Pancreatology 19 (2019) 751–759

Contents lists available at ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

# OCIAD1 promoted pancreatic ductal adenocarcinoma migration by regulating ATM

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ARTICLE INFO

*Article history:*
Received 2 May 2018
Received in revised form
29 December 2018
Accepted 14 January 2019
Available online 31 January 2019

*Keywords:*
OCIAD1
ATM
PDAC migration

ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive neoplastic disease, characterized with poor outcomes and a 5-year survival rate less than 5%. Dysregulation or dysfunction of immune response factors contribute to cancer development. In this study, we found that OCIAD1 is high expressed in pancreatic cancer gene chip, and verified OCIAD1 associating with cancer malignancy in specimens from patients with PDAC. OCIAD1 down-regulation inhibited PDAC cell lines migration and vice versa. Further analysis of pancreatic cancer gene chip found OCIAD1 high expression was associating with low ATM expression. Then we proved that OCIAD1 regulated ATM to affect the migration of PDAC. Thus we concluded that high OCIAD1 levels in PDAC promoted tumor cells migration. OCIAD1 exerted its effects by regulating ATM.


## Introduction

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive neoplastic disease, characteristic with poor outcomes and an estimated 5-year overall survival rate of less than 5% [1]. Despite improvements in surgical techniques and adjuvant medical therapy, these figures have not changed in over four decades, with the mortality approaching the incidence. According to the GLOBOCAN 2012, the age standardized rate (ASR) of pancreatic cancer incidence data is 4.9 per 100000 in men, and 3.6 per 100000 in women. ASR mortality rate is 4.7 per 100000 in men, and 3.4 per 100000 in women. Worldwide, the age-standardized rate (ASR-W) for the incidence and mortality of pancreatic cancer is 4.2% and 4.0%, respectively [2]. Combination of surgical resection and chemoradiation therapy is currently the only treatment that may improve five-year survival rates from <4% to 25–30% [3,4]. To explore effective medication, the molecular mechanism of PDAC needed a better understanding. Accumulating data had showed that immune response factors were involved in the pathogenesis of PDAC [5].

OCIAD1 (ovarian cancer immunity antigen domain protein 1) was one of the immune response factors [5]. It located on chromosome 4P11 [6]. Studies had found that the overexpression of OCIAD1 in ovarian cancer and thyroid cancer could improve the function of integrins and cell extracellular matrix interactions in migration and metastasis, thereby regulated cells behavior in cancer [7,8]. In this study, we investigated the role of OCIAD1 in PDAC. We found that high OCIAD1 level in PDAC gene chips, and verified the high expression in PDAC patient's malignant tissues. We then investigated the role of OCIAD1 in PDAC cell lines by loss and gain-

*Abbreviations:* OCIAD1, Ovarian cancer immunoreactive antigen domain protein 1); ATM, Ataxia telangiectasia-mutated); PDAC, Pancreatic ductal adenocarcinoma.

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https://doi.org/10.1016/j.pan.2019.01.009
1424-3903/

of-function approaches. The predicted targeted genes of OCIAD1 were also considered and validated. Our data helps to elucidate the role of OCIAD1 in PDAC.

## Materials and methods

### *Bioinformatics analysis*

We implemented Biological information analysis in R computer language and GSEA analysis in gene chip of pancreatic cancer to analyze the normal and abnormal expression of genes, screened out the high expressions. Gene chip data sources were GSE32676 which included 42 cases of pancreatic ductal adenocarcinoma and 7 cases of non-pancreatic cancer, GSE21654 which included 22 pancreatic cancer cell lines, and GSE42952 which included 33 cases of pancreatic ductal adenocarcinoma.

### *Patients*

Surgical specimens from 16 PDAC patients and matched tumor-adjacent normal tissues were obtained from the Department of General Surgery, Changhai Hospital, Second Military Medical University (Shanghai, China) in September 2015. All patients gave signed, informed consent for their tissues to be used for scientific research. Ethical approval for the study was obtained from Second Military Medical University (Shanghai, China). Diagnosis were made based on pathological and/or cytological evidence. The histological features of the specimens were evaluated by senior pathologists according to the World Health Organization classification criteria. Tissues were obtained before chemotherapy and radiotherapy and were immediately frozen and stored at −80 °C prior to analysis. Patient's complete clinical data were electronically recorded.

### *Cell culture*

AsPC-1, BxPC-3, SW1990, CAPAN cell lines were obtained from the Cell Bank of Chinese Academy of Science (Shanghai, China) and cultured in DMEM medium (Hyclone, South Logan, UT, USA) supplemented with 10% fetal bovine serum (Hyclone), 2 mM L-glutamine and 100 μg/mL penicillin/streptomycin (Bio Light, Shanghai, China) as described in previous studies.

### *Cell migration assay*

Cell migration assay was performed using a Transwell chamber with 6.5 mm diameter polycarbonate filters with 8-μm pore size according to the manufacturer's instructions. We added $2 \times 10^4$ cells transfected with OCIAD1 siRNA for 48 h in culture media containing 0.5% FBS to the inserts. The lower chambers were filled with culture media containing 1% FBS and the cells were allowed to migrate for 16 h. The migrated cells were counted manually, and the experiments were repeated at least 3 times.

### *Western blotting*

Cells were lysed by RIPA lysis buffer purchased from Beyotime Biotechnology (Shanghai, China). The protein extracted from cells was separated by 10% SDS-PAGE gel and transferred to polyvinylidene fluoride membrane. The membranes were then incubated with indicated primary and secondary antibodies conjugated to horseradish peroxidase. Finally, the blots were incubated with Super Signal West Pico chemoluminescent substrate and visualized using the GenegGnome HR Image Capture System.

### *Immunohistochemistry*

Briefly, 4-μm thick sections were cut and anti-OCIAD1 antibody (Sigma-Aldrich) was applied. Subsequent counterstaining was performed with hematoxylin. Immunostaining results for OCIAD1 were evaluated using a semi-quantitative scoring system as described previously, which calculated the staining intensity and the percentage of positive cells. Immunohistochemistry (IHC) staining was scored according to the following criteria: −, 0–10% of the nucleated cells stained, +, 10–40% stained, ++; 40–70% stained and +++, 70–100% stained. ATM expression was considered to be observed when score ≥ +. Alternatively, IHC score of ATM expression was (− ~ +) and (++ ~ +++), which represented low and high expression, respectively.

