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RAPID COMMUNICATION

Inhibition of ALDH2 by disulfiram leads to synthetic lethality via ROS strikes twice in *ARID1A*-deficient cholangiocarcinoma

Synthetic lethality describes an interaction whereby the co-occurrence of two mutations leads to cell death but one mutation alone does not, which can be exploited for cancer therapeutics.[1] Due to lacking effective non-surgical treatment and early clinical diagnosis markers, patients have high mortality and low overall survival rates in cholangiocarcinoma (CCA).[2] Based on our previous predicted results in CCA (SLOAD, http://tmliang.cn/SL), *ARID1A*: *ALDH2* is a pair gene with synthetic lethal. Thus, the involvement of ARID1A in maintaining genome stability makes it a potential candidate for synthetic lethal therapy of ARID1A-deficient tumors. ALDH2 belongs to aldehyde dehydrogenase (ALDH) family. Endogenous aldehyde accumulation is considered as an important source of cellular reactive oxygen species (ROS).[3] The synthetic lethal interaction between *ARID1A* and *ALDH2* was detected in 11 cancer types (Table S1). Simultaneously, the synthetic lethal interaction was also screened in another research.[4] Finally, the common *ARID1A*:*ALDH2* gene pair in the two datasets was selected for further experimental validation. We cultured CCA cells (HCCC9810 and HuCCT1) *in vitro* and constructed the stable cell lines of *ARID1A* knockout and cooperate with ALDH2 inhibitor (disulfiram, DSF) to explore whether there was synthetic lethality between *ARID1A* and *ALDH2*. Cell cloning experiment showed that there were no significant changes on growth rate of CCA cells after *ARID1A* knockout or treatment with DSF. However, after treatment with DSF, there was markedly inhibition on the survival of CCA cells in the case of *ARID1A* knockout ($P < 0.05$) (Fig. 1A).

The apoptosis of CCA cells in different DSF treatment groups were also detected by flow cytometer. The results demonstrated that the two *ARID1A* knockout CCA cell lines did not show obvious apoptosis than that in WT CCA cells. Compared with DSF treatment in WT CCA cells, treatment of DSF combined with *ARID1A* knockout resulted in more serious apoptosis (Fig. 1B). Furthermore, higher levels of PI with red fluorescence and Annexin V-FITC with green fluorescence were detected in *ARID1A* knockout CCA cells treated with DSF (Fig. 1C). These results indicated that DSF combined with *ARID1A* knockout promoted the apoptosis of CCA cells.

Subsequently, we detected the expression of Bcl2 protein related to apoptosis signaling pathway. The results showed that the expression of Bcl2 was down-regulated in the two *ARID1A* knockout cells after treated with DSF ($P < 0.05$). The expression of Bax in *ARID1A* wild-type cells treated with DSF was up-regulated, but Bax protein accumulation and expression level reached the peak in *ARID1A* knockout CCA cells after treated with DSF ($P < 0.05$) (Fig. 1D). Then, we found that cleaved Caspase3 accumulated at a highest level in the cells co-treated with DSF and *ARID1A* knockout ($P < 0.05$), indicating that the apoptosis process was faster in the two CCA cells co-treated with DSF and *ARID1A* knockout, which was consistent with the results of flow cytometry. Moreover, after treating with disulfiram in the two *ARID1A* knockout cell lines, the cleaved PARP1 protein was significantly accumulated ($P < 0.05$) (Fig. 1D).

Then, we added DSF to the cell culture medium and cultured WT HCCC9810 and KO HCCC9810, WT HuCCT1 and KO HuCCT1 cells, incubated the cells together with DCFH-DA, and then collected the cells for detection the ROS content by flow cytometer. The results showed that ROS content increased in the two *ARID1A* knockout CCA cell lines than that in *ARID1A* WT CCA cell line, resulting in the first stage ROS accumulation, which is the first ROS strike on CCA cells. Subsequently, the content of ROS in *ARID1A* knockout CCA cells was further elevated by DSF, resulting in second stage accumulation, which is the second ROS strike ($P < 0.05$) (Fig. 1E). We found that co-treated with DSF, ROS

