IN THE SPOTLIGHT

Revealing ARID1A Function in Gastric Cancer from the Bottom Up

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Summary: In this issue of *Cancer Discovery*, Lo and colleagues use CRISPR-based genome engineering in primary human gastric organoids to reveal the functional consequences of *ARID1A* loss in the early stages of gastric cancer. They show that *ARID1A* disruption is not tolerated in wild-type organoids, but in the context of *TP53* loss, leads to WNT suppression, mucinous metaplasia, enhanced tumorigenicity, and selectively toxicity to BIRC5/Survivin inhibition.

See related article by Lo et al., p. 1562 (1).

In the past decade, CRISPR/Cas9 systems have revolutionized cancer genetics and disease modeling, providing new avenues to understand disease-associated mutations. Similarly, the advent of self-renewing, three-dimensional (3-D) "organoid" cultures has provided tractable systems to interrogate cell and tissue responses to genetic and pharmacologic perturbation. Together, these two technologies create a flexible and scalable platform for the development of high-fidelity *ex vivo* models to interrogate gene function in normal and disease states, and to accelerate drug discovery and validation. In this issue of *Cancer Discovery*, Lo and colleagues (1) demonstrate the power of combining human 3-D culture systems with targeted genome editing, building bottom-up cancer models to understand the contribution of ARID1A mutations to gastric tumorigenesis. They show that loss of *ARID1A* drives hyperplastic, mucinous growth during the early stages of transformation, and creates a genotype-specific dependency on FOXM1-BIRC5/Survivin that can be targeted therapeutically (Fig. 1).

ARID1A is a subunit of the SWI/SNF complex, a genome caretaker involved in chromatin remodeling and DNA repair, and an apparent tumor suppressor across a wide range of cancer types. In gastric cancer, *ARID1A* is mutated in nearly one third of all tumors and is associated with poor prognosis (2), yet exactly how it contributes to this disease has been unclear. Even with a basic understanding of normal gene function, defining the precise consequences of gene disruption during transformation can be tricky. For suspected tumor suppressors, it relies on comparing large numbers of wild-type (WT) or mutant samples, and manipulating or reintroducing the target in already-transformed cells. Such approaches are even more complicated for genes like *ARID1A*, which appear to have pleiotropic effects in different settings. Due to its role as a general chromatin modifier, the consequences of *ARID1A* loss are as varied as the cancers in which the mutations occur. In the pancreas, *ARID1A* loss has been reported to support tumorigenesis by increasing MYC expression and enhancing KRAS-driven transformation, whereas loss during the early stages of transformation may block KRAS-mediated lineage disruptions (3, 4). A similar dual role is seen in other tissues. In the intestine, *ARID1A* loss suppresses WNT signaling, causing stem cell and crypt loss, but can also support the progression to malignancy in the colon (5, 6). In the liver, ARID1A supports tumor growth in early stages yet acts to restrict progression at later stages of disease (7). These many examples, though completely unique in tissue type and genetic context, converge on the idea of ARID1A as a context-dependent tumor suppressor. Such varied responses across different tissues and contexts underscore the importance of investigating gene function in specific disease types and stages.

One strategy for interrogating the impact of tumor suppressor loss on cancer growth is to build cancer models "from the bottom up." Constructing models in this way allows control over both the timing of gene disruption and the combination of oncogenic events, essentially providing a means to "watch" transformation as it happens. This was the approach taken by Lo and colleagues. Starting with genetically WT human gastric organoids, they used Cas9-based editing to first delete *TP53* (disrupted in ~50% gastric cancers) and then mutate *ARID1A*. Interestingly, Lo and colleagues were unable to maintain *ARID1A*-mutant organoids with intact p53 function. This observation is consistent with context-dependent roles reported in mouse pancreas (4), intestine (5, 6), and liver (7), and suggests a general requirement for ARID1A function in normal cells or nascent tumors that is overcome during transformation.

In *TP53* knockout (KO) organoids, *ARID1A* loss drove hyperproliferation and loss of apical–basal polarity. Consistent with the *in vitro* features of malignancy, *ARID1A/TP53* double KO (DKO) organoids showed efficient engraftment in immunocompromised mice and evidence of high-grade dysplasia within the tumors. A standout feature of the *ARID1A*-mutant organoids and tumors was a strong mucinous phenotype associated with intestinal metaplasia not seen in p53-mutant cells or gastric epithelium from which