### *Knockdown and overexpression experiments*

Low expression of OCIAD 1 in ASPC-1 and BXCP-3 cells were constructed by infection of AX2 lentivirus, ANTI306 virus as control. OCIAD1 was overexpressed instantaneously in SW1990 and CAPAN cells, which were transfected with the plasmid (pCDNA3.0-OCIAD1). The control group was empty plasmid (pCDNA3.0) and the transfection reagent was Lipofectamine 2000. All the cells were transfected after cell passaged 48 h.

### *Statistical analysis*

Data was presented as the mean ± s.d from at least three independent experiments. The differences between groups were analyzed using two-tailed Student's *t*-test when only two groups were compared. The Wilcoxon matched-pairs signed rank test was used to determine if there was a statistically significant difference in the expression of OCIAD1 and ATM between matched pairs. The differences between groups were analyzed using ANOVA when three or more than three groups were compared. Correlation analysis was performed by two-tailed Person's correlation coefficient analysis. Patients' survival was determined by Kaplan-Meier analysis. Statistical analyses were performed using SPSS software (version 17.0). $P < 0.05$ was considered statistically significantly different.

## Results

### *Screened and selected OCIAD1*

We used NCBI gene expression information chip GSE32676 which included 42 pancreatic ductal adenocarcinoma and 7 non-malignant pancreatic and R language bioconductor packages for analysis. The results showed that there were a lot of abnormal gene expressions in pancreatic cancer tissue. Differentially expressed genes screened were shown in the volcano, with the cutoff P-value < 0.05 and log2 fold change [Log2(FC)] ≥ 1, 850 up-regulated genes indicated by red dots; 1149 down-regulated represented by green dots (Fig. 1). Furthermore, we got the expression changes in gene expression information chip GSE21654 which included 22 pancreatic cancer cell lines. And listed out the significant change expressions of 100 mRNA, including OCIAD1 (Fig. 2). The most significant change expressions of gene are: AFAP1 AS1, BHLHE41, UNC5B AS1, PPFIBP1, HIST3H2A, CD93, ADCY1, ADCYAP1, LIFR (Table 1).

### *High OCIAD1 level in PDAC tissues was correlated with the severity of tumor*

To investigate whether up-regulation of OCIAD1 in PDAC tissues

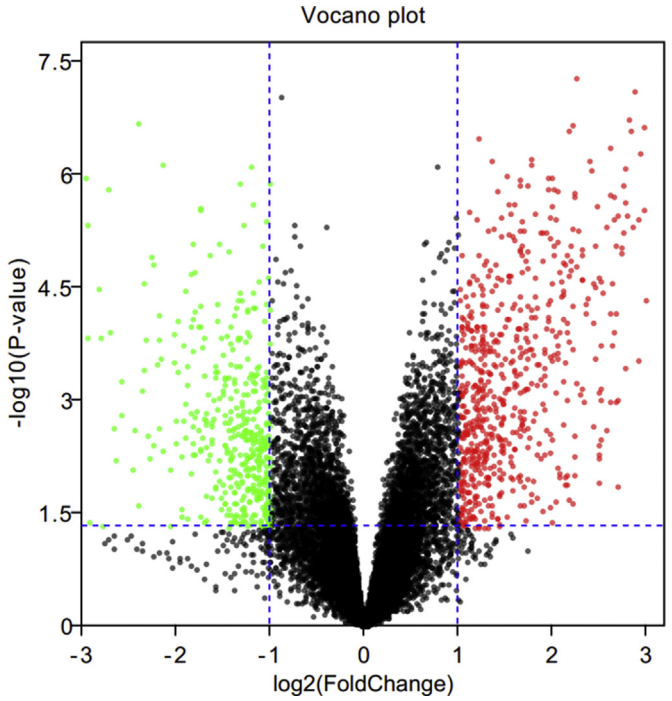


**Fig. 1.** Biological information analysis Gene chip GSE32676 shows abnormal mRNA expression in pancreatic cancer. Differentially expressed genes screened were shown in the volcano, with the cutoff P-value < 0.05 and log2 fold change [Log2(FC)] ≥1, 850 up-regulated genes indicated by red dots; 1149 down-regulated represented by green dots.

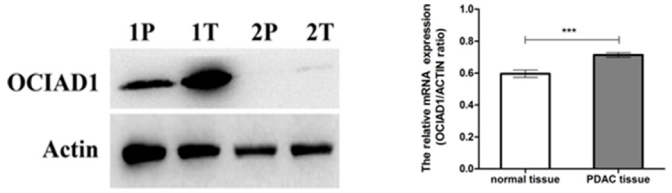


**Fig. 2.** The expression of OCIAD1 gene in 16 pairs of tissue samples was analyzed by RT-PCR. The results showed that the expression of OCIAD1 gene was up-regulated in pancreatic cancer tissues compared with normal tissues (*t*-test, P < 0.001).

**Table 1**
The most significant change expression of genes in GSE32676.

| | logFC | P.Value | The average expression quantity | |
|---|---|---|---|---|
| | | | PDAC | nonmalignant |
| OCIAD1 | 0.352936 | 0.030813 | 5.98812104 | 5.635184567 |
| AFAP1-AS1 | 2.517314 | 2.60E-05 | 6.647443139 | 4.130129025 |
| BHLHE41 | 1.192607 | 0.001724 | 5.813484905 | 4.620877736 |
| UNC5B-AS1 | 1.055536 | 0.002669 | 4.948368305 | 3.89283234 |
| PPFIBP1 | 1.037381 | 0.000859 | 8.555154628 | 7.51777363 |
| HIST3H2A | 1.012956 | 0.000873 | 7.501476948 | 6.488521278 |
| CD93 | −1.04343 | 0.004023 | 9.324955046 | 10.36838468 |
| ADCY1 | −1.36279 | 0.000725 | 5.774833899 | 7.13762177 |
| ADCYAP1 | −1.38425 | 0.009211 | 4.612473033 | 5.996726101 |
| LIFR | −1.57931 | 0.000418 | 7.540287473 | 9.119597355 |

was correlated with prognosis of PDAC patients, 16 pairs of PDAC tissues and matched tumor-adjacent normal tissues were collected from 16 patients who had undergone tumor resection. OCIAD1 level was assayed by RT-PCR and western bolt. The RT-PCR results showed that the expression of OCIAD1 gene was up-regulated in pancreatic cancer tissues compared with normal tissues (*t*-test,

