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Роль белка интегрина альфа 2 (ITGA2) в прогрессировании рака поджелудочной железы

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Резюме

Введение: Рак поджелудочной железы — относительно редкий тип рака, хотя он часто очень агрессивен и дает большие метастазы в другие части тела. Исследование потенциального генного маркера или генно-таргетной терапии может улучшить ранний прогноз и/или лечение пациента.

* **Иллюстрации**
1–4 —
на цветной
вклейке
в журнал
(стр. IV).

Цели: В этом исследовании мы идентифицируем интегрин альфа 2 (ITGA2) как потенциальную мишень в ингибировании прогрессирования рака поджелудочной железы.

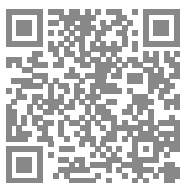
Материалы и методы: Анализ клеточного цикла, уровень экспрессии генов и анализ пролиферации клеток используются в этом исследовании в качестве исследовательских методов. Двусторонний t-критерий Стьюдента используется для сравнения изучаемых групп.

Результаты: Анализ клеточного цикла трансформированных клеточных линий выявил увеличение фазы G0/G1 и наступление в клетках остановки клеточного цикла (покоя) после подавления экспрессии ITGA2. С другой стороны, подавляя эффект ITGA2, мезенхимально-эпителиальный переход и возможность миграции клеточных линий за счет ингибирования экспрессии метастатического маркера виментина. Кроме того, ITGA2 может манипулировать микроокружением опухоли путем подавления белков внеклеточного матрикса (ECM-белков) LAMB3 и LAMC2.

Заключение: Снижение регуляции ITGA2 снижает пролиферацию клеток, вызывает остановку клеточного цикла и снижает вероятность метастазирования при раке поджелудочной железы.

Ключевые слова: ITAG2, рак поджелудочной железы, покой, ECM-белки, LAMB3, LAMC2

EDN: TCKBGG





The Role of Integrin Subunit Alpha 2 (ITGA2) in Pancreatic Cancer Progression

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Summary

Background: Pancreatic cancer is a relatively uncommon type of cancer, although it is often very aggressive and highly metastasizes to other parts of the body. Investigating a potential gene marker or gene targeted therapy can improve the patient's early prognosis and/or treatment.

Objectives: In this study, we identify Integrin Subunit Alpha 2 (ITGA2) as a potential target in inhibiting pancreatic cancer progression.

Materials and Methods: Cell cycle analysis, gene expression level, and cell proliferation assay are implanted in this study as investigational methods. Two-tailed student's t test is used to compare between the studied groups.

Results: Cell cycle analysis for the transformed cell lines revealed increasing in G0/G1 phase and entering the cells the cell cycle arrest (quiescence) after knocking down ITGA2 expression. On the other hand, knocking down the ITGA2 effect, the mesenchymal to epithelial transition and the migration possibility of the cell lines by inhibiting the expression of metastatic marker vimentin. Furthermore, ITGA2 can manipulate the tumor microenvironment by downregulating extracellular matrix proteins (ECM-proteins) LAMB3, and LAMC2.

Conclusion: ITGA2 downregulation reduces the cell proliferation, induces the cell cycle arrest, and reduce the possibility of metastasis in pancreatic cancer.

Keywords: ITAG2, Pancreatic Cancer, Quiescence, ECM-proteins, LAMB3, LAMC2

* Illustrations 1–4 to the article are on the colored inset of the Journal (p. IV).

Introduction

Pancreatic cancer is the fourth leading cause of cancer-related deaths in the United States due its low survival rate.^[1] There are number of treatment options for pancreatic cancer, including surgery, chemotherapy, radiotherapy, immunotherapy, and targeted therapy. Early diagnosis is important to the treatment processes and prevent the tumor from spreading to other parts of the body.^[1,2] Using present and potential pancreatic cancer markers can improve the chances of early diagnosis and survival rate.^[1]

