Structural Affinity of CAP1 and AC isoforms in Pancreatic Cancer Cells

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INTRODUCTION

Cancer is a disease where the body's cells divide continuously and spread into surrounding tissues causing harm. Of all the different cancers, pancreatic cancer is one of the major unsolved health problems **(1)**. Adenylyl cyclase (AC)/adenosine 3',5' cyclic monophosphate (cyclic AMP) pathway has shown to participate in the development of pancreatic pre-neoplastic lesions **(2)**. Cyclic AMP is a cellular second messenger that is critical in signal transduction pathways because it links membrane receptors and their ligands to the activation of internal cellular enzymatic activity and gene expression **(3,4).** It also plays an important role in multiple cancer cellular processes, including migration and invasion. In a previous study, we determined that AC3 is highly expressed in human pancreatic cancer tissues when compared to healthy tissues **(5).** Along with AC3, we determined, in previous studies, that AC1, AC4 and AC7 are also expressed in human pancreatic cancer cells **(5).** Regardless the mechanism by which AC/cyclic AMP pathway inhibits cell migration and invasion, our lab studies the participation of proteins physically interacting with transmembrane ACs. Of the identified proteins, our interest focuses on adenylyl cyclase associated protein -1 (CAP1), which is a scaffolding protein that regulates actin filament formation, cell migration and invasion **(6)**. CAP1 binds to G-actin and inhibits a spontaneous

HYPOTHESIS AND OBJECTIVE

Hypothesis: Because AC3 is highly expressed in human pancreatic cancer tissues, we hypothesize that, of the 4 AC isoforms that were studied, AC3 has the highest affinity for CAP1.

Objective: Determine in which extent CAP1 interacts with the transmembrane AC isoforms AC1, AC4, AC3 and AC7.

MATERIALS AND METHODS

Theoretical data: Homology modeling: To determine which AC isoform has a higher affinity for CAP1, we studied the amino acid sequences of AC/CAP1 complexes based on previous observations in *Saccharomyces Cerevisiae* **(8).** Amino acid sequences of CAP1 and AC isoforms from human, rat and *Saccharomyces Cerevisiae* were obtained from Protein databases of NCBI sequence viewer.

Experimental data: To determine which AC isoform has a higher affinity for CAP1, we used sequential co-immunoprecipitation.

Pancreatic cancer cell lines: The cell lines used were the HPAC cells and the PANC-1 cells. Both cell lines were purchased from American Type Culture Collection (ATCC) in 2014. HPAC cell line will be maintained in a 1:1 mixture of Dulbecco's Modified Eagle's Medium (DMEM)/Ham's F12 medium (GIBCO/Life technologies) (containing 1.2 g/L sodium bicarbonate, 2.5 mM L-glutamine, 15 mM HEPES and 0.5 mM sodium pyruvate supplemented with 0.002 mg/mL insulin, 0.005 mg/mL transferrin, 40 ng/mL hydrocortisone, 10 ng/mL epidermal growth factor and 5 % fetal bovine serum. PANC-1 cell line was maintained in DMEM medium (GIBCO/Life technologies) with 10% fetal bovine serum.

Sequential co-immunoprecipitation:

- Wash PANC1 and HPAC cells once with PBS.
- Lyse the cells with 400 uL immunoprecipitation buffer containing 50 mM TRIS HCl, pH 7.5, 150 mM sodium chloride, 5 mM EDTA, 10 mM sodium pyrophosphate, 25 mM β-glycerophosphate, 0.1 % Triton X-100, 1mM PMSF, 10 ug/mL leupeptin, 10 ug/mL aprotinin, 1mM sodium vanadate, 25 mM sodium fluoride, 1mM dithiothreitol.
- Rotate lysate overnight with rabbit anti-AC1, anti-AC3, anti-AC4 or anti-AC7 antibody.
- