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Research

AURKA inhibition shows promise as a therapeutic strategy for ARID1A-mutant colorectal cancer

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Abstract

Purpose Mutations in ARID1A frequently occur in colorectal cancer (CRC) cells. However, there are currently no clinical treatment options specifically addressing this aberration. The preliminary in vitro experiments revealed a synthetic lethal interaction between ARID1A and Aurora kinase A (AURKA) in colorectal cancer (CRC) cells.

Methods We collected samples from 80 CRC patients and evaluated the efficacy of AURKA inhibitor (AURKAi) using the ATP-tumor chemosensitivity assay (ATP-TCA) on untreated ARID1A-proficient (ARID1A +) and ARID1A-deficient (ARID1A-) CRC patient samples. In addition, we validated this result by a clonogenic assay. Additionally, we examined the effects of AURKA inhibitors on cell cycle progression and apoptosis in ARID1A + and ARID1A- CRC patient samples using flow cytometry.

Results The results showed that AURKAi selectively inhibited the growth of ARID1A- CRC cells. Furthermore, AURKA inhibitors significantly increased G2/M arrest and induced apoptosis in ARID1A- cells.

Conclusion We believe that AURKAi hold promise as potential therapeutics for ARID1A mutation colorectal cancer patients.

Keywords Colorectal cancer · ARID1A · AURKA · Ex vivo · SWI/SNF

Abbreviations

CRC	Colorectal cancer
AURKA	Aurora kinase A
SWI/SNF	Wwitch/sucrose non-fermenting
ARID1A	AT-rich interaction domain 1A
AJCC	American joint committee on cancer
ATP-TCA	ATP-tumor chemosensitivity assay
OS	Overall survival

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1 Introduction

Mutations in various subunits of the switch/sucrose non-fermenting (SWI/SNF) remodeling complex have been identified across multiple cancer types, including colorectal cancer (CRC) [1, 2]. Among these subunits, ARID1A (also known as BAF250A) plays a crucial role in the SWI/SNF complex, which functions as an ATPase chromatin remodeling and tumor suppressor entity [3]. Dysregulation or loss of function in this complex is implicated in genomic instability observed in tumor development [4]. Notably, mutations in the ARID1A gene are present in approximately 10% of CRC cases [1], with recent findings suggesting a strong correlation between ARID1A loss and CRC progression and metastasis [5]. Given the high frequency of ARID1A mutations and loss of expression in cancer, therapeutic strategies have focused on exploiting synthetic lethality [6]. Synthetic lethality refers to the phenomenon where the simultaneous loss of two genes leads to cell death, whereas the loss of either gene alone is viable [7]. This approach has been utilized in cancer therapy, particularly in targeting tumors with loss-of-function mutations in tumor suppressor genes [8]. By disrupting synthetic lethality targets, selective lethality in cancer cells harboring tumor suppressor mutations can be induced.

Recent epigenetic drug screening has revealed that inhibitors targeting aurora kinase A (AURKA) act as synthetic lethality partners of ARID1A [9]. AURKA, a mitotic serine protein, is involved in various biological processes, including promoting mitotic entry from the G2 phase through the nuclear localization of CDC25C [9]. AURKA overexpression is associated with tumorigenesis and genetic instability in multiple cancers, including lung, pancreas, and CRC, and correlates with poor outcomes in lung and colon cancer patients [10]. These findings highlight AURKA as a significant therapeutic target.

AURKA inhibitors have made some progress in clinical research over the past decade, particularly in the treatment of various cancer types such as breast cancer and small cell lung cancer [11, 12]. MLN8237 (alisertib) is one of the main AURKA inhibitors and has been evaluated for its efficacy and safety in several clinical trials [13]. However, regarding ARID1A-deficient colorectal cancer, although specific clinical trial information is limited, preliminary studies have suggested that AURKA inhibitors might have potential therapeutic effects in this context [10]. For instance, in tumors deficient in ARID1A, inhibition of AURKA can lead to cell cycle arrest and apoptosis, potentially inhibiting tumor growth.

