Histone demethylase KDM5D drives sex-specific differences in colorectal cancer

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Introduction

Sex-specific differences in cancer outcomes are observed in CRC, with males typically being more adversely affected than females. While sex differences have been correlated with risk factors and sex hormone differences[1], molecular and biological mechanisms underlying such correlations have not been identified nor genetically validated. In this regard, the Y chromosome contains a small number of protein-coding genes that, except for sex-determination genes, have received limited attention as potential determinants of cancer hallmarks and clinical outcomes.

The most common genetic alterations in sporadic human CRC include activating mutations of the *KRAS* oncogene and inactivating mutations or deletion of the *APC* and *TP53* tumor suppressors[2]. A GEMM of CRC with these signature alterations, "iKAP", recapitulates the evolution of CRC from adenoma to adenocarcinoma to invasive and metastatic diseases, and established that KRAS mutation (KRAS*) drives CRC invasiveness and metastasis and its extinction eliminates invasive and metastatic diseases[3,4].

Aim

Integrating GEMM platform and patient data analyses to identify key genes regulating mutant KRAS-driven sex differences in CRC invasion and metastasis.

Materials and Methods

1. Sex-stratified Kaplan-Meier survival analyses, mutation analyses and transcriptomic analyses in CRC patients, iKAP GEMM tumors and CCLE database.
2. Transcriptomic (RNA-seq) and epigenomic (ChIP-seq and Hi-ChIP) analyses of iKAP tumors and derived cancer cell lines.
3. Histopathological studies and IHC of GEMM tumors.
4. Molecular biology studies (western blot, qPCR, IF and co-IP) of iKAP cell lines.
5. Cell culture-based functional studies (luciferase reporter assay, migration assay, cell junction permeability assay and flow cytometry) in iKAP cell lines.
6. Transmission electron microscopy of GEMM tumors.
7. T cells killing assay using iKAP cell lines and OT-1 CD8+ T cells

Results

1. KDM5D is correlated with sex-specific KRAS* CRC metastasis.

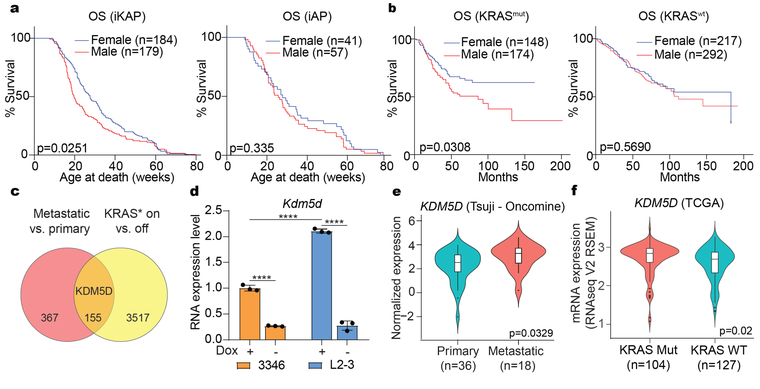


Figure 1. a, Overall survival (OS) analyses of iKAP and iAP mice. **b**, OS analyses of CRC patients from Colorectal Cancer Subtyping Consortium. **c**, Intersecting analysis of differentially expressed genes in male iKAP tumors. Fold change > 1.5 and p-value < 0.05. **d**, qPCR for *Kdm5d* expression in male iKAP cell lines derived from primary tumor (3346) and matched liver metastasis (L2-3). **e**, **f**, Expression of *KDM5D* in male CRC patients.

2. *Kdm5d* transgene expression promotes tumor invasiveness and metastasis.

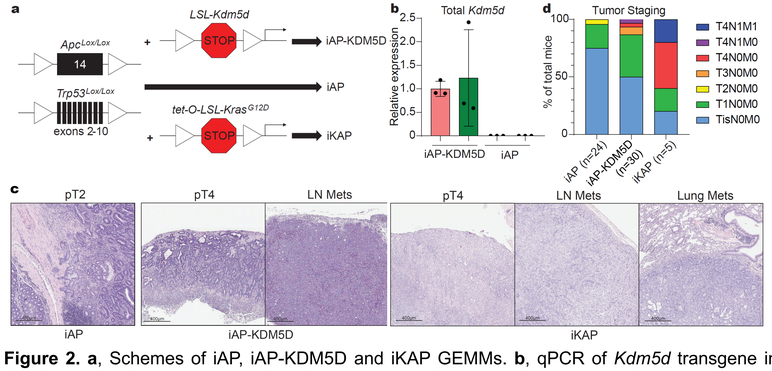


Figure 2. a, Schemes of iAP, iAP-KDM5D and iKAP GEMMs. **b**, qPCR of *Kdm5d* transgene in FFPE tissues of primary tumors from female mice. **c**, Representative images of the most invasive primary tumors and/or LN and distal metastases in GEMMs. iAP: female; iAP-KDM5D: female; iKAP: male. **d**, Percentage of TNM stages of tumors.

