Helicobacter pylori infection causes expansion of metaplastic pit cells

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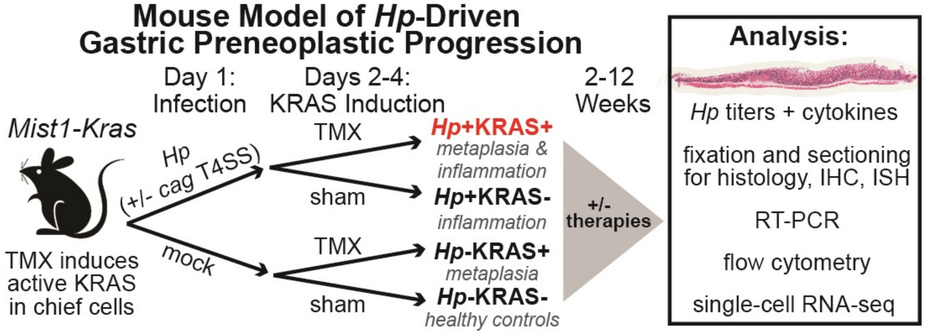
Background & Significance

Gastric cancer is the fifth most common cancer and fourth-leading cause of cancer deaths worldwide. More than 80% of gastric cancer is attributable to stomach infection with *Helicobacter pylori* (*Hp*), a bacterium that infects half of humans. However, the specific mechanism(s) through which *Hp* infection leads to cancer are not fully understood.

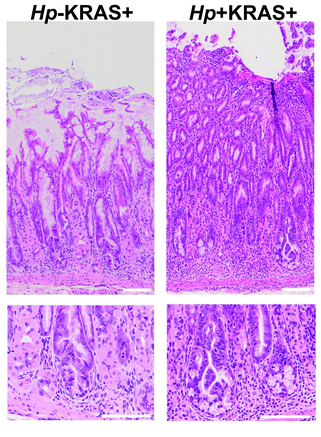
Hp does not cause cancer in wild-type mice for unknown reasons, so mouse models use additional perturbations like oncogene expression and/or chemical carcinogens. In *Mist1-Kras* mice, tamoxifen induces expression of a constitutively active *Kras* allele in the gastric chief cells.



Sustained *Hp* infection promotes preneoplastic progression in KRAS+ mice

I used immunohistochemistry (IHC) with quantitation of staining, gene expression profiling and tissue scoring by a veterinary pathologist to demonstrate that *Hp* infection plus active KRAS exacerbates human disease phenotypes compared to *Hp* or KRAS alone.

Disease Parameter	Metric	*Hp*+ KRAS-	*Hp*- KRAS+	*Hp*+ KRAS+
Parietal cells	Pathology scoring	-	-	- -
	H,K-ATPase+ cells	-	-	- -
Metaplasia	CD44v9+ glands	None	++	+++
	TFF3+ glands	None	+++	++
	MUC2+ glands	None	+	+
Dysplasia	Pathology scoring	None	Mild	Moderate
	TROP2+ glands	None	+	++
Proliferation	KI-67+ cells	++	++	+++
Inflammation	# of immune cells	++	+	+++
Variant pit cells	scRNA-seq	Rare	Rare	Many
	MUC4+ glands	+	+	+++

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Sustained *Helicobacter pylori* infection accelerates gastric dysplasia in a mouse model

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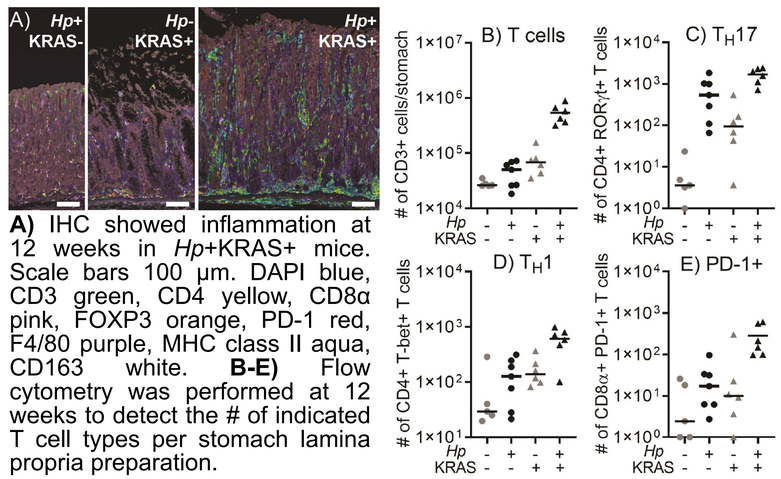
Hp+KRAS+ mice have severe inflammation marked by T cell infiltration

