COMMENTARY

ARID1A mutation: A new target for efficient cancer immunotherapy

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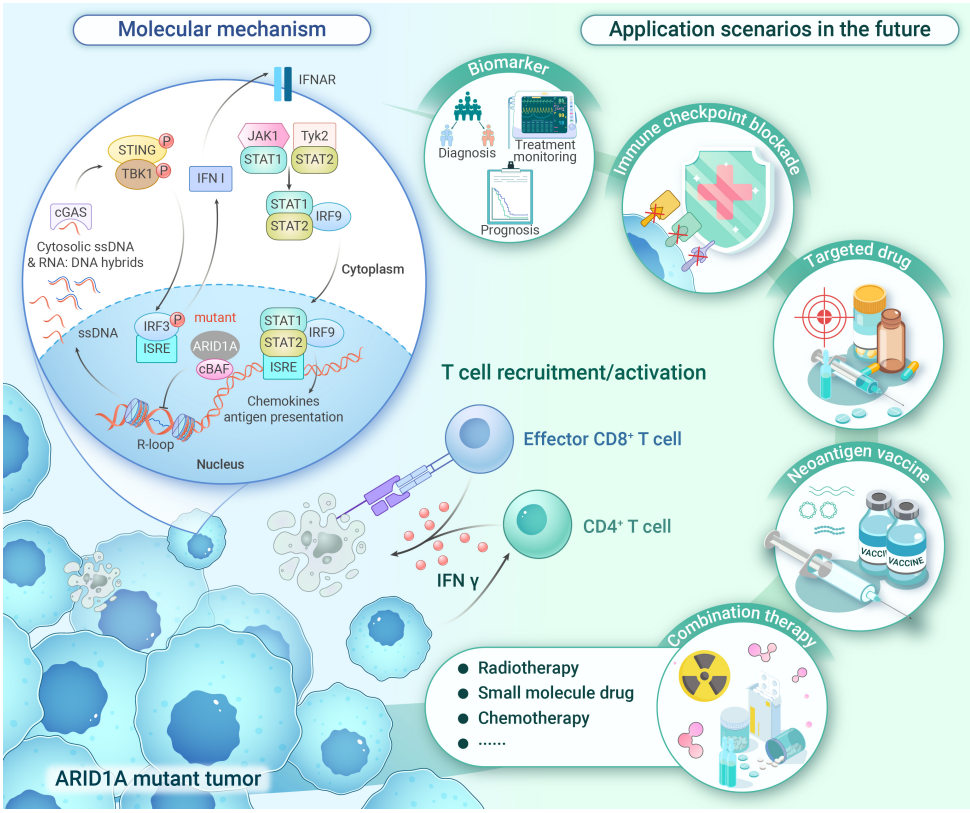

Figure 1. Molecular mechanisms and application scenarios of ARID1A-mutant tumor For molecular mechanisms of immune activation in ARID1A-deficient tumor, ARID1A deletion causes R-loop formation within the genome during DNA replication and translation in the nucleus, resulting in an accumulation of single-stranded DNA (ssDNA) and ssDNA-RNA-hybrids in the cytoplasm. ssDNA and these complexes are recognized by the cGAS-STING pathway, triggering the activation of the TBK1-IRF3 pathway, phosphorylating IRF3 (p-IRF3) and translocating into the nucleus, which in turn stimulates the production of type I interferon of tumor cells. This process also enhances the expression of chemokines and antigen-presenting factors in tumor cells, recruits $CD8^+$ and $CD4^+$ T cells, and ultimately activates anti-tumor immunity (Left). The future application scenarios of ARID1A mutation in cancer therapy, such as biomarker, immune checkpoint blockade, targeted drug, neoantigen vaccine and combination therapy (Right). Note: $CD4^+$ T cells, Cluster of differentiation 4 positive T lymphocytes; $CD8^+$ T cells, Cluster of differentiation 8 positive T lymphocytes; ARID1A, AT-rich interactive domain-containing protein 1A; cDC1, conventional class I dendritic cells; IFN γ, Interferon gamma; ssDNA, single-stranded DNA; cBAF, canonical BAF; IRF3, interferon regulatory factor 3; IRF9, interferon regulatory factor 9; cGAS, cyclic GMP-AMP synthase; TBK1, TANK Binding Kinase 1; STING, stimulator of interferon genes; IFN I, type I interferon; IFNAR, type I IFN receptor; JAK1, Janus kinase 1; Tyk2, Tyrosine Kinase 2; STAT1, signal transducer and activator of transcription 1; STAT2, signal transducer and activator of transcription 2.

