The dosage-dependent role of *Arid1a* in gastric development and tumorigenesis

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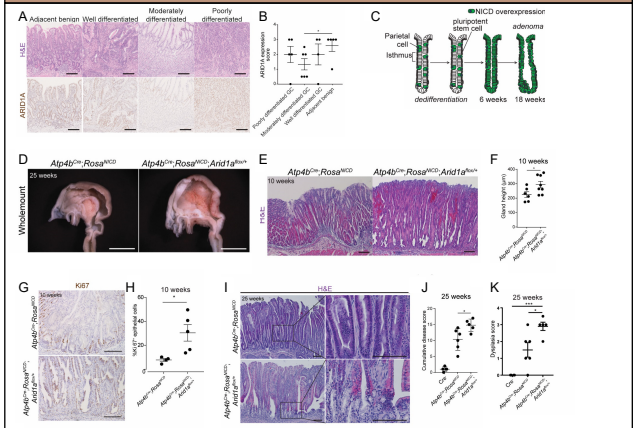
Introduction

Gastric cancer (GC) is the third most deadly cancers, accounting for an estimated 8% of all cancer mortality in 2019 (Ferlay et al., 2019). One striking feature of the GC genome is a high incidence of mutations altering chromatin modifiers. Indeed, mutations in genes for BRG1- or HBRM-associated factors (BAF) chromatin remodeling complex proteins (ARID1A mutations at 14-31%, ARID1B at 9%) and Nucleosome Remodeling Deacetylase (NuRD) complex proteins (CHD3 at 8%, CHD4 at 9%) are collectively very common (Cerami et al., 2012; Gao et al., 2013; Wang et al., 2011).

AT-rich interaction domain 1A (**ARID1A**), a subunit of the BAF complex, has been identified as the second most mutated gene after TP53 in GC (Cancer Genome Atlas Research, 2014). In GC, recent clinical studies have shown that ARID1A deficiency is associated with poor prognosis and lymph node metastasis (Ajao et al., 2015; Han et al., 2016; Inada et al., 2015; Yang et al., 2016). Several cell culture studies have provided some evidence supporting its role as a tumor suppressor in GC. Knockdown of ARID1A in GC cell lines promoted proliferation, migration and survival (Nagl et al., 2005; Yan et al., 2014; Yang et al., 2018). However, its *in vivo* role remains unclear.

Heterozygous loss of *Arid1a* promote tumor progression

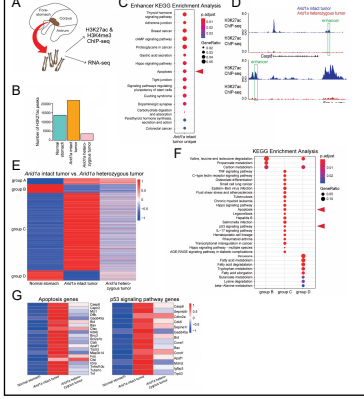


Promotion of gastric tumor progression by *Arid1a* heterozygosity.

(A) Analysis of human GC samples shows loss of ARID1A protein expression in moderately differentiated tumors.
(B) Quantification of ARID1A protein level shows significant loss of ARID1A expression in moderately differentiated tumors compared to benign tissue.
(C) Diagram outlining adenoma formation in the Notch-driven gastric adenoma model.
(D) Wholemount images of the gastric lumen comparing *Arid1a* heterozygous adenoma mice to adenoma mice with intact *Arid1a*, showing larger tumors in the former.
(E) Histological analysis of *Arid1a* intact and *Arid1a* heterozygous gastric adenoma shows increased progression in heterozygous mice.
(F) Quantification of gastric gland height confirms a significant increase in gland height of *Arid1a* heterozygous tumors compared to *Arid1a* intact tumors at initial stages of tumor progression.
(G) Staining of proliferative marker, Ki67 staining, shows increased expression in *Arid1a* heterozygous tumors compared to *Arid1a* intact tumors at 10 weeks.
(H) Quantification of Ki67+ cells in the epithelium of *Arid1a* intact and *Arid1a* heterozygous tumors confirms significant increase in the number of proliferative cells in *Arid1a* heterozygous tumors at 10 weeks.
(I) Histological analysis of *Arid1a* intact and *Arid1a* heterozygous gastric adenoma at late-stage (25 weeks) shows increased tumor progression in *Arid1a* heterozygous mice.
(J) Histopathology scoring of late-stage tumors shows a significant increase in the cumulative disease score of *Arid1a* heterozygous tumors compared to *Arid1a* intact tumors.
(K) Histopathology scoring of dysplasia using Table S1 shows no significant difference between *Arid1a* intact and heterozygous tumors at an early stage. At late stages, *Arid1a* heterozygous tumors have a significantly higher dysplastic index compared to *Arid1a* intact tumors.

Scale = 100µm. *$P<0.05$, ***$P<0.001$

Heterozygous loss of *Arid1a* suppress p53 signaling



Chromatin and gene expression analyses of gastric adenomas with and without Arid1a heterozygosity.

(A) Experimental overview of chromatin and transcriptomic analyses performed using normal (wildtype) stomachs, *Arid1a* intact and Arid1a heterozygous gastric tumors.

(B) Analysis of H3K27ac peaks shows a drastic reduction in peak number in *Arid1a* heterozygous tumors compared to Arid1a intact tumors.

(C) Pathway enrichment analysis of top 2000 enhancers unique to *Arid1a* intact tumor exhibits an enrichment in apoptosis pathway (red arrow).