**Table 2**
The statistics of OCIAD1 expression in pancreatic carcinoma patients and clinical data.

| | N (n = 16) | Upregulation | Normal | $\chi^2$ | P |
|---|---|---|---|---|---|
| Sex | | | | 0.291 | 1.00 |
| Male | 8 | 5 | 3 | | |
| Female | 8 | 6 | 2 | | |
| Age | | | | 0.291 | 1.00 |
| <=60 | 8 | 6 | 2 | | |
| >60 | 8 | 5 | 3 | | |
| Location | | | | 0.259 | 1.00 |
| head | 11 | 8 | 3 | | |
| Body/tail | 5 | 3 | 2 | | |
| Differentiation | | | | 1.68 | 0.51 |
| well + moderate | 13 | 8 | 5 | | |
| poor | 3 | 3 | 0 | | |
| UICC clinical stage | | | | 5.66 | 0.03 |
| I | 9 | 4 | 5 | | |
| IIa+b | 7 | 7 | 0 | | |
| T -status | | | | 0.485 | 1.00 |
| T2 | 15 | 10 | 5 | | |
| T3 | 1 | 1 | 0 | | |
| N-status | | | | 4.36 | 0.04 |
| 0 | 10 | 5 | 5 | | |
| 1 + 2+x | 6 | 6 | 0 | | |

P < 0.001). The RT-PCR results showed that 11 of 16 pairs showed a higher OCIAD1 level in PDAC tissues than in matched tumor-adjacent normal tissues (Fig. 2). And as showed in Table 2, statistical analysis revealed that higher OCIAD1 expression levels in PDAC tissues was significantly correlated with the severity of tumor, expression levels of stageIIa+b was significant higher than stage I (p = 0.03) and expression levels of N-status(1 + 2+x) was significant higher than N-status(0) (p = 0.04).

*OCIAD1 down-regulation inhibited PDAC cell lines migration*

According to the GEO dataset of GSE36133 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE36133), which test 917 cell lines' transcriptome, mRNA levels of OCIAD1 was higher in AsPC-1(11.463),BxPC-3(10.915) than SW1990(10.848),CAPAN(10.899). So we down-regulated OCIAD1 in both AsPC-1 and BxPC-3, and up-regulated in CAPAN and SW1990 cell lines. So we down-regulated the level of OCIAD1 by OCIAD1 AX2 transfection in AsPC-1 and BxPC-3 cell lines (Fig. 3A). We found OCIAD1 AX2 transfection down-regulated the OCIAD1 level in the two cell lines (Fig. 3B). 48 h after OCIAD1 AX2 transfection, AsPC-1 and BxPC-3 cells migration were assayed by Transwell at the indicted time point; two-tailed Student's *t*-test was used to assay the difference between migrations at 72 h. We found OCIAD1 AX2 transfection inhibited cells migration in AsPC-1 and BxPC-3 (Fig. 3C).

*OCIAD1 up-regulation promoted PDAC cell lines migration*

Then we up-regulated the level of OCIAD1 by OCIAD1 mimics transfection in CAPAN and SW1990 cell lines. The effectiveness of transfection was verified by RT-PCR(Fig. 4A) and protein levels (Fig. 4B). 48 h after OCIAD1 mimics transfection, CAPAN and SW1990 cells migration were assayed by Transwell at the indicted time point; two-tailed Student's *t*-test was used to assay the difference between migration at 72 h. Data revealed that OCIAD1 mimics transfection promoted CAPAN and SW1990 cell lines migration (Fig. 4C).

