

## Harnessing mutual exclusivity between *TP53* and *ARID1 A* mutations

Shuai Wu<sup>a</sup>, Nail Fatkhutdinov<sup>a,b</sup>, and Rugang Zhang<sup>a</sup>

<sup>a</sup>Gene Expression and Regulation Program, The Wistar Institute, Philadelphia, PA, USA; <sup>b</sup>Kazan Federal University, Kazan, Russia

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Epithelial ovarian cancer (EOC) remains the most lethal gynecological malignancy in the developed world. EOC is composed of multiple separate diseases. Ovarian clear cell carcinoma (OCCC) and ovarian endometrioid carcinoma (OEC) are the second and third deadliest subtypes of EOC. Notably, OCCC is associated with the worst prognosis compared to other EOC subtypes when diagnosed at advanced stages. OCCC typically has a low response rate to platinum-based standard of care for EOC, and there is currently no effective therapy for this disease.



The past few years have brought unprecedented progress in understanding the genetics of EOC, but cures remain elusive due to the lack of insights into mechanisms that can be targeted to develop new therapies. The discovery of somatic *ARID1 A* mutations in over 50% OCCC and 30% OEC represents a major advance in these histosubtypes.<sup>1,2</sup> Over 90% of the *ARID1 A* mutations observed in EOC are frame-shift or non-sense mutations that result in loss of ARID1 A expression. Notably, loss of ARID1 A correlates with late-stage disease and predicts early recurrence. Thus, there is an even greater need for targeted therapy that is selective for *ARID1 A*-mutated EOC. Mutations in *ARID1 A* and *TP53* are typically mutually exclusive in EOC.<sup>3</sup> However, therapeutic strategies that harness this genetic characteristic have not been explored. A recent study demonstrates that *ARID1 A*-mutated EOC depend on HDAC6 activity, and inhibition of HDAC6 promotes apoptosis in *ARID1 A*-mutated cells in a p53-dependent manner.<sup>4</sup> This work explains, at least in part, the mutual exclusivity between *ARID1 A* and *TP53* mutations because it indicates that *ARID1 A* mutation contributes to inactivation of p53's apoptosis-promoting function. In addition, these studies suggest potential translation of these findings by repurposing clinically applicable HDAC6 inhibitors for *ARID1 A*-mutated EOC.

ARID1 A epigenetically regulates gene expression via the SWI/SNF chromatin-remodeling complex by controlling gene accessibility. SWI/SNF complexes contribute to both gene activation and repression in a context-dependent manner.<sup>5</sup> Previous studies demonstrated that SWI/SNF promoted transcriptional activation of tumor suppressor genes that are repressed by EZH2.<sup>6</sup> In contrast, ARID1 A reduced the promoter activity of *HDAC6* gene, thus acting as a direct transcriptional repressor. Mechanistically, it was shown that lysine 120

acetylated p53 (p53K120 Ac), a pro-apoptotic posttranslational modification, is a direct target for HDAC6 deacetylase activity. Thus, *ARID1 A*-mutated tumors have elevated levels of HDAC6, which in turn represses apoptotic function of p53 by reducing p53K120 Ac levels. The apoptosis induced by HDAC6 inhibition correlates with an increase of mitochondria localization of p53K120 Ac and a decrease in mitochondria membrane potential (Fig. 1). In both xenografts and genetically engineered mouse models of OCCC, pharmacological inhibition of HDAC6 using a clinically applicable drug ACY1215 induced apoptosis selectively in *ARID1 A*-mutated, but not wildtype, OCCC tumors. Indeed, ACY1215 improved the survival of mice bearing *ARID1 A*-mutated OCCC.