3. KDM5D impairs tight junction through epigenetically repressing AMOT.

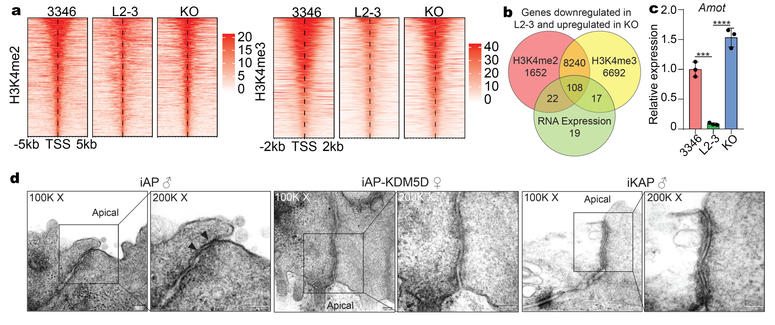


Figure 3. a, Heatmaps of H3K4me2/3 levels around transcription start sites (TSSs) in iKAP cells. **b**, Intersecting analysis of differential genes. **c**, qPCR of *Amot* in iKAP cells. **d**, Representative images of transmission electron microscopy imaging of tight junctions between epithelial cells in primary tumors from GEMMs. Tumors were collected on 102-104 days after induction.

4. KDM5D represses MHC class I antigen processing and presentation.

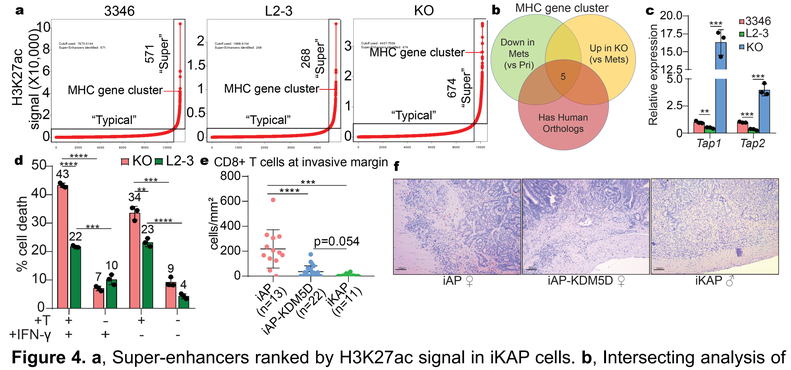
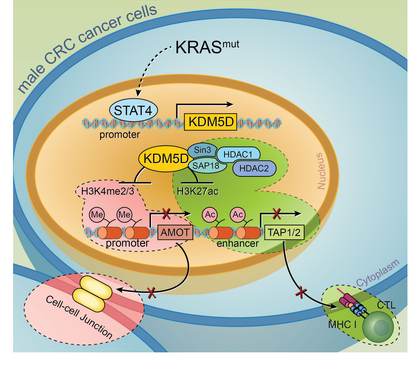


Figure 4. a, Super-enhancers ranked by H3K27ac signal in iKAP cells. **b**, Intersecting analysis of all MHC cluster genes that were downregulated in L2-3 vs. 3346 and upregulated in KO and that have human orthologs. **c**, qPCR of *Tap1* and *Tap2* in iKAP cells. **d**, Percentage of dead iKAP cells (GFP+ for iKAP cells and SYTOX Blue+ for dead cells) in CD8+ T cell killing assay. iKAP cells were pretreated with 2 mg of ovalbumin protein and +/- 10ng/ml IFN-γ for 24 hours. iKAP cells were re-plated +/- activated OT-1 CD8+ T cells at the ratio of effector: target = 10: 1. **e**, Quantitation of the number of CD8+ cells at the tumor invasive front detected by IHC in primary tumors with comparable invasiveness (later pTis and pT1). **f**, Representative images of IHC for CD8 at the tumor invasive front.

Conclusion



Discussion

Though KDM5D and its X chromosome paralog KDM5C share structural and functional similarity, we have excluded the possibility that KDM5D and KDM5C function coincidentally in KRAS* CRC with the evidence that (i) *KDM5C* is not regulated by KRAS*, (ii) *KDM5C* is not differentially expressed in CRC primary vs. metastatic tumors, and (iii) KDM5C does not regulate KDM5D downstream genes.

References

1. Kim, S. E. *et al.* Sex- and gender-specific disparities in colorectal cancer risk. *World J Gastroenterol* **21**, 5167-5175, doi:10.3748/wjg.v21.i17.5167 (2015).
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