AC3 Has an Inhibitory Effect on the Cell Cycle and Enhances Staurosporine-Induced Apoptosis in Pancreatic Cancer Cells. Participation of R-Smads.

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INTRODUCTION

- Pancreatic cancer is one of the most lethal human malignancies. It is estimated that 40,560 individuals will die from pancreatic cancer in 2015[1]. Adenylyl cyclase (AC) is an enzyme responsible for converting ATP into cAMP. An increase in cAMP levels can inhibit cell proliferation and migration in pancreatic cancer cells.
- Previously, we found that five of the ten AC isoforms are expressed in two pancreatic adenocarcinoma cell lines, HPAC and PANC-1. Two of the AC isoforms, AC1 and AC3, were highly expressed in pancreatic tumor tissue (~ 2.5 fold) in comparison to adjacent non-tumor tissue.

Rationale: Because AC1 inhibited cell proliferation, two mechanisms can be involved: an inhibition of cell cycle and/or an increase in the cell death.

Hypothesis: AC1 inhibits cell proliferation by decreasing the cell cycle and by increasing the cell death via apoptosis.

- **Objectives:**
  1. Confirm whether or not AC1 inhibits cell proliferation of pancreatic cancer cell lines HPAC and PANC-1 measuring BrdU incorporation.
  2. Study whether or not AC1 promotes apoptosis of pancreatic cancer cell lines HPAC and PANC-1.
  3. Study whether AC1 activates PKA pathway by studying the phosphorylation of cAMP-response element-binding protein (CREB) by Western-blotting.

METHODS

- **Knock-down AC1 and AC3:** HPAC and PANC-1 cells were treated with siRNA AC1 (100 nM) (Ambion) or siRNA AC3 (100 nM) (Ambion) for 48h. Experiments were carried out as indicated below:

1.BrdU Incorporation: Cells were stimulated with forskolin (20 uM) and IBMX (1mM) for 48h, and incubated with BrdU (10 uM)[2] for 12h, followed by fixation with 4% formaldehyde. Cell membranes were permeabilized and DNA denatured with 2N HCl in 0.5% Triton X-100. Blocking was achieved with 1% BSA, followed by 1h incubation with AlexaFluor-488 anti-BrdU antibody at room temperature. Nuclei were stained with Hoechst 33342 stain and percent incorporation was quantified by counting nuclei displaying BrdU in captured images. Immunofluorescence was viewed with a Zeiss Axiovert 200 microscope with a X10 objective lens.

2. Apoptosis detection: Samples were stimulated with forskolin (20 uM) and staurosporine (1 uM) for 12 hours. AC1 and AC3 were knocked out using siRNA specific to the isoforms. An ELISA assay was conducted to measure the amount of active caspase-3 proteins present in the cell lines.

3. PKA Pathways: HPAC and PANC-1 cells were stimulated with forskolin (20 uM) and IBMX (1mM) for 12 min and lysed using a cell lysis. Both CREB and Smad phosphorylation were analyzed by Western-blotting.

RESULTS

1. FSK evoked a maximum BrdU incorporation at 48h. Whereas in the absence of AC1, FSK-stimulated cells displayed a slight increase in BrdU incorporation, FSK-stimulated cells showed a significant increase in incorporation in the absence of AC3.

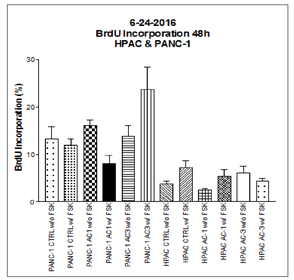
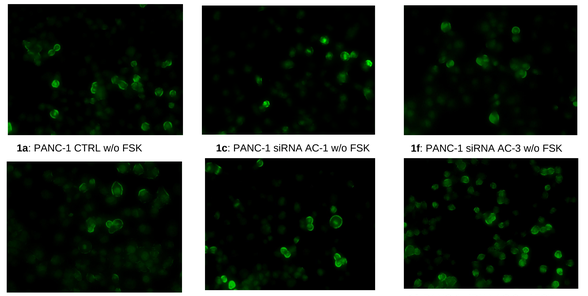


Figure 1: The absence of AC-3 in FSK-stimulated PANC-1 cells results in a significant increase in BrdU incorporation.