*Select OCIAD related molecules and verified ATM*

Statistical information from the database GSE32676 (https://

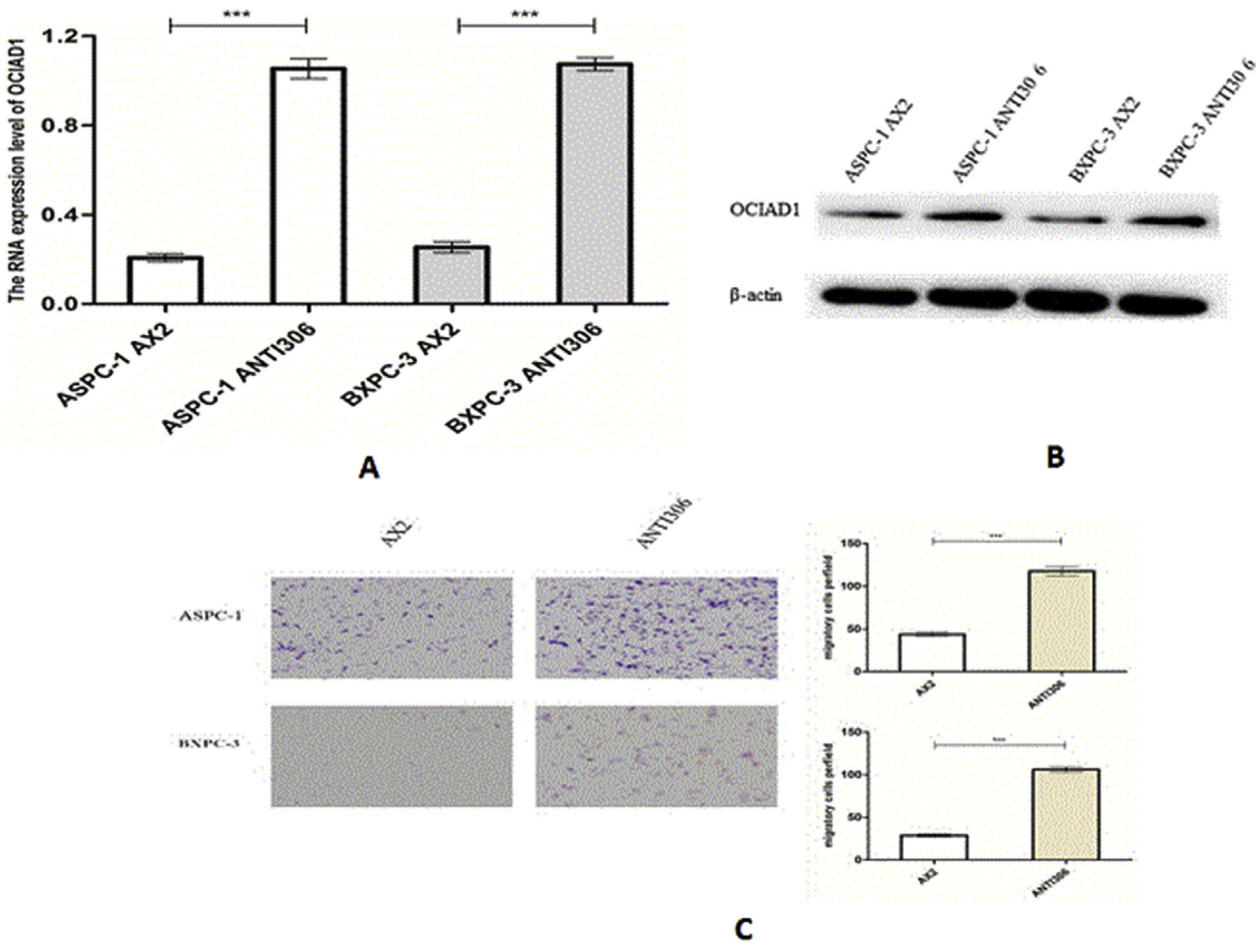


**Fig. 3.** *OCIAD1 down-regulation inhibited PDAC cell lines migration*. Down-regulated the level of OCIAD1 by AX2 transfection in AsPC-1 and BxPC-3 cell lines, then the level of OCIAD1 were assayed and verified successful by RT-PCR (Fig. 3A) and western bolt (Fig. 3B). 48 h after OCIAD1 mimics transfection, AsPC-1 and BxPC-3 cells migration were assayed by Transwell at the indicted time point; two-tailed Student's *t*-test was used to assay the difference between migration at 72 h (Fig. 3C). All data are mean ± s.d. of three separate experiments. *P < 0.05.

www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE32676) with 32 samples was used. Many genes were screened out (data not showed), including ataxia telangiectasia-mutated (ATM) and anaplasticlymphoma kinase(ALT). Correlation analysis of OCIAD1 with ATM, Pearson correlation coefficients R = −0.473, p-value = 0.006; Correlation analysis of OCIAD1 with ALK, Pearson correlation coefficients R = 0.476, p-value = 0.006(Table 3 and Fig. 5A and B).

After we did the Validation of OCIAD1 downregulation (left)/upregulation (right) in various cell lines either at the mRNA or protein level (Fig. 6). Then down-regulated OCIAD1 expression in AsPC-1 and BxPC-3 cells, the results showed that the mRNA and protein expression ALK in AsPC-1 cells significantly increased (P < 0.05), but not significantly increased in BxPC-3 cells (P > 0.05). The mRNA and protein expression of ATM in AsPC-1and BxPC-3 cells were both significantly increased (P < 0.05) (Fig. 6A). Up-regulated OCIAD1 expression in SW1990 and CAPAN cells, the mRNA expression of ALK in SW1990 cell increased significantly (P < 0.05), the mRNA expression of ALK in CAPAN cell decreased significantly (P < 0.05), the protein expression of ALK in SW1990 and CAPAN cells increased significantly (P < 0.05). The mRNA expression of ATM in SW1990 and CAPAN cells was both significantly lower (P < 0.05), and the protein expression of ATM in SW1990 and CAPAN cells decreased no obvious (Fig. 6B). The results showed that there is a close relationship between OCIAD1 and ATM.

*OCIAD1 regulate ATM expression effects pancreatic cancer cell migration*

The protein levels show no difference of ATM kinase after treatment with ATM kinase inhibitor different concentrations (0 mM, 0.1 mM, 1.0 mM, 10 mM) and different treated time schedule (24 h). After cell treated, the levels of total and phosphorylated ATM were detected by Western blotting. ACTIN was used as a loading control (Fig. 7) [9–11].

The expression of OCIAD1 was down-regulated by OCIAD1 AX2 transfection in AsPC-1 and BxPC-3 cell lines, then treated the experimental cells with ATM kinase inhibitor KU55933. 48 h after treatment, cellular migration was assayed by Transwell. We found the cells migration ability significantly increased (P < 0.05) (Fig. 7A). Up-regulated the level of OCIAD1 by OCIAD1 mimics transfection in CAPAN and SW1990 cells, and treated CAPAN and SW1990 cells with ATM kinase activator Chloroquine, 48 h after treatment, cellular migration was assayed by Transwell. We found the cells migration ability significantly decreased (P < 0.05) (Fig. 7B). The outcome meant that down-regulated OCIAD1 expression could inhibit cell migration and it worked through ATM kinase phase.