Given these preliminary results, investigating the application of AURKA inhibitors in ARID1A-deficient colorectal cancer patient samples is particularly important. To evaluate the synthetic lethality concept of AURKA inhibitors and ARID1A deficiency in colon cancer, the AURKA inhibitor Alisertib were used to exert synergistic effects on ARID1A- CRC patient samples, demonstrating their efficacy. Further clinical studies can validate their efficacy and safety in this specific context, providing new treatment options for patients with ARID1A-deficient colorectal cancer.

2 Methods

2.1 Patient sample

CRC tissues were collected from 80 consenting patients after surgical colorectal resection, all of whom had no prior exposure to chemotherapy or radiotherapy. Each tissue sample was harvested within one-hour post-surgery. Part of each sample was allocated for the ATP-TCA test in the lab, while the remaining tissue was preserved in formalin for IHC processing. All patient samples used in our study were obtained with informed consent. We adhered to strict protocols to ensure that patients were fully informed about the purpose of the research and their rights. Consent forms were reviewed and approved by our Institutional Review Board (IRB ID: TJ-C20220526), and all participants provided written consent prior to sample collection. Samples were de-identified to protect patient confidentiality and were stored securely to prevent unauthorized access. We followed all applicable guidelines for the ethical use and disposal of human tissues. We implemented stringent privacy measures to safeguard patient information. Access to patient data and tissue samples was restricted to authorized personnel only, and all data was anonymized to ensure confidentiality. Our study procedures were reviewed and approved by the IRB to ensure that they met all ethical requirements.

2.2 Clonogenic survival assay

During the surgical procedure, the harvested tissues were immediately placed in McCoy's 5A Medium containing 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin (Invitrogen Corp, Carlsbad, CA) on ice, and were promptly transported to the laboratory. The fresh tissue was diced into approximately 1 mm pieces using scissors and then subjected to digestion with 3 ml of Collagenase A (0.4 mg/ml) (Roche Applied Science, Indianapolis, IN) at 37 °C for 1 h with periodic agitation. The reaction was halted with 20 µl of EDTA (500 mM). The digested tissue was then quickly processed in a homogenizer and the resulting mixture was filtered through a 70 µm mesh strainer (BD Falcon, San Jose, CA) to remove any remaining tissue debris. The cells were washed with PBS, centrifuged to pellet them, and subsequently cultured in McCoy's 5A Medium supplemented with 10% FBS and 1% penicillin–streptomycin in a humidified incubator set at 37 °C with 5% CO_2. Cells were then plated in triplicate in 6-well culture dishes, each with an area of 9.6 cm^2. They were given 4–6 h to adhere before being treated with 20 nM and 35 nM Alisertib. Following this, they were incubated for an additional 10–14 days at 37 °C with 5% CO_2. Subsequently, the cells were fixed and stained using a solution of 96% ethanol and 15% Giemsa, and then rinsed with distilled water.

2.3 Immunohistochemistry (IHC) analysis

The ARID1A protein levels in 57 primary CRC samples were assessed using IHC, as previously described [4, 14]. Briefly, each tissue section underwent deparaffinization, rehydration, blocking of endogenous peroxidase, and antigen retrieval. This was followed by an overnight incubation at 4 °C with the ARID1A (PSG3) antibody (sc-32761, Santa Cruz Biotechnology, Inc., CA, USA). Sections were then rinsed with PBS containing 0.1% Tween-20, incubated with a secondary antibody against mouse antigens for 30 min, and treated with streptavidin-HRP. Color development was achieved using Diaminobenzidine tetrahydrochloride, with hematoxylin for counterstaining. Two independent evaluators, blinded to the clinical and pathological details of the patients, reviewed and scored the stained sections. Over 1000 cancer cells per slide were examined to evaluate staining intensity and the proportion of stained cells. ARID1A expression was considered positive if more than 60% of cells were stained, and negative if 60% or fewer were stained.