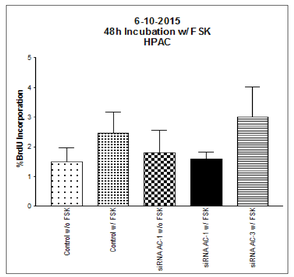
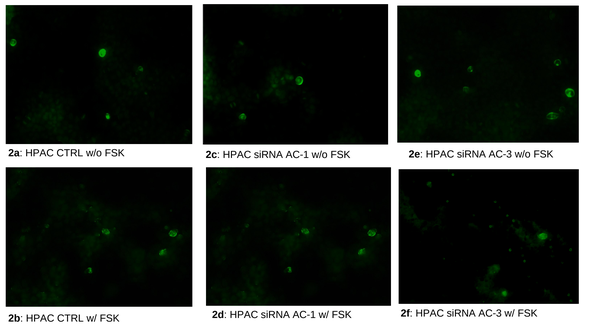


Figure 2: The absence of AC-3 in FSK-stimulated HPAC cells results in a slight increase in BrdU incorporation.



2. FSK caused negligible effect on caspase-3 activation. However, the combined effect of FSK and staurosporine, a pro-apoptotic agent, led to an increase in active caspase-3. The lack of AC3 significantly impaired the effect of FSK in both cell lines.

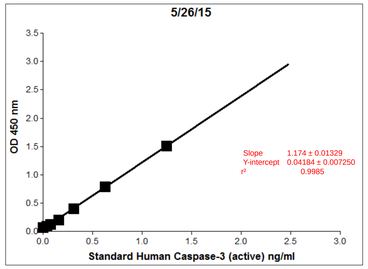


Figure 3: The following standard curve were obtained for the various standards of caspase 3 (active).

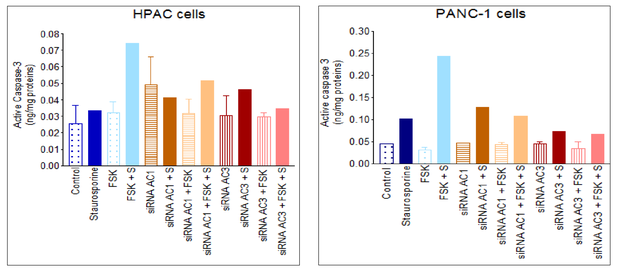
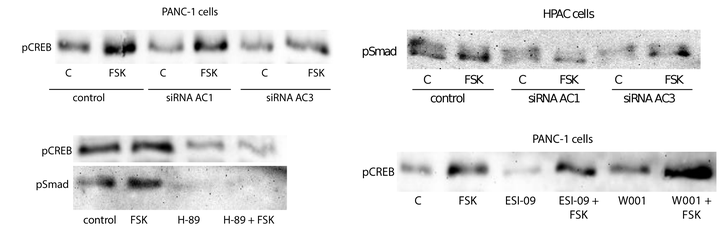


Figure 4: Forskolin (FSK) enhances staurosporine-induced apoptosis in HPAC and PANC-1 cells via AC1 and AC3. n=1-3 experiments.

RESULTS

3. Using Western-blotting our data showed that CREB was phosphorylated by AC3, whereas Receptor-Regulated Smads (R-Smads) (Smads 1, 5 and 9) were phosphorylated by AC1. The fact that AC3, but not AC1, mediated the phosphorylation of CREB was confirmed by using the AC1 inhibitor, W001. The PKA inhibitor, H-89, abolished the effect of forskolin, whereas the Epac inhibitor, ESI-09, did not modify it.



CONCLUSIONS

- We hypothesize that AC3 may have an inhibitory effect on the rate of cell cycle of pancreatic cancer cells; further experiments are required to better understand the significance of AC1 on proliferation.
- While FSK alone does not modify apoptosis, FSK enhances staurosporine-induced apoptosis in both HPAC and PANC-1 cells via AC1 and AC3 activation.
- Forskolin-stimulated-AC3 phosphorylates CREB, whereas forskolin stimulated-AC1 phosphorylates R-Smads (Smads 1, 5 and 9). Forskolin induced-phosphorylation was mediated by PKA, but not Epac1.

REFERENCES

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[2] Guo L, Sans MD, Hou Y, Ernst SA, Williams, JA. C-Jun/AP1 is required for CCK-induced pancreatic Cell dedifferentiation and DNA synthesis in vitro. Am J Physiol Gastrointest Liver Physiol 302: G1381-1396, 2012.

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