Immunotherapy has revolutionized cancer treatment, with immune checkpoint blockade (ICB) being a prominent approach of immunotherapy, that activates durable and curative anti-tumor immune responses by inhibiting checkpoint proteins on T cells. However, only approximately 30% of patients experience favorable outcomes with ICB.[1] Identifying molecular biomarkers that can reliably predict ICB response is therefore of significant clinical importance, with implications for prognosis and therapeutic strategies.

About 20% of human cancers contain mutations in subunits of the switch/sucrose non-fermentable (SWI/SNF) complex.[2] The canonical BAF (cBAF) complex subunit AT-rich interactive domain-containing protein 1A (ARID1A) is the most frequently mutated SWI/SNF subunit and is one of the most frequently mutated tumor suppressor genes, with inactivating mutations found in 8-60% various solid tumors.[2] Moreover, *ARID1A* mutations have been implicated in diverse biological processes, including cancer immune response, DNA damage checkpoint regulation etc. The clinical trials of ICB have identified a significant enrichment of *ARID1A* mutations in patients who respond to immunotherapy across various solid tumors,[3] indicating that *ARID1A* mutations could be a predictive biomarker for ICB response. However, the molecular mechanisms by which *ARID1A* mutations enhance ICB efficacy remain unclear.

Recently, researchers from the Salk Institute investigated the impact of *ARID1A* mutations on ICB therapy and the mechanisms underlying anti-tumor immunity.[2] Using *ARID1A* knockout (*ARID1A*-KO) mice, they observed reduced tumor growth that was attributed not to the inhibition of tumor cell proliferation but rather to induced a robust anti-tumor immune response. In contract, the tumor suppression effects were absent in adaptive immunodeficient mice. Notably, *ARID1A*-KO tumors displayed an increase in $CD8^+$ T cells, which expressed tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and granzyme B (GZMB).

Using RNA sequencing and gene enrichment analysis of *ARID1A*-KO tumor cells, investigators established a set of 57 interferon-stimulated genes (ARID1A-IFN) containing antigen presentation (*Tap1*, *Psmb8*, *Psmb9*), IFN-responsive transcription factors (*Stat1*, *Stat2, Irf9*), and T-cell recruitment (*Cxcl10*). Moreover, the ARID1A-IFN set was also significantly upregulated in patients with *ARID1A*-mutant tumors. Most *ARID1A* mutations are nonsense or frameshift mutations, leading to loss of function, with a smaller proportion being missense mutations. By further analyzing The Cancer Genome Atlas (TCGA) data from patients with colorectal, gastric, and uterine cancer, researchers found that nonsense and frameshift mutations, but not missense mutations, resulted in reductions in ARID1A protein levels and significant increases in the ARID1A-IFN signature and immune cytolysis score. Next, it was revealed that high ARID1A-IFN signature scores were significantly correlated with prolonged survival only in colon cancer patients with *ARID1A* mutations, but not in those without. Meanwhile, a significant positive correlation was identified between high ARID1A-IFN signature and complete response to anti-PD-1 therapy in patients with gastric cancer. Overall, these results demonstrate a significant correlation between high ARID1A-IFN profiles and beneficial clinical outcomes, suggesting that ARID1A expression could be used as a biomarker to predict the sensitivity of ICB treatment, providing a theoretical foundation for prioritizing immunotherapy in patients with *ARID1A* mutations. However, it is important to note that the "ARID1A-IFN signature" was derived from a limited variety of ARID1A-deficient cancer cell lines (melanoma and colon cancer). Meanwhile, the significant associations between high ARID1A-IFN signatures and positive clinical outcomes have been validated in some ARID1A-mutated cancers characterized by nonsense and frameshift mutations (e.g., colorectal, gastric, and uterine cancers). In the future, expression of the ARID1A-IFN signature needs to be further evaluated in a broader range of cancers, like non-small cell lung cancer (NSCLC) with

