主論文の要旨

# ATAD2 is associated with malignant characteristics of pancreatic cancer cells

（ATAD2 は膵臓癌細胞株の悪性化に関連している）

名古屋大学大学院医学系研究科　分子総合医学専攻
病態内科学講座　消化器内科学分野
（指導：後藤 秀実　教授）
刘　乃榕

## Background

Pancreatic cancer is one of the most aggressive human cancers and the leading cause of cancer-related death. Gemcitabine is the current standard treatment for advanced or resected pancreatic cancer, but the drug only provides modest survival benefits. To develop novel therapeutic strategies for pancreatic cancer, it is crucial to gain further insight into the molecular mechanisms supporting the malignant characteristics of pancreatic cancer.

ATAD2 (ATPase family AAA domain containing protein 2), which is also known as ANCCA (AAA+ nuclear co-regulator cancer associated) is highly conserved in a wide range of species and is associated with numerous biological processes, including cell proliferation, migration and invasion. ATAD2 has been shown to regulate gene expression or chromatin modification in corporation with multiple transcription factors. Accumulating studies have demonstrated that ATAD2 is associated with progression of numerous cancers. For example, high expression of ATAD2 is a strong predictor of poor prognosis of breast cancer. ATAD2 promotes proliferation and survival of breast cancer cells by regulating expression of B-Myb, EZH2 or ACTR/AIB1. High expression of ATAD2 has been also reported in endometrial carcinoma, hepatocellular carcinoma, cervical cancer and lung cancer. These studies clearly show that ATAD2 is critical for cancer progression and is also a promising target for cancer therapy. We examined whether ATAD2 has any critical roles for the progression of pancreatic cancer and show that ATAD2 is important for proliferation, invasion and drug resistance of pancreatic cancer cells.

## Materials and Methods

siRNAs were transfected using Lipofectamine RNAiMAX. Cell invasion and migration were assessed using modified Boyden chambers. Proliferation of siRNA-transfected cells was evaluated using Cell Count Kit 8. To evaluate anchorage independent growth, siRNA-transfected cells were cultured in 0.36% agar in RPMI for 2 weeks.

## Results

*ATAD2 knockdown promotes apoptosis*

To determine a role of ATAD2 in pancreatic cancer progression, expression of ATAD2 in pancreatic cancer cells was examined. ATAD2 was expressed in multiple pancreatic cancer cell lines at similar levels (Fig. 1A). KP4 and PK9 cell lines were used for further analysis. Transfection of two different siRNAs sufficiently reduced expression level of ATAD2 in both cell lines (Fig. 1B). Depletion of ATAD2 significantly reduced proliferation of either KP4 or PK9 cells

(Fig. 1C). In addition, ATAD2 knockdown promoted apoptosis of both cell lines (Fig. 1D).

*ATAD2 knockdown suppresses cell migration and invasion*

Invasion and migration of ATAD2-depleted cells were examined using modified Boyden chambers. KP4 and PK9 were transfected with siRNAs and, 72 h later, cells were suspended and placed in the upper chambers of the filters. The cells were allowed to migrate for 6 h to the bottom surface of the filter coated with fibronectin. Migration of both KP4 and PK9 cells was delayed by ATAD2 knockdown (Fig. 2A). To determine cell invasive ability, we used matrigel-coated Boyden chambers. ATAD2 depletion significantly suppressed the invasion of both cell lines as well (Fig. 2B).

*ATAD2 depletion suppresses anchorage-independent growth*

One of the malignant characteristics of cancer cells is the ability to grow in the absence of cell adhesion to extracellular matrix, which is called anchorage-independent growth. To determine the ability of cells to grow in the absence of cell adhesion, siRNA-transfected cells were cultured in soft agar for two weeks, and then the formation of colonies was examined. ATAD2 depletion significantly suppressed anchorage-independent growth of both KP4 and PK9 cells (Fig. 3).

*ATAD2 depletion sensitizes cells to gemcitabine*

Gemcitabine is used for pancreatic cancer treatment. We tested if ATAD2 knockdown sensitize cancer cells to the drug. siRNA-transfected cells were cultured in the presence of different concentrations of gemcitabine, and cell growth was assessed. ATAD2-depleted cells were more sensitive to gemcitabine than control siRNA-transfected cells (Fig. 4A). To further confirm this result, TUNEL assay was performed to assess apoptosis. siRNA-transfected cells were cultured in the presence of 1 μM gemcitabine, and then apoptotic cells were examined. We observed more apoptotic cells among the ATAD2-depleted cells than the control cells, indicating that ATAD2 knockdown sensitizes pancreatic cancer cells to gemcitabine.

## Discussion

Previous studies indicated that ATAD2 suppression facilitated P53 and P38-dependent apoptosis of hepatocellular carcinoma cells. ATAD2 was also essential for the survival and proliferation of breast cancer cells. ATAD2 was

required for the expression of members of the kinesin families, such as KIF4A, KIF20A, and KIF23, that are essential for the progression of mitosis. As a result, depletion of ATAD2 induced apoptosis of both drug-sensitive and drug-resistant cancer cells. Together with our results, ATAD2 appears to be essential for the survival of multiple cancer cells.

ATAD2 depletion increased the sensitivity of pancreatic cancer cells to gemcitabine. Although gemcitabine is often used for pancreatic cancer treatment, its effect is far from satisfactory. Accumulating studies have demonstrated that chemical inhibitors for AAA+ proteins are useful for cancer treatment. p97, a member of the AAA+ family of proteins, is essential for the regulation of protein homeostasis. Inhibition of p97 by small chemicals induced unfolded protein responses and promoted apoptosis in multiple cancers. Thus, chemical inhibitors that disrupt the enzymatic activity of ATAD2 may be useful for the treatment of pancreatic cancer in combination with gemcitabine. In addition to the AAA+ domain, ATAD2 has a conserved domain called the bromodomain, which is essential for the binding of ATAD2 to acetylated histone. Recent studies reported that some chemicals can specifically inhibit the bromodomain of ATAD2, further showing that ATAD2 may be a promising target for the development of chemical inhibitors. Our results and other studies clearly indicate that the combination therapy of gemcitabine and ATAD2 inhibitors may be one of the potential therapeutic strategies for the treatment of pancreatic cancers.

## Conclusion

ATAD2 is expressed in pancreatic cancer cells. Depletion of ATAD2 suppressed proliferation, migration, invasion and anchorage-independent growth of pancreatic cancer cells. In addition, ATAD2 depletion sensitized pancreatic cancer cells to gemcitabine. Our results show that ATAD2 is associated with malignancy of pancreatic cancer cells.