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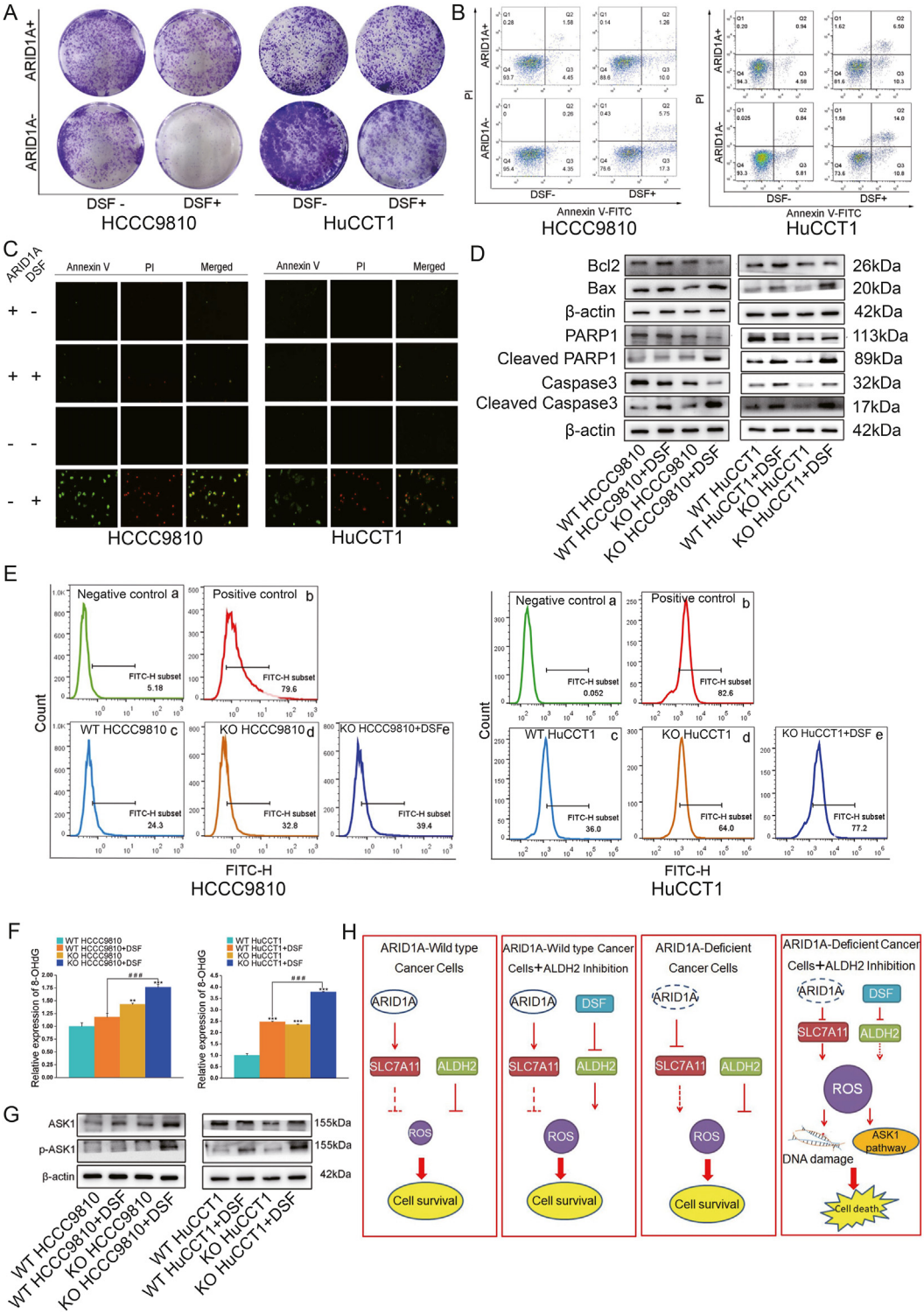

Figure 1 Inhibition of ALDH2 by disulfiram leads to synthetic lethality via ROS strikes in *ARID1A*-deficient cholangiocarcinoma. **(A)** Colony formation of *ARID1A*-WT and *ARID1A*-KO on HCCC9810 (0.05 μM DSF) and HuCCT1 (0.15 μM DSF) cells. DSF promotes apoptosis of *ARID1A*-KO CCA cells indicated by flow cytometry **(B)** and fluorescent staining **(C)**. **(D)** Expression of apoptosis-related proteins in *ARID1A*-KO CCA cells induced by DSF treatment. **(E)** *ARID1A*-KO combined with DSF significantly elevates ROS level in CCA cells. DNA damage **(F)** and ROS/ASK1 signaling pathway **(G)** are induced by ROS accumulation in *ARID1A* knockout CCA cells. **(H)** A schematic model for synthetic lethality of *ARID1A*-deficient CCA to inhibition of *ALDH2*.

accumulation in the *ARID1A* knockout cells was more than twice as high as that in the wild-type cells ($P < 0.05$), especially in the *ARID1A* knockout HuCCT1 cells after treating with DSF (Fig. 1E).

ROS accumulation can destroy DNA structure, lead to genome instability and induce apoptosis. The content of DNA damage marker 8-OHdG was detected by ELISA. The results showed that the content of 8-OHdG in *ARID1A* knockout cells was higher than that in wild-type CCA cells. In addition, the content of 8-OHdG in *ARID1A* knockout CCA cells combined with DSF was significant higher than that in other groups ($P < 0.05$) (Fig. 1F).

ROS has been proved to activate the signal pathway of apoptosis signal-regulated kinase 1 (ASK1). We then detected the phosphorylation level of ASK1 in different treatment groups. The results showed that the phosphorylation level of ASK1 in DSF-treated plus *ARID1A* knockout CCA cells was significantly higher than that in DSF-treated plus *ARID1A* wild-type CCA cells ($P < 0.05$) (Fig. 1G).

According to our findings, *ARID1A* knockout or DSF treatment of *ARID1A* wild-type CCA cells could increase the intracellular ROS level, cells could still tolerate the ROS content. However, after DSF treating *ARID1A* knockout CCA cells, the intracellular ROS content would further increase and accumulate to toxic concentration, resulting in intolerance, causing more serious DNA damage, activating endogenous apoptosis mediated by ASK1 signaling pathway, and then leading to cell death (Fig. 1H). Further, we highlight the potential value for repurposing DSF in CCA precision treatment.

Conflict of interests

The authors have declared no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.02.005.

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