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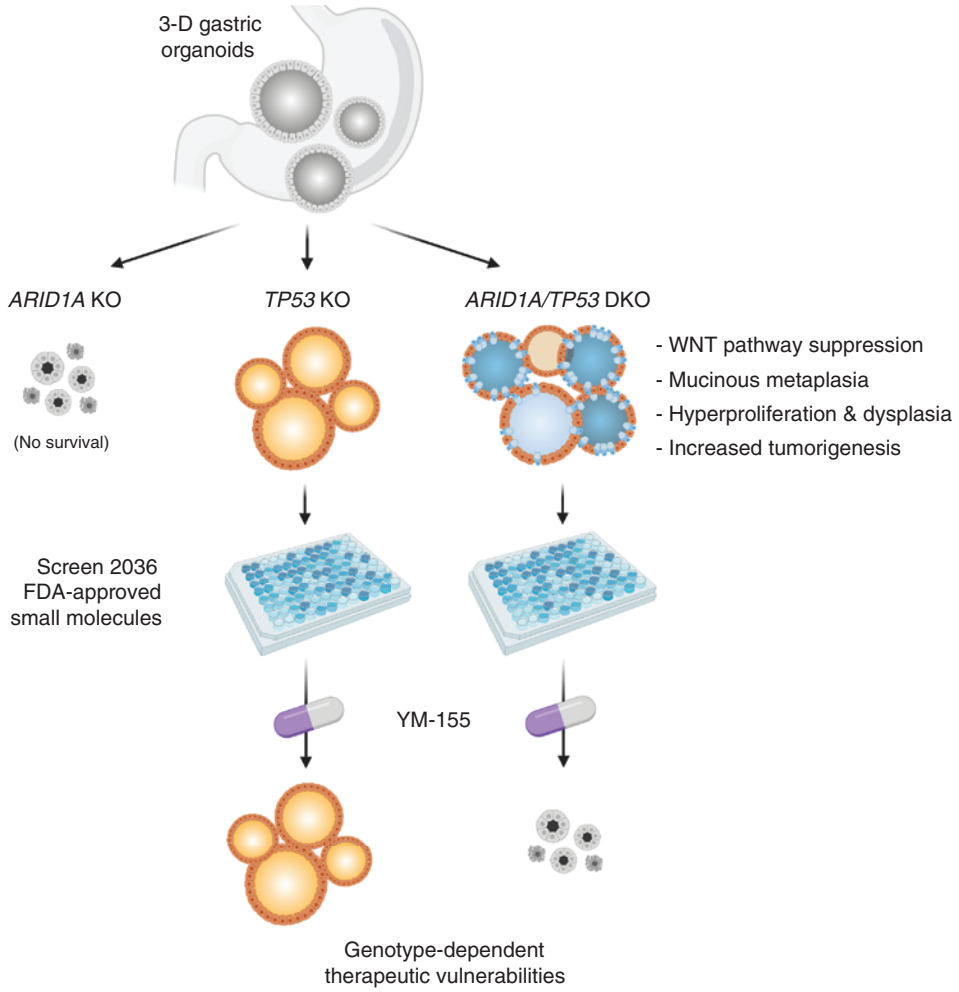

Figure 1. Dissecting the function of ARID1A in gastric cancer through engineered organoid models. Lo et al. used Cas9-mediated editing to create mutations in *ARID1A*, *TP53*, or both *ARID1A* and *TP53*. *ARID1A*-mutant organoids were unable to survive beyond 2 weeks, whereas *TP53* KO and *ARID1A*/*TP53* DKO organoids could be propagated continuously. DKO organoids showed increased proliferation, mucinous intestinal metaplasia, and increased tumor growth *in vivo* relative to *TP53* KO cultures. Screening of both *TP53* KO and DKO organoids against a library of FDA-approved compounds identified YM-155, a BIRC5/Survivin inhibitor that was selectively toxic to ARID1A-mutant cells.

the organoids were derived. Similar features were seen across a panel of nearly 200 human gastric cancers, whereby low ARID1A expression correlated with mucinous features. Lo and colleagues further showed that the mucinous behavior, but not hyperproliferation, was due to suppression of WNT signaling following *ARID1A* loss; hence, WNT reactivation could block mucinous differentiation without altering hyperplastic growth. Of note, oncogenic WNT pathway alterations are seen in 25% to 30% of gastric tumors and are often associated with *ARID1A* mutations. Though not directly tested in this study, it will be interesting in the future to determine how well these or similar "bottom-up" organoid models predict the multifaceted behavior of human cancers carrying complex combinations of these recurrent genetic lesions.

Lo and colleagues took advantage of the genetically defined gastric organoids platform they created to hunt for specific therapeutic vulnerabilities associated with *ARID1A* loss. To do this, they screened a library of more than 2,000 FDA-approved small-molecule compounds in both *TP53* KO and *TP53/ARID1A* DKO organoid clones. These screens revealed a small number of compounds with antiproliferative properties including some previously identified targets. Lo and colleagues focused on YM-155, a BIRC5/Survivin inhibitor, which showed increased potency against ARID1A-mutant organoids, and directly linked to the transcriptional induction of *BIRC5* seen in ARID1A-mutant cells. Despite a clear and robust effect in engineered 3-D organoids, treatment of isogenic 2-D cancer cell lines with YM-155 showed no increased sensitivity linked to *ARID1A* loss. So, what underlies this difference? Certainly, it is possible that loss of ARID1A confers acute cell dependencies during the early stages of tumorigenesis that are overcome as cells acquire additional genetic and epigenetic alterations. Indeed, as ARID1A functions as a broad chromatin modifier, it could create transcriptional/epigenetic scenarios that evolve as tumors progress. It is equally possible that engineering *ARID1A* mutations in already transformed cells (as the authors did in this case) does not create the same signaling dependencies as when those mutations occur and are selected for during

tumorigenesis. Finally, it is plausible that culture conditions (2-D vs. 3-D) and media influence the outcome of drug treatments, as has been demonstrated in other settings. Whatever the underlying reason, the engineered organoid models described by Lo and colleagues provide fertile ground to explore YM-155 sensitivity, either via the direct engineering of additional genetic events associated with gastric cancer progression or through unbiased genetic screens.

In all, Lo and colleagues describe an elegant dissection of ARID1A function and reveal that in gastric cancer this enigmatic tumor suppressor interfaces with multiple oncogenic pathways to promote tumorigenesis. Their work showcases the speed and feasibility of genome engineering in human organoids to understand the impact of recurrent cancer-associated mutations and build models for preclinical target assessment. As has been described in colorectal organoids, expansion of this platform could create complex genetic models with increased mutational diversity, providing a setting to understand how specific mutational events contribute to treatment response and resistance (8–10). In addition, a parallel "bottom-up" engineering of organoids derived from different organs would provide a controlled context to interpret the varied cellular responses to disruption of genes such as *ARID1A* that have both genotype- and tissue-dependent functions.

Authors' Disclosures

L.E. Dow reports personal fees from Mirimus Inc outside the submitted work. No disclosures were reported by the other author.

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