## Discussion

In this study, we performed bioinformatics analysis to screen

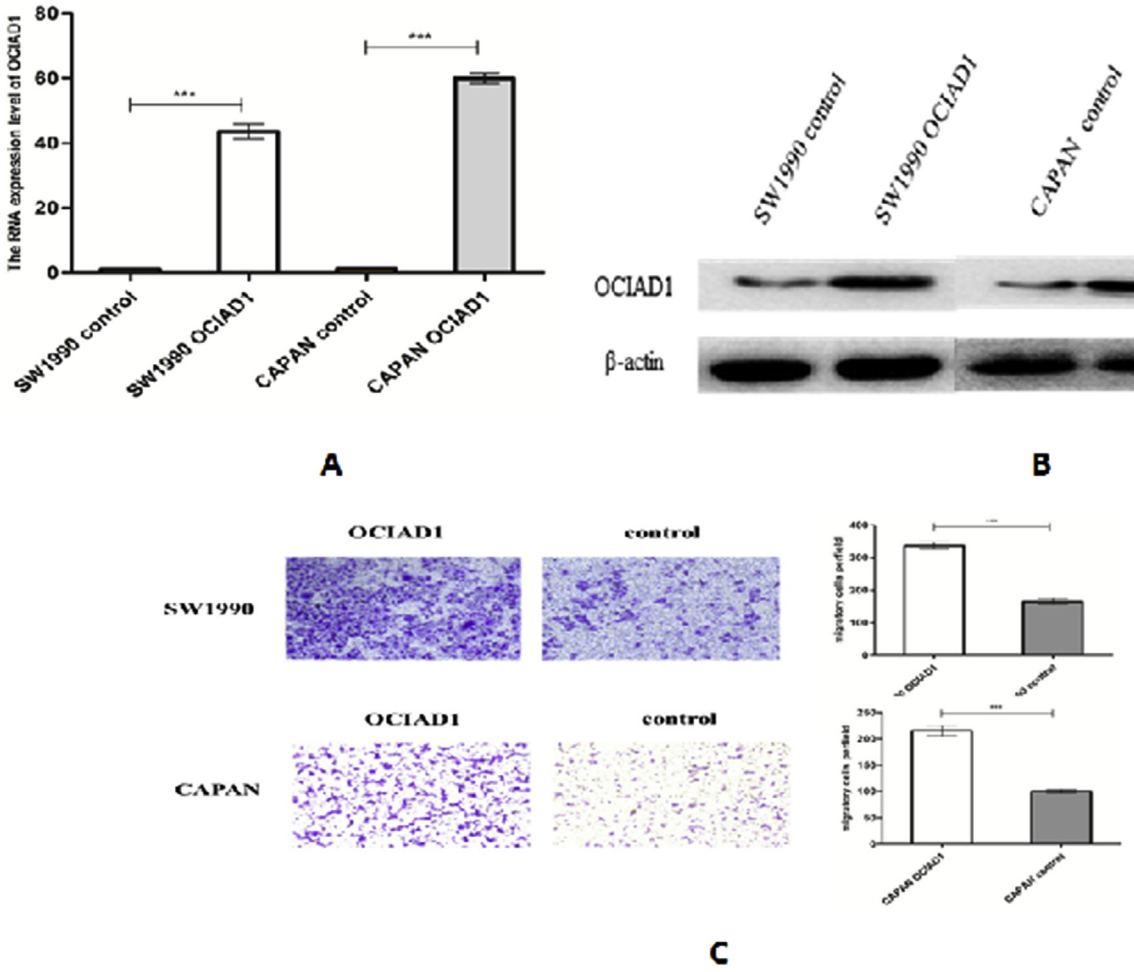


**Fig. 4.** *OCIAD1 up-regulation promoted PDAC cell lines migration*. Up-regulated the level of OCIAD1 by *OCIAD1* mimics transfection in CAPAN and SW1990 cell lines, The effectiveness of transfection was verified successful by RT-PCR (Fig. 4A) and western bolt (Fig. 4B). 48 h after OCIAD1 mimics transfection, CAPAN and SW1990 cells migration were assayed by Transwell at the indicted time point; two-tailed Student's *t*-test was used to assay the difference between migration at 72 h (Fig. 4C). All data are mean ± s.d. of three separate experiments. *P < 0.05.

**Table 3**
Statistical information from the database GSE32676.

| Sample(32) | OCIAD1 | ATM | ALK |
|---|---|---|---|
| GSM811004.CEL | 6.702073 | 5.756737 | 4.608821 |
| GSM811005.CEL | 6.004132 | 6.212674 | 3.860109 |
| GSM811006.CEL | 5.857258 | 5.322135 | 4.128178 |
| GSM811007.CEL | 5.55417 | 6.024653 | 3.721522 |
| GSM811008.CEL | 5.610789 | 5.511238 | 4.173407 |
| GSM811009.CEL | 6.01132 | 5.910282 | 4.519979 |
| GSM811010.CEL | 5.928413 | 6.449317 | 4.25241 |
| GSM811011.CEL | 5.340697 | 6.457723 | 3.880642 |
| GSM811012.CEL | 6.431414 | 5.390816 | 4.234046 |
| GSM811013.CEL | 5.630989 | 5.63787 | 4.026713 |
| GSM811014.CEL | 6.035356 | 6.690574 | 3.697467 |
| GSM811015.CEL | 6.529494 | 5.17933 | 4.330371 |
| GSM811016.CEL | 5.959982 | 6.299939 | 4.037782 |
| GSM811017.CEL | 5.687878 | 5.868047 | 3.916004 |
| GSM811018.CEL | 5.675815 | 6.295484 | 3.834151 |
| GSM811019.CEL | 5.483201 | 6.657236 | 3.914609 |
| GSM811020.CEL | 6.284926 | 5.752188 | 4.321019 |
| GSM811021.CEL | 6.242559 | 5.518651 | 4.074351 |
| GSM811022.CEL | 6.141066 | 5.468656 | 4.087901 |
| GSM811023.CEL | 5.864884 | 5.932291 | 4.095562 |
| GSM811024.CEL | 6.354527 | 5.679255 | 4.336147 |
| GSM811025.CEL | 6.313502 | 5.597836 | 4.232272 |
| GSM811026.CEL | 5.678926 | 6.340382 | 3.88834 |

(*continued on next page*)

**Table 3** (*continued*)

| Sample(32) | OCIAD1 | ATM | ALK |
|---|---|---|---|
| GSM811027.CEL | 5.666084 | 5.946283 | 4.017749 |
| GSM811028.CEL | 6.71357 | 5.046102 | 4.278952 |
| GSM811029.CEL | 6.05934 | 5.945717 | 4.600246 |
| GSM811030.CEL | 5.923305 | 5.977567 | 3.981451 |
| GSM811031.CEL | 5.866433 | 7.953947 | 3.985837 |
| GSM811032.CEL | 5.340009 | 5.952736 | 4.472 |
| GSM811033.CEL | 5.410814 | 8.023354 | 3.875619 |
| GSM811034.CEL | 5.327194 | 6.33687 | 4.094679 |
| GSM811035.CEL | 5.519195 | 5.528741 | 4.261351 |

pancreatic cancer genes chip and selected the significant different expression genes, and proved in clinical test samples that OCIAD1 was significantly up-regulated expression. Combined with clinical data analysis, we found that high expression of OCIAD1 was associated with clinical staging. The high expression of OCIAD1 meant the high malignant degree. Our data may add a new biomarker in molecular profiles associated with the diagnosis of PDAC patients. Further research showed OCIAD1 down-regulation inhibited PDAC cell lines migration and up-regulation promoted PDAC cell lines migration. Related molecules were screened out and ATM was verified. Further tests indicated that ATM was regulated by OCIAD1. Thus we concluded that high OCIAD1 level in PDAC promoted tumor cells migration, contributed to the malignant degree of PDAC patients, and exerted its role by regulating ATM.