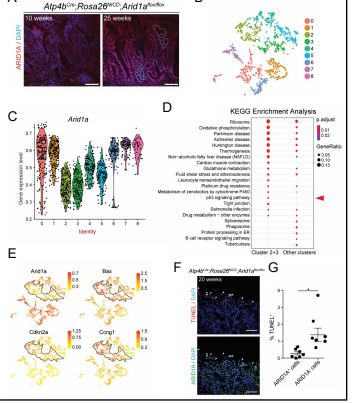
(D) Genes involved in apoptosis display a loss in H3K27ac peak signal at enhancer regions of *Arid1a* heterozygous tumors compared to *Arid1a* intact counterparts.

(E) A global heatmap of all dysregulated genes between wildtype stomachs, *Arid1a* intact tumors and *Arid1a* heterozygous tumors identifies 4 groups of dysregulated genes.
Group A and B are genes upregulated and downregulated in all tumor samples, respectively, regardless of *Arid1a* status.
Group C are genes upregulated in *Arid1a* intact tumors only, and group D are genes that are only downregulated in *Arid1a* intact tumors.

(F) Pathway enrichment analysis dysregulated genes identifies enrichment of apoptosis and p53 signaling genes (red arrows) in group C.

(G) A higher resolution heatmap of p53 signaling pathway and apoptosis related genes highlights the upregulation of these genes in *Arid1a* intact but not in *Arid1a* heterozygous tumors when compared to wildtype gastric tissue.

Homozygous loss of *Arid1a* confers competitive disadvantage via activation of p53



Single-cell analysis of Arid1a knockout gastric adenoma epithelium.

(A) Immunofluorescence images of ARID1A staining in *Arid1a* deleted gastric adenomas at early and late stages demonstrate re-population of adenomatous glands by ARID1A+ cells at 25 weeks. Dotted lines indicate epithelial cells without ARID1A expression at 25 weeks.

(B) Clustering analysis of single-cell RNA-seq data from *Arid1a* deleted gastric adenomas identifies 9 clusters of cells with distinct expression profiles.

(C) Violin plots depicting *Arid1a* expression among the 9 clusters of cells identifies clusters 2 and 3 to have lower *Arid1a* expression compared to the other clusters.

(D) Pathway enrichment analysis of clustering markers found an enrichment of the p53 signaling pathway (red arrow) in clusters 2 and 3, but not in the other clusters.

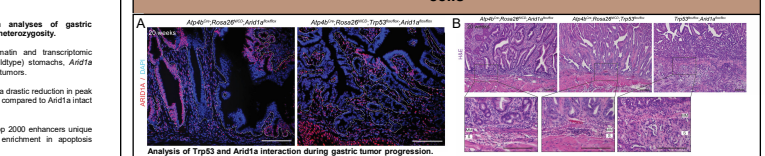
(E) Feature plots of the single-cell RNA-seq data show a negative correlation between *Arid1a* expression and the expression of genes related to p53 signaling and apoptosis.

(F) Representative images of TUNEL and ARID1A immunofluorescence staining in *Arid1a* homozygous gastric adenomas; white arrowheads indicate TUNEL+ ARID1A- cells.

(G) Quantification of TUNEL+ cells confirms an increase in the percentage of TUNEL+ cells in the ARID1A- cell population compared to the ARID1A+ cell population.

Scale = 100µm. *$P<0.05$

Trp53 deletion partially rescue fitness disadvantage in *Arid1a* homozygous cells



Analysis of Trp53 and Arid1a interaction during gastric tumor progression.

(A) ARID1A staining in gastric adenomas with the deletion of *Arid1a* alone or *Arid1a* and *Trp53* together shows increased number of *Arid1a* deleted cells when *Trp53* is deleted; the white dotted lines outline the boundary between the epithelium and mesenchyme.
(B) Histological analysis of the gastric layers from mice with single or double deletion of Arid1a and Trp53, highlighting an example of submucosal invasion in the doubly deleted mice; the white dotted line demarcates the muscularis mucosa (MM) and the submucosa (S)
Scales = 100 µm

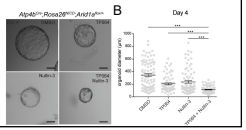
Combinatorial treatment of epigenetic inhibitor and p53 agonist inhibit tumor organoid growth

Combinatorial drug treatment of gastric tumor organoids.

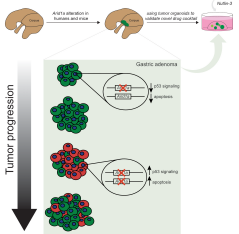
(A) *Arid1a* heterozygous tumor organoids on day 4 of combinatory treatment of 1.5 µM TP064 and/or 2 µM Nutlin-3 or DMSO shows reduction of organoid size in the doubly treated group.

(B) Quantification of the diameter of Arid1a heterozygous tumor organoids after 4 days of treatment with either 1.5µM TP064 and/or 2µM Nutlin-3 shows a significant reduction in organoid size after simultaneous treatment of TP064 and Nutlin-3 compared to DMSO and single compound treatment. Experiments were repeated twice with organoids obtained from different mice.

Scale = 100 µm. ***$P<0.001$



Summary



We generated a clinically relevant gastric tumor model with an Arid1a heterozygous deletion.

Arid1a heterozygous tumors exhibit a global loss of active enhancer marks and downregulation of the p53 and apoptotic pathway genes, leading to enhanced tumor growth and progression.

Homozygous deletion of Arid1a in gastric tumors conferred a competitive disadvantage through abnormal activation of the p53 pathway.

Combinatorial treatment consisting of an epigenetic inhibitor and a p53 agonist synergistically inhibits the growth of organoids established from Arid1a heterozygous tumors.

Future directions

How does mutations in *Arid1a* cooperate with environmental factors such as bacterial infection during gastric tumorigenesis?

How does mutations in multiple BAF complex genes interact with other each other to promote gastric tumor development/progression?