2.4 ATP-tumor chemoresistance assay (ATP-TCA)

The ATP-TCA was performed following previously described methods [4]. Solid tumor tissues were finely minced and digested with collagenase (1.5 mg/ml) at 37 °C overnight (approximately 12 h). Debris and red blood cells are removed using Ficoll-Hypaque solution (density 1.077) through density gradient centrifugation. Centrifugation conditions: 400 × g, 20 min, room temperature. Cells are resuspended in serum-free CAM medium and supplemented with the following antibiotics and antifungal agents: gentamicin (50 µg/ml), penicillin–streptomycin (100 U/ml), metronidazole (10 µg/ml), and amphotericin B (2.5 µg/ml). Cell viability and count are determined using trypan blue exclusion. Cell suspensions are prepared at 200,000 cells/ml for solid tumors and 100,000 cells/ml for malignant effusions. Polypropylene round-bottom 96-well plates (Corning-Costar, High Wycombe, UK) are used for the experiment, with 200 µl of CAM medium added to each well. Inhibitors are added at six levels (5 nM to 200 nM) in triplicate. Inhibitor concentration are based on prior pharmacokinetic and biological response data [15]. Each 96-well plate includes a medium-only control (MO) and a maximum inhibitor concentration control (MI) for complete cell kill and zero ATP reading. Plates are incubated at 37 °C, 5% CO_2, and 100% humidity for 6 days. After incubation, cells are lysed using Tumor Cell Extraction Reagent (DCS Innovative Diagnostik Systeme). ATP levels are measured using a microplate luminometer with a luciferin-luciferase assay (MPLX, Berthold, Pforzheim, Germany).

2.5 Cell cycle

The cell cycle analysis was performed following previously described methods [16, 17]. Cells were seeded in six-well plates at densities ensuring 70–80% confluence at examination. After settling, cells were treated with 20 nM Alisertib for 48 h, washed twice with PBS, trypsinized, and centrifuged at 800 × g for 5 min. Cells were fixed in 70% ethanol for 2 h at 4 °C, washed twice with ice-cold PBS, and resuspended in PBS containing 50 µg/ml RNase at 37 °C for 30 min. They were then stained with 50 µg/ml propidium iodide in darkness at 4 °C for 30 min. Flow cytometric analysis was performed using a BD FACSCalibur flow cytometer (BD Biosciences, San Diego, CA, USA), and data were analyzed with ModFit software (Verity Software House, Inc., Topsham, ME, USA).

2.6 Statistical analysis

Data are presented as mean ± standard deviation. Statistical analyses were performed using SPSS 14.0 and SAS software (version 14.1, SAS Institute, Cary, NC, USA). Statistical significance was defined as a p-value less than 0.05. The $\chi 2$ test was used to examine the relationship between ARID1A expression and clinicopathological features, depending on the data type.

3 Results

3.1 Clinicopathologic characteristics and ARID1A expression in patient samples of colorectal *cancer*

Tissue samples from 80 individuals diagnosed with CRC were evaluated for ARID1A expression (Fig. 1A). As indicated in Table 1, 9 out of 80 tumors (11.25%) were ARID1A negative. The patients' clinical and pathological characteristics are concisely presented in Table 1. It is notable that there was no correlation between the loss of ARID1A expression and factors such as gender, age, tumor location, TNM stage, or tumor size. However, a statistically significant difference in ARID1A expression was observed concerning pathological differentiation ($P < 0.05$). These findings suggest that CRC patients lacking ARID1A expression tend to have poorly differentiated tumors.