Only a few research has been done about OCIAD1, was reported in 2007 that OCIAD1 acted by controlling sells ability to adhere extracellular matrix proteins collagen1 and laminin10/11 [12]. OCIAD1 was a key player in generating ovarian cancer recurrence,

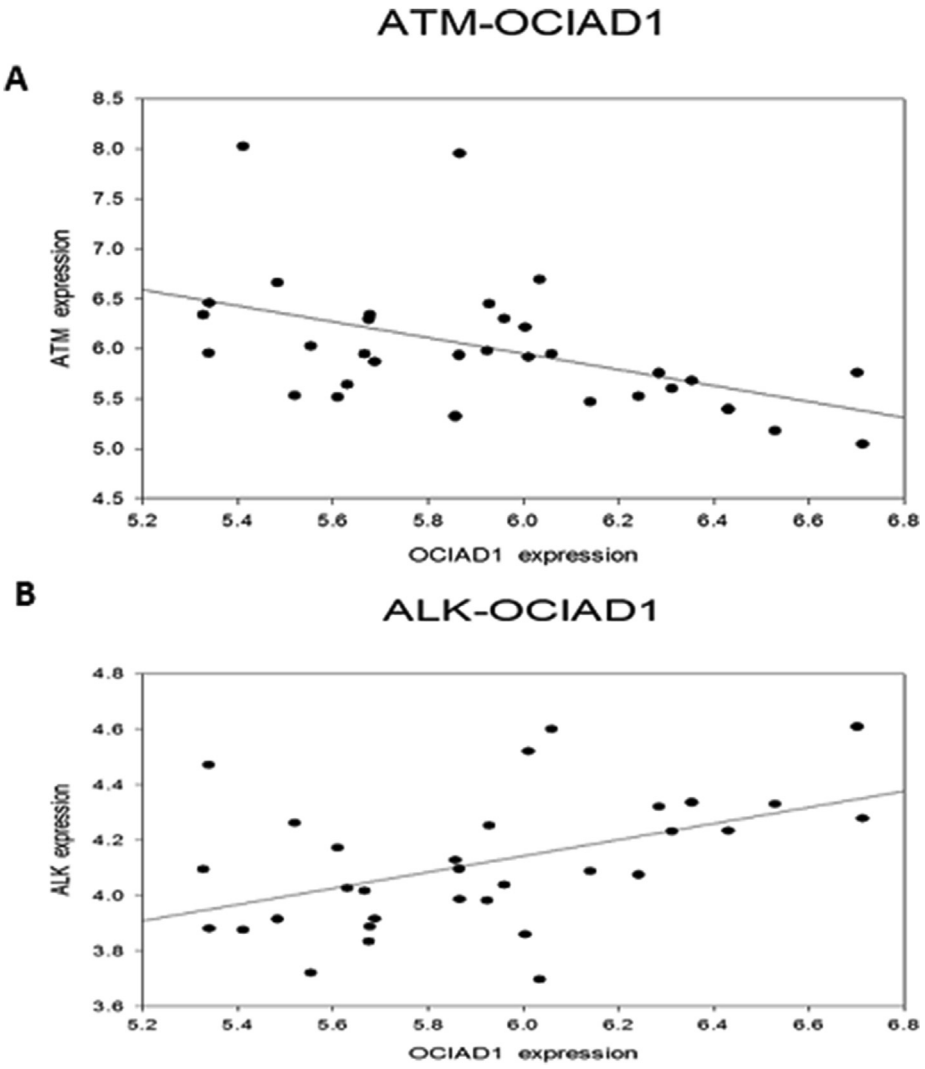


**Fig. 5.** Statistical information from the database GSE32676 with 32 samples, the results showed that there were negative correlation between OCIAD1 and ATM, negative correlation between OCIAD1 (Fig. 5A) and ATM, positive correlation between OCIAD1 and ALK (Fig. 5B).