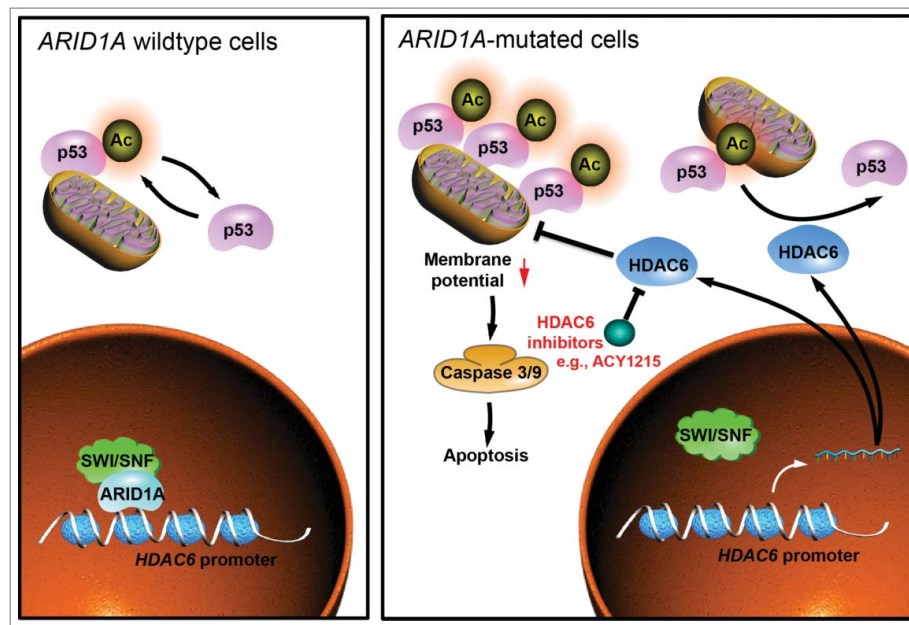
Beyond EOC, the mutual exclusivity between *ARID1 A* and *TP53* mutations was observed in a number of other major cancer types such as prostate, stomach, colon and uterine. It will be interesting to determine whether HDAC6 inhibition is equally effective in these cancers with mutated *ARID1 A* and wildtype *TP53*. Interestingly, in contrast to ARID1 A inactivation, knockdown of BRG1, the catalytic subunit of the SWI/SNF, did not affect sensitivity to HDAC6 inhibition. This is consistent with the notion that ARID1 A targets the SWI/SNF complex to specific genomic loci.<sup>5</sup> ARID1 A exists in multiple different complexes based on mutual exclusivity among different subunits including the catalytic subunits.<sup>5</sup> Thus, the observed differences between ARID1 A and BRG1 inactivation may reflect the unique ARID1 A-dependent genomic distribution of the SWI/SNF complex and the fact that BRG1 loss can be compensated by the mutual exclusive catalytic subunit BRM.

Despite the great progress in exploring checkpoint blockade immunotherapy, biomarkers of response to immune checkpoint blockade remain to be fully explored. Of proposed biomarkers, microsatellite instability (MSI) is particularly interesting in the context of *ARID1 A*-mutated cancers, because ARID1 A loss is often associated with MSI in endometrial and gastric cancers. In addition, a recently study showed that OCCC with MSI had higher levels of CD8+ and PD-1+ tumor infiltrating lymphocytes compared to MSI-negative OCCC.<sup>7</sup> Thus, *ARID1 A*-mutated cancers may be susceptible to anti-PD-1/PD-L1 immunotherapies. HDAC6 has been reported to positively regulate PD-L1 expression, enhance

**CONTACT** Rugang Zhang, PhD  [rzhang@wistar.org](mailto:rzhang@wistar.org)  Gene Expression and Regulation Program, The Wistar Institute, Room 312, 3601 Spruce Street, Philadelphia, PA 19104.

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**Figure 1.** Schematics of molecular mechanism by which HDAC6 inhibition is selectively against *ARID1A* mutation in a p53-dependent manner.

T-cell activation and improve function of antigen-presenting cells. These data raise the possibility that HDAC6 inhibition in combination with PD-1/PD-L1 blockade is potentially a promising strategy for *ARID1A*-mutated cancers. This strategy will not only eliminate *ARID1A*-mutated tumor cells in a p53-dependent manner but also restore anti-tumor immunity. This synergistic combination could maximize the therapeutic efficacy, minimize the toxicity and possibility of developing resistance.

In summary, these findings demonstrate that targeting HDAC6 activity represents a novel therapeutic strategy for *ARID1A*-mutated EOC. HDAC6 inhibitor ACY1215 displayed minimal toxicities in clinical studies, supporting high translational potential of our study. This approach takes advantage of *ARID1A* mutational status and mutual exclusivity between *ARID1A* and *TP53* mutations. Considering that *ARID1A* shows one of the highest mutation rates among epigenetic regulators across all human cancers, these findings should have far reaching implications for the future development of epigenetic therapeutic strategies. Combinational approaches and, in particular, synergistically utilizing epigenetic and immune therapies warrant further exploration for *ARID1A*-mutated cancers.

## Abbreviations

EOC	epithelial ovarian cancer
MSI	microsatellite instability
OVCC	ovarian clear cell carcinoma
OEC	ovarian endometrioid carcinoma
p53K120 Ac	lysine 120 acetylated p53

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