3.2 AURKAi sensitizes primary tumors of CRC patients with ARID1A deficiency

The sensitizing effect of AURKAi on colorectal cancer, with and without ARID1A expression, was assessed in an ex vivo experiment. Cells from primary tumors exhibiting both ARID1A expression patterns underwent testing using an ATP-based tumor chemosensitivity assay (ATP-TCA). Among the 80 colorectal cancer specimens, 68 completed the ATP-TCA test, while 12 were excluded due to low cell count. All 68 cases, including 7 negative and 61 positive colorectal cancer specimens, were utilized to construct a dose–response curve for AURKA inhibitor (Alisertib) via the ATP-TCA method (Fig. 1B, demonstrating better cell viability on the y-axis). The findings unequivocally illustrate that the ARID1A- group exhibits significantly greater sensitivity to Alisertib compared to the ARID1A + group, with IC50 values of 35.4 nM (95% CI: 32.05 to 42.17) and 20.94 nM (95% CI 17.35 to 25.55) respectively ($p < 0.001$). We next confirmed the anti-proliferative effect of Alisertib at different concentrations (20 nM and 35 nM) using a clonogenic assay (Fig. 1C and D for 20 nM, and Fig. 1E and F for 35 nM). The results show that ARID1A- Ex Vivo CRC cells are more sensitive to Alisertib. We also observed that increasing the concentration of Alisertib reduced colony formation in ARID1A + Ex Vivo CRC cells, although the effect was more pronounced in ARID1A- Ex Vivo CRC cells.

3.3 Impact of AURKAi and ARID1A deficiency on cell cycle and apoptosis

AURKA is a member of the highly homologous family of serine/threonine kinases, pivoting in cell cycle and division regulation. Therefore, we assessed the impact of AURKAi on cell cycle distribution and apoptosis concerning its synthetic lethality effect in the context of ARID1A deficient. As a result, AURKAi leads to an increase of cells in the G2/M phase in ARID1A mutant cells, but an increase of cells in the S phase in ARID1A wild-type cells (Fig. 2A). In addition, we found out that inhibition of AURKA selectively induced apoptosis in ARID1A – Ex Vivo CRC cells (Fig. 2B).

4 Discussion

Colorectal cancer (CRC) ranks among the most prevalent malignancies globally, accounting for nearly 1,850,000 cases annually and representing the second leading cause of cancer-related deaths [9, 18]. Approximately 881,000 individuals succumb to CRC each year, constituting 9.2% of all fatal cancer cases worldwide. CRC patients with locoregional lymph node involvement exhibit a 5 year overall survival (OS) rate of 70%, whereas those with distant metastases have