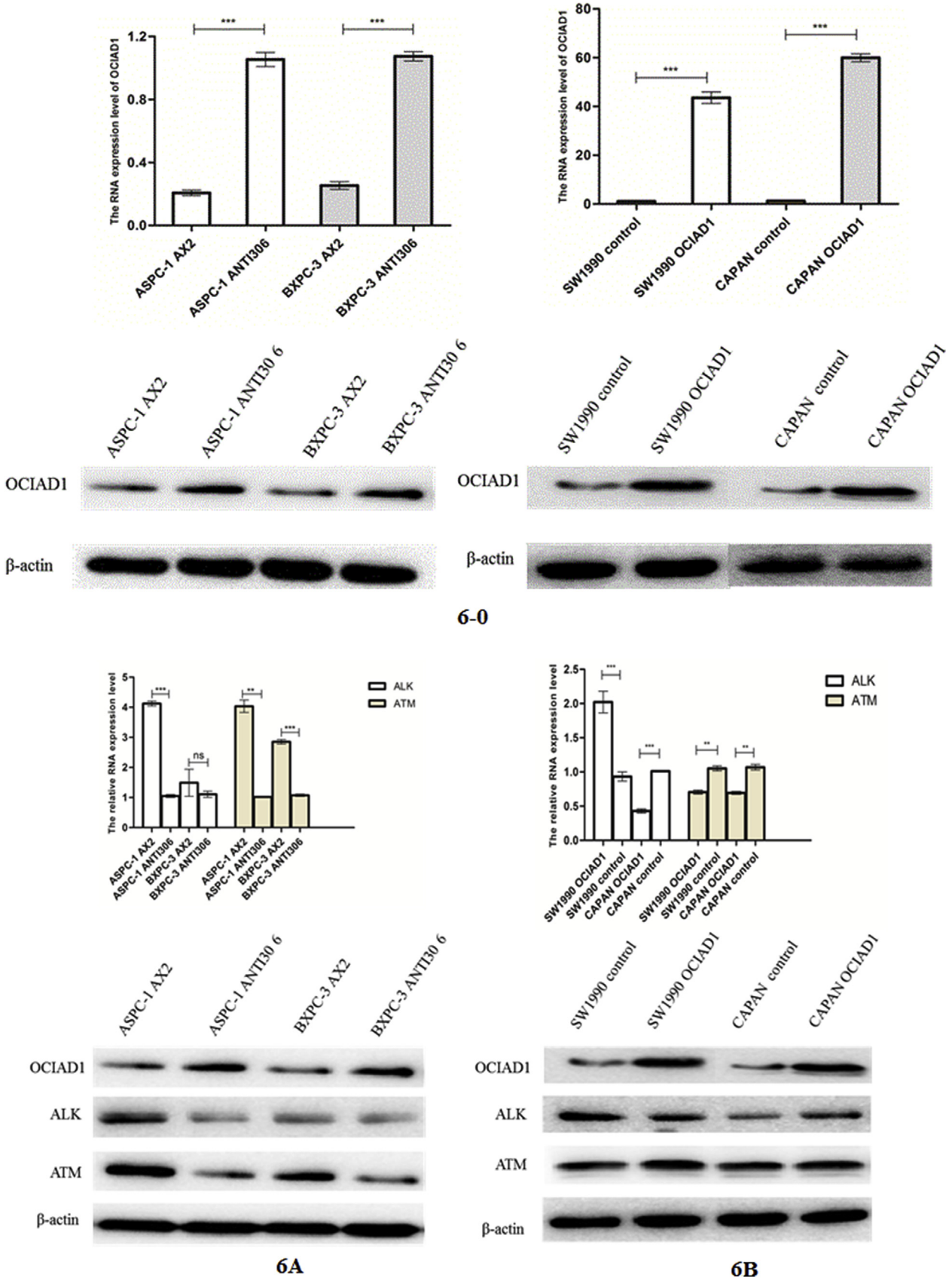


**Fig. 6.** Validation of OCIAD1 downregulation (left)/upregulation (right) in various cell lines either at the mRNA or protein level (Fig. 6). Down-regulated OCIAD1 expression in AsPC-1 and BxPC-3 cells, the results showed that the expression ALK in AsPC-1 cells significantly increased ($P < 0.05$), but not significantly increased in BxPC-3 cells ($P > 0.05$). The mRNA and protein expression of ATM in AsPC-1and BxPC-3 cells were both significantly increased ($P < 0.05$) (Fig. 6A). Up-regulated OCIAD1 expression in SW1990 and CAPAN cells, the mRNA expression of ALK in SW1990 cell increased significantly ($P < 0.05$), the mRNA expression of ALK in CAPAN cell decreased significantly ($P < 0.05$), the protein expression of ALK in SW1990 and CAPAN cells increased significantly ($P < 0.05$). The mRNA expression of ATM in SW1990 and CAPAN cells was both significantly lower ($P < 0.05$), and the protein expression of ATM in SW1990 and CAPAN cells decreased no obvious (Fig. 6B).

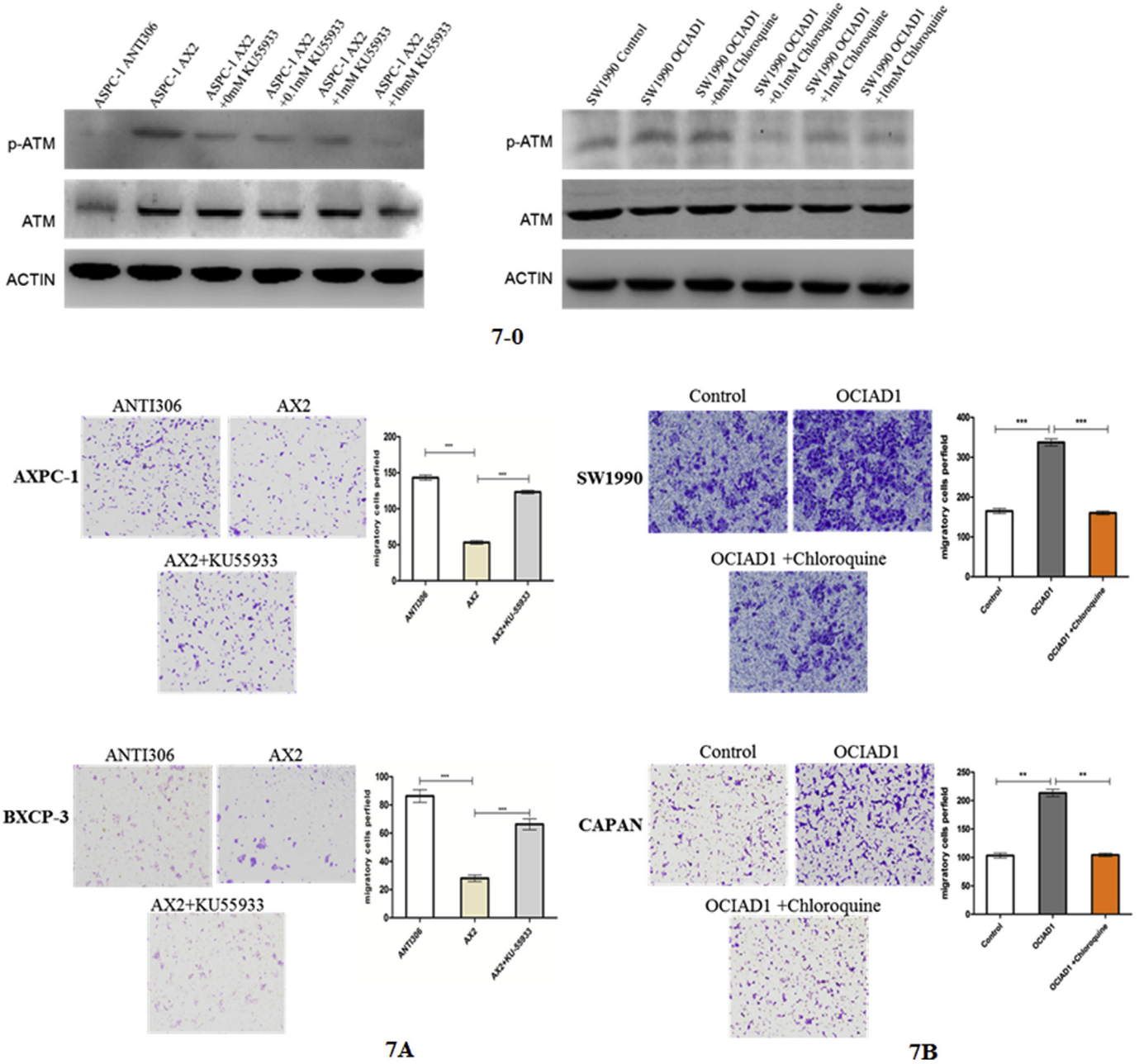


**Fig. 7.** The protein levels show no difference of ATM kinase after treatment with ATM kinase inhibitor different concentrations (0 mM, 0.1 mM, 1.0 mM, 10 mM) and different treated time schedule (24 h). (Fig. 7–0). Down-regulated OCIAD1 by OCIAD1 AX2 transfection in AsPC-1 and BxPC-3 cell lines and treated the cells with ATM kinase inhibitor KU55933. 48 h after treatment, the cellular migration assay showed the cells migration ability obviously increased ($P < 0.05$) (Fig. 7A). Up-regulated OCIAD1 by OCIAD1 mimics transfection in CAPAN and SW1990 cells, and treated the cells with ATM kinase activator Chloroquine, 48 h after treatment, cellular migration assay showed the cells migration ability obviously decreased ($P < 0.05$) (Fig. 7B).

functionally regulated by LPA and MKK6 signaling [13]. It also was reported to interact with ROP18 to associate with DNA, transcriptional regulation, translation modification, protein degradation and cell adhesion [14]. ATM was the product of the gene mutated in the human genetic disorder ataxia-telangiectasia, characterized by hypersensitivity to ionizing radiation and defective cell cycle checkpoints. It was clear that the role of ATM in DNA damage recognition, cell cycle control [15]. ATM-dependent cellular response to DNA double strand breaks played a pivotal role in the maintenance of the integrity of the genome. A proper DNA damage required amplification of the ATM-dependent damage signal by recruiting the DNA damage checkpoint factors to the site of chromatin[16].