predominant missense mutations. Moreover, the sensitivity of the ARID1A-IFN signature as a biomarker may vary depending on tumor type, differentiation, tumor stage, tissue architecture, and histological grade. To solve the above challenges, it is necessary to integrate multiple modalities, including gene editing, organoid culture, systems biology, small molecule screening, and artificial intelligence, which will allow for a comprehensive assessment of the accuracy, sensitivity, and reproducibility of the ARID1A-IFN signature as a pan-cancer therapeutic target and predictive molecular marker.

In this study, Maxwell and colleagues[2] first demonstrated that ARID1A deletion in mouse models triggers anti-tumor immunity through R-loop-derived cytoplasmic DNA and the STING-IFN-I pathway, which elucidates the molecular mechanisms underlying the enhanced anti-tumor immunity and immunotherapeutic response clinically observed in *ARID1A*-mutant tumors (Figure 1), providing insights into clinical findings where *ARID1A* mutations correlate with enhanced ICB responsiveness. Specifically, ARID1A deletion promotes the formation of R-loops during DNA replication and transcription, resulting in the accumulation of ssDNA and RNA-DNA hybrids in the cytoplasm. These hybrids are detected by the cGAS-STING pathway, triggering the production of IFN-I, which in turn recruit $CD8^+$ and $CD4^+$ T cells, activating anti-tumor immunity. In addition, ARID1A-deficient tumor cells can produce neoantigens or increase chemokine expression to promote T-cell recruitment in tumor tissues. The ARID1A-IFN gene signature, driven by R-loop-derived cytoplasmic DNA and the cGAS-STING pathway, could serve as a biomarker for predicting and prioritizing immunotherapy efficacy in *ARID1A*-mutant cancers. Moreover, ARID1A itself can be employed as a therapeutic target, as targeting ARID1A or inhibiting SWI/SNF complex can induce R-loop-driven ARID1A-IFN, transforming immunologically "cold" tumors into "hot" tumors. This offers novel strategies for tumor ICB therapy, which greatly promotes the research of cBAF inhibitors and ARID1A-targeted therapies in the context of ICB.

ARID1A-mutant tumors also implicated in other signaling pathways, including the mevalonate pathway, glutathione (GSH) metabolism, PI3K-AKT-mTOR pathway, and NFκB pathway. In ARID1A inactivated ovarian clear cell carcinoma, mevalonate pathway inhibitors, such as simvastatin, have been shown to inhibit tumor growth in mice, enhance anti-tumor immunity through pyroptosis, and synergize with ICB.[4] Besides, targeting GSH metabolic pathway by APR-246/buthionine sulfoxide and PI3K/AKT/mTOR signaling by EZH2 or 3-MA could induce ferroptosis, disulfur death or autophagy in ARID1A-deficient tumor. These results highlight that *ARID1A* mutation-associated pathways can be targeted to induce distinct forms of cell death in tumor cells and inhibit tumor growth. Tumor cells undergoing ferroptosis or pyroptosis release tumor antigens and danger-associated molecular patterns (DAMPs), which can activate immune cells and enhance immune responses. Moreover, tumor neoantigens induced by *ARID1A* mutations may serve as direct targets for immunotherapy or be employed in neoantigen vaccines to potentiate anti-tumor immunity.