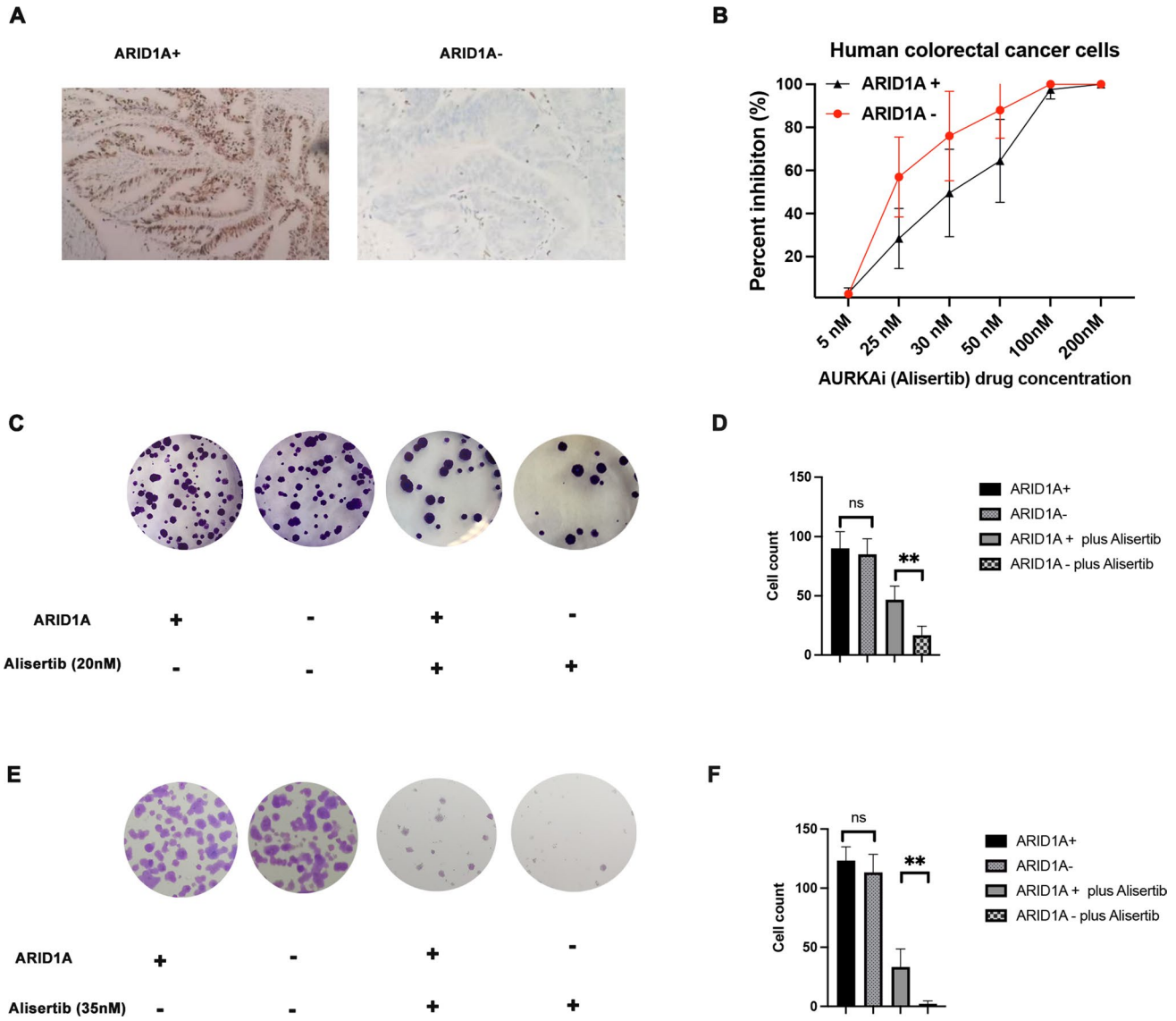

Fig. 1 Effect of AURKAi in CRC patients' samples. **A**: illustration of immunohistochemistry (IHC) results showing the presence or absence of ARID1A expression in CRC clinical tumor. **B**: evaluation of the impact of the AURKAi alisertib on ex vivo CRC patient cells using the ATP-Tumor Chemosensitivity Assay. The assay measured ATP activity in ex vivo cells treated with alisertib at concentrations ranging from 0 to 10 nM in CRC patient cells with (+) and without (-) ARID1A expression. X-axis is the test drug concentration percentage, and the Y-axis is the tumor growth inhibition percentage. **C**: results of the colony formation assay of ARID1A + and ARID1A—Ex Vivo CRC cells in 20 nM alisertib; **D**: percentage of colonies formed of ARID1A + and ARID1A—Ex Vivo CRC cells in 20 nM alisertib. **E**: results of the colony formation assay of ARID1A + and ARID1A—Ex Vivo CRC cells in 35 nM alisertib; **F**: percentage of colonies formed of ARID1A + and ARID1A—Ex Vivo CRC cells in 35 nM alisertib

a markedly poorer prognosis, with a 5 year OS of only 12% [19]. Given the unfavorable outcomes associated with CRC, it is imperative to elucidate novel molecular mechanisms underlying CRC development to enhance survival rates.

Loss of ARID1A expression is a common phenomenon across various cancers [20–22]. Recent research has demonstrated that nearly 60% of breast invasive ductal carcinomas exhibit reduced ARID1A expression, correlating with the most aggressive breast cancer phenotypes [21]. Another study indicated that ARID1A loss occurs in 30% of gastric cancer cases and is linked with poor clinical outcomes [20]. The current evidence showed that ARID1A as a tumor suppressor gene pivotal in the progression of numerous cancers. Nonetheless, limited research has been conducted on the frequency and clinical significance of ARID1A loss in CRC, with existing findings being inconclusive [20, 23, 24]. First of all, the incidence of ARID1A mutations in colorectal cancer (CRC) varies widely across different studies.

