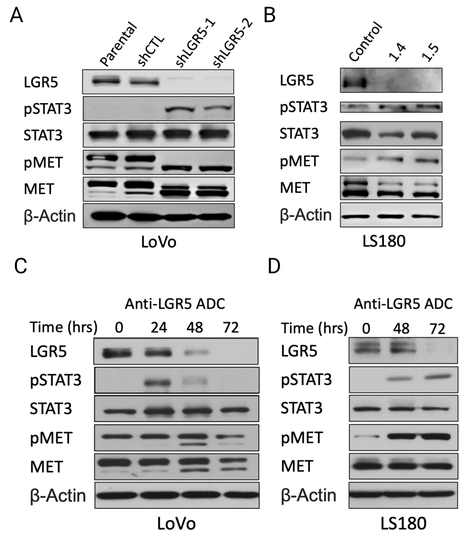

Figure 3. (A) Western blot of changes in MET and STAT3 in LoVo control and LGR5 KD cells. (B) Western blot of changes in MET and STAT3 in LS180 control and LGR5 CRISPR KO cells. (C-D) Western blots of MET and STAT3 phosphorylation in (C) LoVo and (D) LS180 cells treated with anti-LGR5 ADC (6.5 nM) at different time-points.

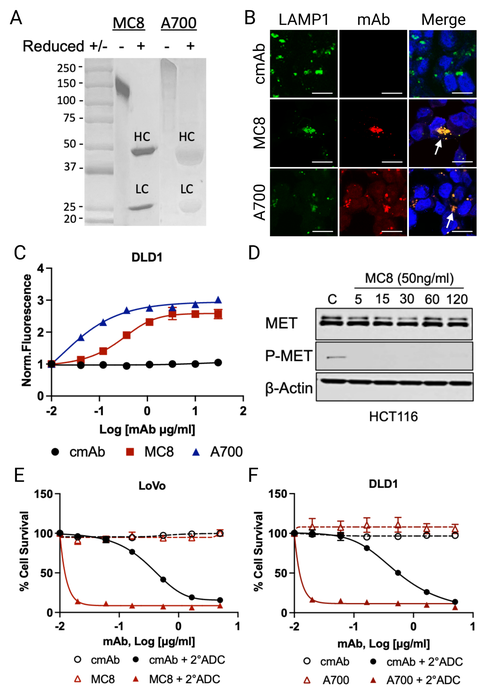

Figure 4. (A) Coomassie blue staining of c-MET monoclonal antibodies (mAbs) MC8 and A700. (B) Immuno-cytochemistry validation of MC8 and A700 binding to human c-MET and internalization into LAMP1$^+$ lysosomes in LoVo cells. (C) Fluorescence cell-based binding assay confirming MC8 and A700 binding to DLD1 cells. (D) Western Blot confirming downstream MET phosphorylation inhibition by MC8. (E-F) Cytotoxicity of (E) MC8 and (F) A700 coincubated with PBD-conjugated secondary mAbs.

DISCUSSION

- In addition to c-MET overexpression in a majority of CRC patients, it's downstream activation upon LGR5 loss makes c-MET a promising therapeutic target.
- Both ABT-700 and MC8 c-MET mAbs demonstrates high binding affinity, internalization and lysosomal colocalization in human colorectal cancer cells
- Ongoing studies include conjugation with different cytotoxic payloads and validation in a wider panel of CRC cell lines and patient derived xenografts.

ACKNOWLEDGEMENTS

- The RNAseq results shown here are in whole or partly based upon data generated by the TCGA Research Network https://www.cancer.gov/tcga.
- This work is funded by NIH/NCI R01CA226894 and R21CA270716, and Cancer Prevention Research Institute of Texas (CPRIT) RP190542 to K.S Carmon.
- Preliminary data obtained from "Loss of LGR5 through plasticity or gene ablation is associated with therapy resistance and enhanced MET-STAT3 signaling in colorectal cancer cells." Posey TA, Jacob J, Parkhurst AN, Subramanian S, Francisco LE, Liang Z, and Carmon KS. bioRxiv doi: 10.1101/2022.03.01.482539