Integrin Subunit Alpha 2 (ITGA2), is a member of integrins family. Integrins are transmembrane proteins mediated the cell adhesion to extracellular matrix (ECM) such as collagen-related proteins.^[3] ITGA2 encodes a heterodimer transmembrane receptor for several collagen-related proteins.^[3,4] ECM-proteins such as laminin subunit beta 3 (LAMB3), laminin subunit gamma 2 (LAMC2), thrombospondin 2 (THBS2), and versican (VCAN) affect cancer cell progression, proliferation, and

metastatic spread.^[5,6] Researchers found that, the expression of ECM-proteins are higher in pancreatic cancer tissue than in normal pancreatic tissue samples. The ECM-proteins triggers pancreatic cancer progression by directly promoting cellular alteration, and enriching tumorigenic microenvironment formation by disturbing stromal-cell behavior.^[6]

ITGA2 has been identified as a potential therapeutic target in several types of cancer. In a study on non-small lung cancer, the research indicates the significant role of ITGA2 in cancer cells adhesion, migration, and the possibility of recurrence. Samples from patients shows high expression of ITGA2 which associate with lower recurrence-free survival. In addition, the ITGA2 overexpression lung cancer cell lines increase cell size with no effect on cell proliferation or invasion.^[7] In vitro study, showed that, ITGA2 is overexpressed in tumor cells and is associated with poor prognosis in pancreatic cancer patients.^[8] The reason for high expression of ITGA2 in pancreatic

cancer is not yet clear.^[2] Furthermore, ITGA2 protein levels are significantly increased in pancreatic cancer tissues compared to non-tumor pancreatic tissues, suggesting that ITGA2 may be a potential clinical therapeutic target for pancreatic cancer. Moreover, ITGA2 interacts with STAT3, up-regulating the phosphorylation of STAT3 and activation of the STAT3 pathway. Activation of the STAT3 pathway plays a critical role in cancer cell progression and the regulation of programmed death-ligand 1 (PD-L1). ITGA2 is also transcriptionally regulating the expression of PD-L1. Therefore; blocking ITGA2 expression can decrease the phosphorylation level of STAT3 and suppress PD-L1 expression in vivo.^[9] These findings suggest that, ITGA2 could serve as a novel target for immune checkpoint blockade therapy and blocking ITGA2 improves tumor immune responses.^[11] In addition, the results of a study on gastric cancer supports the ITGA2 implication in the programmed cell death. Researchers use ITGA2 antibody on ITGA2 high expression cell line which induced apoptosis by up-regulating the RhoA-p38 MAPK

signaling to endorse the expressions of Bim, Apaf-1 and Caspase-9, but not Ras and Bax/Bcl-2 pathway.^[10] Interestingly, ITGA2 overexpression is associated with the most common KRAS mutation in cancer. The accumulation of KRAS, TP53, CDKN2A, and SMAD4 mutations is involved in the carcinogenic mechanism of cancer.^[11] Understanding the mechanisms of ITGA2 in pancreatic cancer progression may have therapeutic implications.^[2] In fact, the combination of ITGA2 silencing and TGF- β treatment results in smaller and lighter tumors in mice compared to individual treatments, suggesting that ITGA2 may play a role in pancreatic cancer progression and could be a potential therapeutic target. ^[12] In this in vitro study, firstly, we are investigating the role of ITGA2 on the proliferation, progression of the cell cycle of pancreatic cancer cell lines. Secondly, we are investigating the effect of ITGA2 expression on tumor microenvironment such as the level of master of the mesenchymal marker expression, and ECM-proteins expression.

Materials and Methods

PANC-1 and MIA PaCa cells were cultured in DMEM growth media supplemented with 10% FBS, pen/strep and gentamicin. Cells were maintained in an incubator at 37 C° and 5% CO₂ in a humidified incubator.

Establishment of knockdown ITGA2 pancreatic cancer cell lines

Transfection is initiated 12 hours after cells have been plated into culture dishes with a confluence of 40%. Lipofectamine is employed to transfect PANC-1 and MIA PaCa cells with siRNA oligos that are carried by psiRNA-h7SK G1. Similarly, the siRNA for control is scrambled (see Table 1). qRT-PCR is employed to assess the transfection efficiency of ITGA2 using primers that target the gene over the course of 90 hours following transfection (Figure. 1).

Table 1.
siRNA oligo
sequence carried by
psiRNA-h7SK G1 to
knockdown ITGA2

Molecule	Sequence
siRNA oligo	5'-AAGAGAGAACAACAGGTGACT-3'

Quantitative PCR (qPCR)

QPCR analysis used to determine RNA expression for each gene by using two sets of primers (forward and reverse). Human ITGA2, Vimentin marker used as a mesenchymal marker, LAMB3, and LAMC2 used as ECM-protein markers. Housekeeping protein Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) used as internal control (see Table 2). Total RNA collected, cDNA synthesized, and amplified using Biorad CFX96.