Table 1 Relationship between ARID1A mutation and clinicopathological characteristics in colon cancer Patients

Characteristics	ARID1A expression		
	Negative (n = 9)	Positive (n = 71)	P
Age			0.521
≤ 70	6	51	
> 70	3	20	
Gender			0.712
Male	5	43	
Female	4	28	
Tumor location			0.844
Right colon	2	21	
Left colon	1	5	
Rectal	6	45	
TNM stage (AJCC)			0.181
I	0	20	
II	2	10	
III	7	41	
Pathologic differentiation			<0.001
Poor	6	8	
Moderate	2	11	
Well	1	52	
Tumor size (cm)			0.509
≤ 5	3	20	
> 5	6	51	
Lymphatic penetration			0.072
Negative	4	53	
Positive	5	18	

AJCC American Joint Committee on Cancer, *TNM* Tumor-node- metastasis, *ARID1A* AT-rich interactive domain 1A. The correlation between ARID1A expression and clinicopathological characteristics was analyzed by χ 2 test

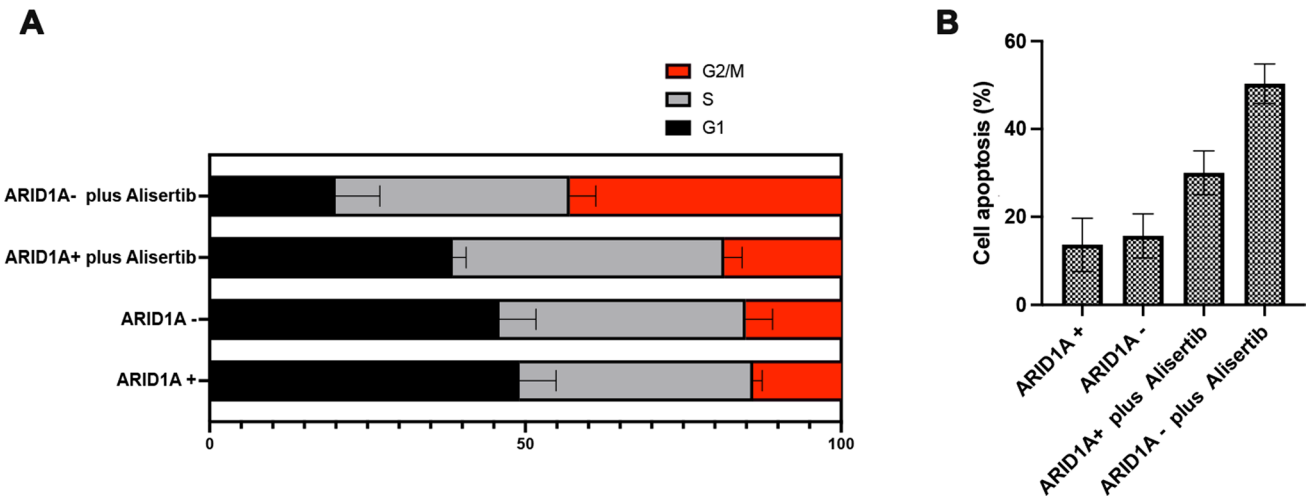

Fig. 2 Effects of AURKAi on Cell Cycle Arrest and apoptosis in ARID1A- CRC patients' samples. **A**: effect of AURKAi on cell cycle progression; **B**: effect of AURKAi on apoptosis

According to a comprehensive review, the frequency of ARID1A variations (including mutations and loss of expression) ranges from 3.6% to 66.7% among different studies [9].

