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Abstract Submission Form

Poster Title: **Regulation of Colorectal Cancer Metastasis by Mesenchymal Niche Cells**

Authors: **Ahmed Bakheet[1,3*], Wen Gao[1,3*], Nardana Esmaeili [1], Dan Cai[3], Nathan Berger[4], William Tse [1,2], Xiaonan Han [1,2,3#]**

Presenter's Name: **Ahmed Bakheet**

Location of Laboratory: **Rammelkamp**

Category: **Cancer Biology/Health Care Research**

Background: CDX2 is a transcription factor expressed in the gastro-intestinal (GI) epithelial (IEC) and stromal cells. *CDX2* along with *APC* can regulate Lgr5 intestinal stem cell (ISC) differentiation to control GI development or neoplasia. *CDX2* or *APC* loss function is associated with CRC advance. However, whether *APC* directly regulates CRC metastasis is not clear. We aim to determine the role of inactive *Apc* gene in Cdx2 GI cells on Lgr5 ISC metastasis.

Methods: Tissue sections were collected from CRC or CRC hepatic-metastatic patients, CDX2, CTNNB1, α-SMA, APC and LGR5 expressions were examined. *Apc* flox mice were crossed with *Lgr5Cre*ER and/or *Cdx2Cre*ER mice to inactivate *Apc* gene in Lgr5 ISCs, Cdx2 IECs or stromal cells. *Cdx2Cre*ER;*Apc* mice were then crossed with *tgfr2* flox mice to deplete *tgfr2* in the *Apc*-deficient Cdx2 cells. GI tract and liver histology was evaluated and the expression of Lgr5, APC, α-SMA, β-catenin, and CDX2 were examined. The colorectal polyps induced by *Apc* inactivation were dissected, total RNA was extracted to perform RNA-Seq and quantitative PCR analyses. The colorectal organoids were differentiated and transplanted into abdominal cavity of recipient mice. Meanwhile, HT-29 cells were transfected with *APC* and/or *CDX2* gRNAs.

Results: CTNNB1 and LGR5 expression were increased in human CRC and hepatic-metastatic CRC while interstitial CDX2 and SMA colocalization were robustly pronounced compared to normal colorectum. TCGA analysis showed the positive correlation of *APC* lower and *CDX2* higher with metastatic rectal cancer. *Apc* depletion in Lgr5 cells in 6-week-old mice led to $Lgr5^{+}$ rectal adenocarcinoma and Lgr5 crypts in liver while depletion of *Apc* in Lgr5 cells in 3-month-old mice only resulted in Lgr5 intestinal adenoma. *Apc* depletion in Cdx2 cells led to rectal fibroma and intestinal adenoma as well as undifferentiated metastatic Lgr5 cells in liver. Notably, *Apc* depletion decreased crypt CDX2 while increased colocalization of CDX2 and α-SMA in the stromal cells of adenocarcinoma, and increased expression of TGFBβr2, β-catenin, and epithelial-mesenchymal transition (EMT) markers (Fibronectin and Vimentin). Finally, *tgfr2* depletion in *Apc*-inactive Cdx2 cells impaired GI cancer cell metastasis to liver. Colorectal orgnoids exhibited cancer phenotypes upon *Apc* depletion in CDX2 or Lgr5 cells. Intra-abdominal xenotransplantation exhibited that organoids with *Apc* deficiency in Lgr5 cells appeared no colonization on or invasion of liver tissues whereas the organoids with *Apc* deficiency in Cdx2 cells invaded liver.

Conclusion: Reduced *Apc* in Cdx2 cells are required for Lgr5 cell transformation to $Lgr5^{-}$ cancer stem cells. Inactive intestinal *Apc* increases Cdx2-α-SMA stromal niche cells and facilitates Lgr5 ISC metastasis to liver partially through TGF-β and/or wnt-dependent EMT mechanisms.