Table 2.
Forward and
reverse primer
sequences used in
qPCR analysis

Molecule	Forward primer sequence	Reverse primer sequence
ITGA2	5'-GGGCATTGAAAACACTCGAT-3'	5'-TCGGATCCCAAGATTTTCTG-3'
Vimentin	5'-GAGAACTTTGCCGTGAAGC-3'	5'-TCCGCAGCTTCTGTAGGT-3'
LAMB3	5'-GGGGGAGATCACAACTTGA-3'	5'-GTGCTGGCAGACACAGACAT-3'
LAMC2	5'-GTCACTGGAGAACGCTGTGA-3'	5'-AGACCCATTTCTGTTGGACAG-3'
GAPDH	5'-ACCCAGAAGACT GTG GAT GG-3'	5'-TTCTAGACGGCAGGTCAGGT-3'

Cell cycle analysis

Cell cycle analysis was performed using flow cytometry of propidium iodide-stained cells. 4X10⁴ of ITGA2 knocked down cell line PANC-1 and MIA PaCa along with control, seeded in six wall plates. Next day, cells washed with 1XPBS and collected by using trypsin. Warm media was added and the cells were centrifuged at 1200 rpm at 4C° for 5 minutes. The supernatant was poured and the cells were washed with 1ml 1XPBS and centrifuged again. The

cells were re-suspended in 0.3 ml 1X PBS and fixed by 0.7 ml of absolute ethanol and incubated at 4°C overnight. 5 µl of 10 mg/ml RNAase A solution was added and incubated

in 37 °C for 1 hour. 10 µl of 1 mg/ml propidium iodide stain added and kept in dark and on ice until analyzed on FACS by reading on 488 nm.

Cell proliferation assay

2X10⁴ cells of ITGA2 knocked down cell line PANC-1 and MIA PaCa along with control cell lines, were seeded in 6 well plate and incubated for 6 hours. The cells were

collected using trypsin followed by washing them with media. Trypan blue dye in Vi-cell XR (cell viability analyzer) used to assess viable cell number.

Statistical analysis

The data were analyzed and significant differences in gene expression were determined by a two-tailed student's t test. P-value ≤ 0.05.

Results

Knocking down ITGA2 upsurges the G0/G1 phase of cell cycle of pancreatic cancer cell lines

Cell cycle analysis reveals increasing in G0/G1 phase in ITGA2 knockdown PANC-1 and MIA PaCa cell lines

compared to the scramble control. However, S phase and G2/M have no significant differences (Figure.2).

Knocking down ITGA2 reduces the pancreatic cancer proliferation rate

Cell proliferation assay show significant reduction in viable cell number in ITGA2 knockdown PANC-1 and

MIA PaCa cell lines compared to the scramble control (Figure.3).

Knocking down ITGA2 in pancreatic cancer cell lines reduces the expression of some ECM- proteins and Vimentin

QPCR results show that ITGA2 knockdown PANC-1 and MIA PaCa cell lines express significantly lower level of master mesenchymal marker vimentin than the scramble control. In addition, ITGA2 knockdown

PANC-1 and MIA PaCa cell lines express significantly lower level of ECM- proteins LAMB3, and LAMC2 compared to the scramble control (Figure.4).

Discussion

Unfortunately, pancreatic cancer is often diagnosed at an advanced stage when it has already spread to other parts of the body, making it more difficult to treat.^[1] This is partly because the pancreas is located deep within the abdomen, making it hard to detect tumors until they have grown large or spread to other organs. Therefore; early diagnosis is critical.^[2] In vitro studies showed that, inhibition of ITGA2 significantly slows down tumor growth, decreases colony formation, and cell viability in pancreatic cancer cells. While overexpression of ITGA2 reverses the effects of ITGA2 inhibition on colony formation and cell viability.^[9] In our study, inhibition of ITGA2 significantly reduce the cancer proliferation rate maybe due to entering the cell the G0/G1 phase. G0/G1 phase considers the resting phase where the cells stop growing, dividing and entering the cell cycle arrest state (quiescence).^[13] Therefore, high level of ITGA2 may consider an inducer for the cancer cells in quiescence state to exit the cell cycle arrest and start growing and dividing.