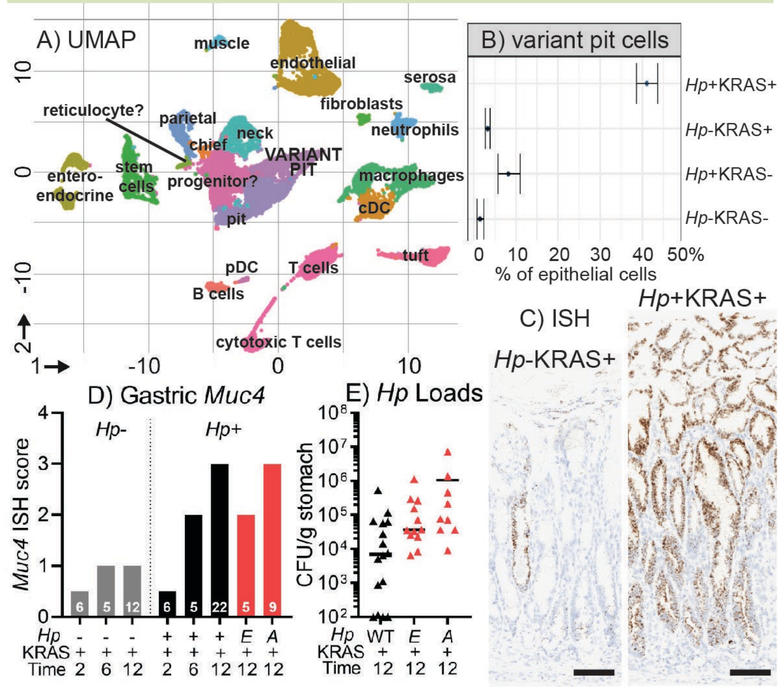
I used IHC with quantitation of staining and flow cytometry to profile gastric inflammation in *Hp* +/-, KRAS +/- mice.



A) IHC showed inflammation at 12 weeks in *Hp*+KRAS+ mice. Scale bars 100 µm. DAPI blue, CD3 green, CD4 yellow, CD8α pink, FOXP3 orange, PD-1 red, F4/80 purple, MHC class II aqua, CD163 white. **B-E)** Flow cytometry was performed at 12 weeks to detect the # of indicated T cell types per stomach lamina propria preparation.

Hp+KRAS+ mice have an expanded population of *Muc4*-expressing pit cells

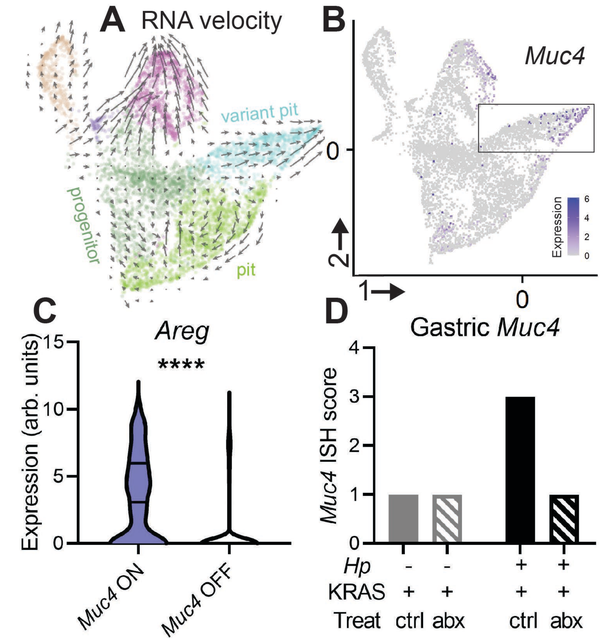
To understand gene expression changes in gastric cell types, I performed single cell RNA-sequencing (scRNA-seq).



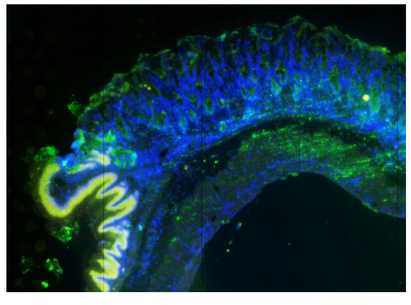
A) scRNA-seq was performed in +/- *Hp*, +/- KRAS mice and gastric cell clusters were identified by UMAP and manually annotated based on gene expression. cDC & pDC, conventional & plasmacytoid dendritic cells. **B)** Variant pit cells comprised the indicated proportions of epithelial cells at 12 weeks. **C)** ISH shows *Muc4* (brown) at 12 weeks. Scale bars, 100 µm. **D)** Median *Muc4* ISH score at 12 weeks is shown and # of mice per group is given in white. **E)** *Hp* titers at 12 weeks. Zeroes are plotted at the limit of detection (100 CFU). *E*, Δ*cagE*; *A*, Δ*cagA*.

Muc4 expression may represent terminal differentiation of variant pit cells

RNA velocity analysis was performed on the scRNA-seq data. RNA velocity predicts cellular state progression by comparing the abundances of unspliced (nascent) and spliced (mature) mRNA within a cell. The resulting vectors can indicate cellular differentiation, maturation and/or proliferation.



A) RNA velocity was determined and the resulting vectors are overlaid onto the central region of the gastric scRNA-seq UMAP, which comprises progenitors (dark green), pit cells (light green), variant pit cells (teal), neck cells (pink), parietal cells (peach) and chief cells (purple). Vectors can indicate cell differentiation, maturation and/or proliferation. **B)** The central region of the gastric UMAP is shown again, with *Muc4*-expressing cells indicated in purple. The box outlines variant pit cells. **C)** Shown is amphiregulin (*Areg*) expression in *Muc4*-positive and *Muc4*-negative variant pit cells. ****, $P < 0.0001$, Mann-Whitney U test. **D)** Mice were treated with antibiotics ('abx') or vehicle ('ctrl') starting at six weeks and euthanized at 12 weeks. The median *Muc4* ISH score is shown.



10x Visium Spatial Gene Expression will be used to investigate the spatial organization of *Muc4*-expressing variant pit cells in the gastric epithelium.

with Jeffery Williams, Stephanie Weaver, and Cassie Sathers

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