Our research found that ARID1A mutations occur in approximately 11% of CRC cases, which align with those of Jones et al. [25], who reported an ARID1A mutation rate of around 10% in CRC. However, Wei et al. [20] discovered that 25.8% of primary CRC tumors exhibited loss of ARID1A protein expression. These findings suggest that while ARID1A loss is not extremely common, it plays a significant role in a subset of CRCs. In addition, Wei et al. [20] identified an association between ARID1A loss and distant metastasis as well as advanced TNM stage in CRC, but found no correlation with age, gender, tumor location, or size. Conversely, Chou et al. [23] discovered a significant association between ARID1A loss and factors such as age, gender, tumor location, and size, yet reported no link with distant metastasis or TNM stage in CRC. These conflicting results prompted us to explore the incidence of ARID1A loss in CRC and its association with clinicopathological parameters. We identified a significant correlation between ARID1A loss and clinicopathological parameters. In our study, we observed that ARID1A expression was associated with pathological differentiation, with ARID1A mutations correlating with poorer pathological differentiation. Our findings are consistent with those of Kim et al. [26], Wei et al.[20], and Inada, Ryo et al. [27] research. The likely reason is that mutations in ARID1A impair DNA repair mechanisms, making cells more prone to mutations and chromosomal instability when dealing with DNA damage. This genomic instability is a significant factor contributing to the high invasiveness and poor differentiation of tumor cells. In addition, the loss of ARID1A leads to dysfunction in cell cycle checkpoints, preventing cells from differentiating properly and resulting in immature tumor cells. These cells exhibit higher proliferation and invasion capabilities. Nonetheless, one study found that abnormalities in ARID1A were linked to a better prognosis [28]. In our study, we did not find a correlation between ARID1A loss and factors such as age, gender, tumor location, and size. This may be due to the relatively small sample size of our study, suggesting that a larger sample size might yield different results. For instance, our findings on lymphatic penetration were very close to the 0.05 significance threshold.

Since ARID1A mutation is relatively common in colorectal cancer (CRC) and its mutations are associated with certain clinicopathological parameters, targeted therapies aimed at ARID1A mutations hold significant clinical relevance. Synthetic lethality is currently a hot topic in gene-targeted therapy [7]. Synthetic lethality occurs when the simultaneous loss or inhibition of two genes results in cell death, while the loss of either gene alone does not. This concept can be particularly useful in cancer therapy, where it allows for the targeting of specific vulnerabilities within cancer cells [7]. In this scenario, targeting a second gene or pathway that the cancer cell becomes dependent on due to the ARID1A mutation can induce synthetic lethality. Recent epigenetic drug screening has revealed that inhibitors targeting aurora kinase A (AURKA) act as synthetic lethality partners of ARID1A [9]. AURKA is involved in cell cycle regulation and mitosis. The combined loss of ARID1A function and AURKA activity creates a situation where cancer cells cannot effectively repair DNA or undergo proper cell division, leading to cell death. This approach can selectively target cancer cells with ARID1A mutations while sparing normal cells, thereby providing a potential therapeutic strategy with reduced off-target effects. Wu et al. [10] found in vitro experiments that ARID1A exhibits a synthetic lethal interaction with AURKA in colorectal cancer (CRC) cells.

The primary goal of our study was to confirm the concept of selective effect of AURKAi on ARID1A deficient tumor cells in a more clinically relevant context, primary tumor cells isolated from surgical material of CRC patients were characterized for ARID1A status, treated with AURKAi and the effect on viability was measured. Our results confirm that there is a synthetic lethal interaction with AURKA and ARID1A-mutation in CRC. The data from the ATP-based tumor chemosensitivity assay (ATP-TCA) reveal that CRC patient cells from ARID1A- tumors were more sensitive to the AURKAi compared to ARID1A + ones. Our colony formation assay is also shown that AURKAi had better effect on ARID1A- cells. Our results are consistent with previous in vitro experiments, which may be due to the following reasons: ARID1A is part of the SWI/SNF chromatin remodeling complex, essential for maintaining genomic stability and proper DNA repair mechanisms. When ARID1A is mutated, these processes are disrupted, making the cancer cells more reliant on other pathways for survival. AURKA plays a critical role in cell cycle regulation and mitosis. Inhibiting AURKA in ARID1A-deficient cells exacerbates their inability to properly manage cell division and DNA repair, leading to increased cell death compared to cells with functional ARID1A [29]. In addition, in ARID1A-deficient cells, the overactivation of the AURKA/PLK1/CDC25 axis leads to abnormalities in cell cycle regulation and division. AURKA inhibitors, by inhibiting PLK1 activity, further exacerbate these cell division abnormalities, resulting in increased cell death [30, 31]. Besides, ARID1A-mutant cells may alter metabolic pathways, becoming more dependent on specific metabolic processes, such as those related to AURKA. Inhibiting AURKA can disrupt these metabolic pathways, leading to an imbalance in cellular energy metabolism and cell death [32]. Some other studies have shown that AURKA may regulate autophagy and apoptosis processes. In ARID1A-deficient cells,