ARID1A plays a significant role in enhancing tumor radio-sensitivity. ARID1A deletion leads to impaired non-homologous end joining (NHEJ) repair, which disrupts the homologous recombination (HR) /NHEJ DNA repair balance. This disruption increases the dependence of tumor cells on PARP-mediated DNA repair and enhances the sensitivity of post-irradiation tumor cells to PARP inhibitors, suggesting that radiotherapy combined with PARP inhibitors may be a promising new therapy for *ARID1A*-KO tumors.[5] For patients without *ARID1A* mutations, ARID1A inhibitors could be employed to block its function and disrupt DNA repair mechanisms. When followed by radiotherapy and PARP inhibition, this approach could improve the anti-tumor efficacy.

Moreover, for tumors without *ARID1A* mutations, autologous tumor cells from patients could be manipulated to create ARID1A-deficient tumor cells in vitro, followed by the preparation of tumor lysates. These lysates could serve as immune adjuvants, promoting chemokine release and enhancing T-cell infiltration. This strategy could transform immune-suppressive tumors into immune-activated tumors, thereby improving the immunogenicity of the tumor and augmenting the anti-tumor effects of other treatments, including ICB, radiotherapy, and chemotherapy. Besides, the cell lysate contains other tumor antigens of the patient, which can be potentially served as an autologous personalized vaccine to inhibit tumor growth alone or combined with other therapeutic techniques.

Therefore, this achievement has profound implications for the selection of clinical oncology treatments, which can not only help clinicians select advantageous patients for ICB treatment based on *ARID1A* mutations, but also further improve the therapeutic efficacy by targeting ARID1A or related molecules in accordance with the molecular mechanism discovered, providing an inspiration for the development of novel ARID1A-related therapies. Nevertheless, there is a paucity of pertinent research on ARID1A deletion in tumors, and further investigation remains to be explored. For instance, it is that not all human tumors with *ARID1A* mutations exhibit the ARID1A-IFN set identified in this study. Future research should investigate the specific contexts in which *ARID1A* mutations induce the ARID1A-IFN signature and assess how well this set predicts ICB response rates in patients with *ARID1A*-mutated tumors. The mechanisms by which ssDNA and RNA-DNA hybrids are released from the R-loops into the cytoplasm also require further investigation to clarify their respective roles in activating the cGAS-STING pathway. R-loop/DNA stress has been identified as a biomarker of ICB response in non-hypermutated cancers, suggesting that tumor cell replication stress may be an independent factor triggering anti-tumor immune response, and this hypothesis needs further validation, especially in tumors without *ARID1A* mutations or hypermutation. ARID1A deficiency can sensitize cancer cells to other drugs, such as ATM or Chk2 inhibitors, DNA damage response inhibitors, and PARP inhibitors, but the optimal dose at which they modulate immune responses needs to be further determined.

Based on the conclusions of ICB clinical trials, this study investigated the effect of ARID1A mutation on ICB response and anti-tumor immunity, and elucidated the relationship and molecular mechanism between *ARID1A* mutation and tumor immune surveillance. The findings offer valuable insights into the molecular mechanisms regulating the enhanced response of human tumors with *ARID1A* mutations to ICB observed in clinical trials, providing methods for clinicians to prioritize ICB therapy. To further advance ARID1A as a therapeutic target, the most direct approach is the development of ARID1A regulatory drugs, such as cBAF inhibitors and SWI/SNF complex pharmacological inhibitors. *ARID1A* mutations also present therapeutic vulnerabilities that can be exploited by molecular inhibitors to suppress tumor growth or enhance ICB efficacy. Meanwhile, mutated ARID1A can interact with these target molecules to cause synthetic lethality and control tumor growth. Lastly, the "ARID1A-IFN signature" has the potential to serve as a biomarker for predicting ICB response or treatment outcomes in cancer patients. The genes within this signature, whether individually or in combination, also offer avenues for the development of multimodal biomarkers for cancer therapy. In conclusion, this study provides novel insights into the potential for new therapeutic approaches targeting *ARID1A* mutations, which could contribute to more effective treatment strategies.

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AUTHOR CONTRIBUTIONS

H.C. and G.G. equally contributed to this manuscript. Both H.C. and G.G. wrote this manuscript. The initial concept for this manuscript was conceived by M.W. and G. Y. All authors reviewed and revised the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.