In our study, we found that high OCIAD1 expression level in PDAC was correlated with low ATM. The performance made this study interesting and owned vital significance. In PDAC, ATM monitored and helped the cells to repair damaged DNA, it was important in the prevention of PDAC malignant activity. High expression of OCIAD1 correlated with low ATM, and affected the function of ATM mediated monitoring and repairs. The more specific mechanism of OCIAD1-ATM action in PDAC needed further investigation. Here we want to say that correlation analysis in Fig. 5

is based on the analysis of mRNA levels of two genes in the tissue. But changes in mRNA and protein levels in Fig. 6 were analyzed and compared in specific cell lines. Due to the kinds of cell type in the tissue, the direction of mRNA and protein changes in one special cell line is not always consistent. Correlation analysis is based on gene chip, and mRNA analysis is based on RT-PCR technology, and the fragments detected by the two methods may be inconsistent. Protein analysis is based on Western Blot technology and detects the expression of this gene at protein level. Therefore, the three results maybe related, not always consistent.

In conclusion, we found high OCIAD1 expression in PDAC genes chip, and contributed to the high PDAC patients malignant degree. OCIAD1 exerted its role by regulating ATM. We hope our study can provide some hint for further studies.

## Conflicts of interest

The authors have declared that no competing interests exist.

## Ethics

Ethical approval was obtained from the ethics ethical committee of The Second Military Medical University of Shanghai. Informed consents were obtained from the patients for publication of this study and any accompanying images.

## Funding

This study was supported by Youth Initial Fund of Second Military Medical University (2013QN07), Natural Science Foundation of Zhejiang Province (LY18H160058) and Doctor Initial Fund of Second Affiliated Hospital & Yuying Children's Hospital of Wenzhou Medical University (216200), China.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pan.2019.01.009.

## References

[1] American Cancer Society. Facts & figures 2018. 2018. Available online: https://cancerstatisticscenter.cancer.org/module/yg6E0ZLc. [Accessed 21 February 2018].
[2] Lin Quan-Jun, Yang Feng, Jin Chen, Fu De-Liang. Current status and progress of pancreatic cancer in China. World J Gastroenterol 2015;21(26):7988–8003.
[3] Hammel P, Huguet F, van Laethem JL, Goldstein D, Glimelius B, Artru P, Borbath I, Bouché O, Shannon J, André T, Mineur L, Chibaudel B, Bonnetain F, Louvet C. LAP07 trial Group.Effect of chemoradiotherapy vs Chemotherapy on survival in patients with Locally advanced pancreatic cancer controlled after 4 Months of gemcitabine with or without Erlotinib:the LAP07 randomized clinical trial. JAMA 2016;315:1844–53.
[4] Khadilkar RJ, Ray A, Chetan DR, Sinha AR, Magadi SS, Kulkarni V, Inamdar MS. Differential modulation of the cellular and humoral immune responses in Drosophila is mediated by the endosomal ARF1-Asrij axis. Sci Rep 2017;7(1):118.
[5] Kulkarni Vani, Rohan J, Khadilkar, Srivathsa MS, SInamdar Maneesha. Asrij maintains the stem cell Niche and controls differentiation during Drosophila lymph gland hematopoiesis. PLoS One 2011;6(11):e27667.
[6] Yang AH, Chen JY, Lee CH, Chen JY. Expression of NCAM and OCIAD1 in well-differentiated thyroid carcinoma: correlation with the risk of distant metastasis. J Clin Pathol 2012;65(3):206–12.
[7] Shetty DK, Inamdar MS. Generation of a heterozygous knockout human embryonic stem cell line for the OCIAD1 locus using CRISPR/CAS9 mediated targeting: BJNhem20-OCIAD1-CRISPR-39. Stem Cell Res 2016;16(2):308–10.
[8] Xavier KB. Bacterial interspecies quorum sensing in the mammalian gut microbiota. C R Biol 2018;341(5):300.
[9] Ivanov VN, Zhou H, Partridge MA, Hei TK. Inhibition of ataxia telangiectasia mutated kinase activity enhances TRAIL-mediated apoptosis in human melanoma cells. Cancer Res 2009;69(8):3510–9.
[10] Hickson I, Zhao Y, Richardson CJ, Green SJ, Martin NM, Orr AI, Reaper PM, Jackson SP, Curtin NJ, Smith GC. Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. Cancer Res 2004;64(24):9152–9.
[11] Sengupta S, Michener CM, Escobar P, Belinson J, Ganapathi R. Ovarian cancer immuno-reactive antigen domain containing 1 (OCIAD1), a key player in ovarian cancer cell adhesion. Gynecol Oncol 2008;109(2):226–33.
[12] Wang C, Michener CM, Belinson JL, Vaziri S, Ganapathi R, Sengupta S. Role of the 18:1 lysophosphatidic acid-ovarian cancer immunoreactive antigen domain containing 1 (OCIAD1)-integrin axis in generating late-stage ovarian cancer. Mol Cancer Ther 2010;9(6):1709–18.
[13] Cheng L, Chen Y, Chen L, Shen Y, Shen J, An R, Luo Q, Du J. Interactions between the ROP18 kinase and host cell proteins that aid in the parasitism of Toxoplasma gondii. Acta Trop 2012;122(3):255–60.
[14] Lavin MF. ATM: the product of the gene mutated in ataxia-telangiectasia. Int J Biochem Cell Biol 1999;31(7):735–40.
[15] Suzuki K, Yamauchi M, Yamashita S. ATM-dependent cellular response to DNA double strand breaks plays a pivotal role in the maintenance of the integrity of the genome. Radiat Protect Dosim 2011;143(2–4):279–83.