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Epigenetic synthetic lethality in ovarian clear cell carcinoma: EZH2 and *ARID1A* mutations

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The components of the Switch/Sucrose non-fermentable (SWI/SNF) complex are mutated in approximately 20% of human cancers. The A/T-rich interacting domain 1A (ARID1A) subunit has one of the highest mutation rates. Most notably, *ARID1A* is mutated in over 50% of ovarian clear cell carcinomas (OCCCs). We reported that inhibition of enhancer of zeste homology 2 (EZH2) is synthetically lethal in *ARID1A*-mutated OCCC.

Genes encoding subunits of the ATP-dependent chromatin-remodeling complex are mutated in many cancer types.[1] Most notably, the A/T-rich interacting domain 1A (*ARID1A*) gene, which encodes a subunit of the Switch/Sucrose non-fermentable (SWI/SNF) chromatin-remodeling complex, is mutated in up to 57% of ovarian clear cell carcinomas (OCCCs).[2,3] Indeed, *ARID1A* is among the genes that show the highest mutation rates across multiple cancer types,[1] including up to 27% of gastric carcinomas, 13% of hepatocellular carcinomas, 13% of bladder carcinomas, 15% of esophageal adenocarcinomas, and 17% of Burkitt lymphomas. In addition to mutation, loss of ARID1A expression has been reported in several cancer types, most frequently in breast and kidney cancers.[4] However, despite the prevalence of *ARID1A* mutations in many cancer types, a rational therapeutic approach to target cancers with *ARID1A* mutations has not yet been explored.

Epithelial ovarian cancer remains the most lethal gynecologic malignancy in the developed world. OCCC ranks second as a cause of death from epithelial ovarian cancer and is associated with a poor prognosis compared to other histologic subtypes. OCCC typically has a low initial response rate to platinum-based standard care, and there is currently no effective therapy for the disease. More than 90% of the *ARID1A* mutations observed in OCCC are frame-shift or nonsense mutations that result in loss of ARID1A protein expression.[3] Notably, loss of ARID1A expression in OCCC significantly correlates with a shorter progression-free survival and is associated with a worse response to chemotherapy compared with ARID1A-positive OCCC. Thus, there is an even greater need for targeted therapy that is selective for *ARID1A*-mutated OCCC. Similarly, *ARID1A* mutation and/or loss of expression have been reported to be a marker of poor prognosis in a number of other cancer types. Thus, new therapeutics based on *ARID1A* mutational status is of high clinical impact.

Cancer mutations that cause a loss of function are not directly druggable with conventional targeted approaches such as antibodies. Synthetic lethality is a phenomenon in which only the simultaneous perturbation of 2 factors results in cell death.[5] Our recent study demonstrates that inhibition of enhancer of zeste homology 2 (EZH2) activity selectively suppresses the growth of *ARID1A*-mutated OCCC cells in a synthetic lethal manner.[6] EZH2 is an epigenetic regulator that silences the expression of its target genes. Notably, EZH2 is often overexpressed in OCCC. Highly specific EZH2 inhibitors (such as GSK126) have been developed, and are now in clinical trials for hematopoietic malignancies.[7] Significantly, the EZH2 inhibitor GSK126 caused the regression of established *ARID1A*-mutated OCCC and decreased the number of disseminated tumor nodules in xenograft models.[6]

Given the recent success in targeting chromatin regulators in cancer, our study has substantial translational potential for the management of *ARID1A*-mutated OCCC. In this context, *ARID1A* mutation status, determined by genome sequence in the upcoming era of precision medicine, or loss of ARID1A protein expression could serve as biomarkers to predict therapeutic efficacy to EZH2 inhibitors. Future studies are warranted to determine whether the observed synthetic lethality between EZH2 inhibition and *ARID1A* mutation extends beyond *ARID1A*-mutated OCCC. Moreover, genetic alterations in components of the SWI/SNF complex are a well-recognized feature of many cancer types. For example, in rhabdoid tumors, a rare childhood cancer, loss of expression of sucrose nonfermenting 5 (SNF5), a non-catalytic core subunit of SWI/SNF, directly upregulates EZH2 expression.[8] Survival of SNF5-deficient cancer cells depends upon the upregulated EZH2.[9] Therefore, it will be critical to determine whether the observed synthetic lethality also applies to mutations in other components of the SWI/SNF complex and to develop companion predictive markers for response to EZH2 inhibitors in these contexts.