ECM-proteins affect pancreatic cancer progression and patient survival by promoting cancer cell

proliferation and metastatic spread.^[6] Researchers showed that, knocking down ITGA2 in pancreatic cancer cells resulted in attenuated migration and invasive abilities.^[3] ITGA2, LAMB3, and LAMC2 expression were inversely correlated with a prolonged patient survival and may be used as potential therapeutic targets in pancreatic cancer.^[6] In our study, the ECM-proteins markers LAMB3, and LAMC2 decrease in the ITGA2 knockdown cell lines. These results suggest the ability of ITGA2 to affect the pancreatic cancer microenvironment toward metastasis. Researchers find that, the silencing of ITGA2 in esophageal squamous cell carcinoma suppresses epithelial to mesenchymal transition.^[14] Our results showed that, vimentin which considers the master mesenchymal marker shows low expression in silencing group compared to the control. These results support the notion of ITGA2 silencing cancer has lower migration and metastatic ability.^[6,7] Our results suggest a potential therapeutic implication of ITGA2 in pancreatic cancer progression and the possibility of using it as prognosis marker.

Conclusion

In this study, the results show a correlation between the loss of ITAG2 expression and the ability of the pancreatic cancer cells for proliferation, manipulating tumor microenvironment, and metastasis. In addition, lowering the level of ITAG2 may be considered as

inducer for the cancer cells to enter quiescence state and halt dividing. Furthermore, the study suggests the possibility of using ITAG2 as a diagnostic marker for both early and late stages of pancreatic cancer, which can be confirmed in a future study.

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Figure. 1: ITGA2 RNA expression in knockdown pancreatic cell lines compared to scramble control.

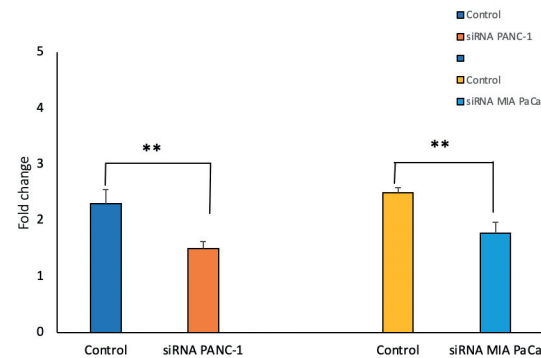


Figure. 2: Cell cycle analysis for ITGA2 knockdown pancreatic cell lines compared to scramble control.

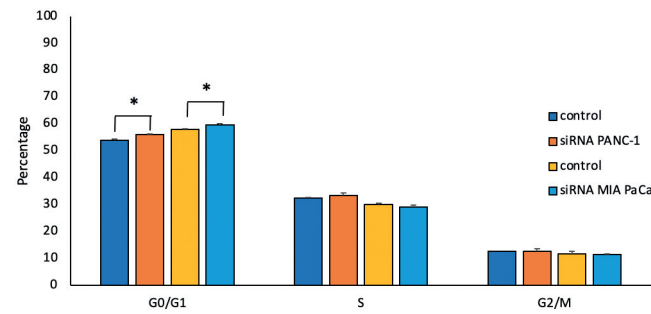


Figure. 3: Cell proliferation assay for ITGA2 knockdown pancreatic cell lines compared to scramble control.

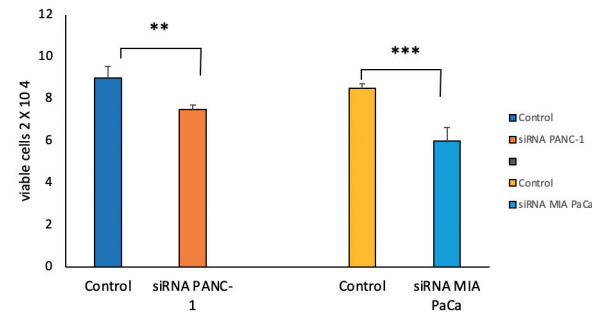


Figure.4: RNA expression of master mesenchymal marker and two ECM-proteins in ITGA2 knockdown pancreatic cell lines compared to scramble control.