these processes might already be disrupted. Therefore, further inhibition of AURKA can trigger stronger autophagy or apoptosis responses, leading to cell death [33, 34].

In our study, we also explored the mechanism of the interaction between AURKA inhibitors and ARID1A mutations. We evaluated the role of cell cycle redistribution and apoptosis. Our results show that AURKAi leads to an increase of cells in the G2/M phase in ARID1A mutant cells, but an increase of cells in the S phase in ARID1A wild-type cells. The reasons for these results may be: ARID1A mutations impair chromatin remodeling functions, which in turn affect the normal operation of cell cycle checkpoints. In these mutant cells, AURKA inhibition may further disrupt G2/M checkpoint control, leading to cell cycle arrest in the G2/M phase. This is because the G2/M checkpoint is particularly sensitive to DNA damage and incomplete DNA replication, and ARID1A mutations make these cells more prone to problems at this checkpoint [35]. Besides, ARID1A mutations can affect DNA damage repair mechanisms, causing these cells to require more time for repair during the G2/M phase. The use of AURKA inhibitors may exacerbate these repair challenges, resulting in a higher proportion of cells being arrested in the G2/M phase [6]. In ARID1A wild-type cells, however, chromatin remodeling functions and cell cycle checkpoints operate normally. When AURKA inhibitors are used, these cells are more likely to arrest in the S phase, as AURKA inhibition directly affects the DNA replication process. This activation of the DNA replication monitoring system leads to S phase arrest to cope with replication stress [22]. In addition, AURKA is involved in the regulation of several S phase-related proteins. In ARID1A wild-type cells, AURKA inhibition may affect the function of these proteins, thereby hindering cell cycle progression through the S phase and increasing the proportion of cells in the S phase [36]. While our results are encouraging, our study has several limitations. Firstly, the sample size was relatively small, involving a limited number of patients. Thus, it will be essential to conduct large-scale, multicenter trials to confirm our findings. Moreover, although ex vivo experiments are crucial for evaluating clinical drugs, further validation through in vivo studies remains necessary.

In conclusion, AURKA has garnered increasing recognition as a promising target for cancer therapy due to its widespread overexpression in various cancer types [37, 38]. Several small-molecule kinase inhibitors, including alisertib, danusertib, MK-5108, and ENMD-2076, have entered clinical trials targeting AURKA for cancer treatment [12, 39–41]. Among these, alisertib (MLN8237) stands out as the most clinically advanced AURKA inhibitor, currently undergoing phase I/II/III clinical investigation for treating leukemia and numerous solid tumors [42]. The clinical efficacy of alisertib varies depending on the tumor type and its potential to enhance progression-free survival and disease stability across various tumor types holds promise [43]. While clinical studies of AURKA inhibitors in hematologic malignancies have progressed rapidly, progress in solid tumor studies has been slower. Hence, there is a pressing need for prompt clinical investigations of AURKA inhibitors for treating solid tumors. In summary, our findings highlight AURKA as a key target gene in ARID1A-deficient CRC cells.

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Data availability The datasets generated and/or analyzed during the current study are not publicly available due to the fact that there are still some related experiments in progress in our group but are available from the corresponding author on reasonable request.

Declarations The authors declare no competing interests.

Ethics approval and consent to participate All experiments involving human participants, human data, or human tissue samples were conducted in accordance with relevant guidelines and regulations. The present study was approved by the ethics committee of MianYang Fulin Hospital in China (IRB ID: TJ-C20220526). Written informed consent was obtained from all participants.

Competing interests The authors declare no competing interests.

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