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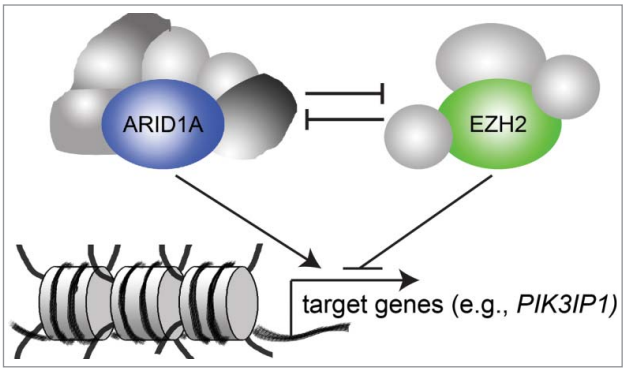

Figure 1. Antagonism between A/T-rich interacting domain 1A (ARID1A) and enhancer of zeste homology 2 (EZH2) underlies the observed synthetic lethality between EZH2 inhibition and *ARID1A* mutations. ARID1A and EZH2 are antagonistic in regulating the expression of the same set of target genes (such as *phosphoinositide 3-kinase interacting protein 1* [*PIK3IP1*]) and ARID1A dominates over EZH2 in determining the expression pattern of these genes.

To elucidate the mechanism underlying the observed synthetic lethality, we profiled changes in gene expression induced by restoration of wild type *ARID1A* or GSK126 treatment in *ARID1A*-mutated cells. This analysis revealed antagonistic roles of ARID1A and EZH2 in regulating a significant number of overlapping genes (**Fig. 1**). Notably, the antagonistic roles of SWI/SNF and polycomb proteins, which include ARID1A and EZH2 respectively, were initially suggested in genetic studies using *Drosophila*. The most interesting novel ARID1A/EZH2 target gene that we identified was *phosphoinositide 3-kinase interacting protein 1* (*PIK3IP1*). Functionally, we demonstrated that PIK3IP1 contributes to the observed synthetic lethality in *ARID1A*-mutated cells treated with EZH2 inhibitor. At the chromatin level, our data indicate that ARID1A dominates in the expression of the ARID1A/EZH2 target genes when both ARID1A and EZH2 are present. In contrast, in the absence of ARID1A, the balance is tipped toward EZH2-dependent silencing of these genes. Consequently, inhibition of EZH2 activity in the absence of functional ARID1A leads to reactivation of target genes such as *PIK3IP1* to trigger apoptosis. As such, EZH2 inhibitors selectively suppress the growth of *ARID1A*-mutated, but not wild type, cells.

In addition to *ARID1A* mutation, the phosphoinositide 3-kinase (PI3K)/protein kinase B (best known as AKT), pathway is often activated in OCCC as a result of gain-of-function mutations in *PIK3CA*, the gene encoding the catalytic subunit of PI3K. Indeed, conditional *Arid1a* knockout together with activation of *phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit* α *isoform* (*Pik3ca*) leads to the development of OCCC in genetic mouse models.[10] Interestingly, the validated ARID1A/EZH2 target gene *PIK3IP1* is a negative regulator of PI3K. These findings suggest that *ARID1A* mutation cooperates with PI3K/AKT signaling to drive OCCC. Thus, a combination of the EZH2 inhibitor together with inhibition of the PI3K/AKT pathway may carry an even greater clinical benefit.

In summary, our recent studies demonstrate that targeting EZH2 activity using clinically applicable small molecule EZH2 inhibitors represents a novel synthetically lethal therapeutic strategy in *ARID1A*-mutated OCCC. Given that mutation and loss of expression of *ARID1A* and genetic alterations in other subunits of the ATP-dependent chromatin remodeling complex are observed at a high frequency in many cancer types, these findings will have far-reaching implications for the future development of epigenetic therapeutic strategies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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