**Metastasis of colon cancer organoids requires Dickkopf-2 to generate cancer cells with Paneth cell properties**

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**Abstract**

**Background:**

Metastasis is the leading cause of cancer-related mortality. Paneth cells provide stem cell niche factors in the homeostatic condition. However, the underlying mechanisms of cancer stem cell niche development in metastatic foci are unclear. Here we report that Dickkopf-2 (DKK2) is essential for the generation of cancer cells with Paneth cell properties during the metastasis of colon cancer.

**Methods:** We developed *Dkk2*-knockout (KO) cancer organoids carrying carcinogenic mutations in *Apc, Kras* and *Tp53* genes. Splenic injection of *Dkk2*-knockout (KO) cancer organoids into C57BL/6 mice was performed to develop liver metastasized colon cancers and the area of liver metastasis was analyzed by live imaging analysis. Bulk RNA sequencing (RNA-seq), single cell RNA sequencing (scRNA-seq) and Ingenuity Pathway Analysis (IPA) were used to identify the target genes of DKK2 in metastatic colon cancer cells. scRNA-seq of the published human patient data was analyzed as well. Assay for transposase-associated chromatin using sequencing (ATAC-seq) and chromatin immunoprecipitation followed by sequencing (ChIP-seq) analysis were conducted to identify the transcription factor regulated by DKK2.

**Results:** Cancer organoid-driven liver metastasis was significantly reduced in the absence of DKK2. Transcriptome analysis showed reduction of Paneth cell markers such as lysozyme and defensin in KO organoids. scRNA-seq analyses of murine liver metastasized colon cancer cells and patient samples identified the presence of lysozyme positive cells with the properties of Paneth cells including enhanced glycolysis. Further analyses of transcriptome and chromatin accessibility identified Hepatocyte nuclear factor 4-alpha (HNF4A) as a downstream target of DKK2. Chromatin immunoprecipitation followed by sequencing analysis revealed that HNF4A binds to the promoter region of *Sox9*, a well-known transcription factor for Paneth cell differentiation.

**Conclusions:** Taken together, DKK2-mediated reduction of HNF4A protein promotes the generation of lysozyme positive cancer cells with Paneth cell properties to develop the stem cell niche in the metastasized colon cancers.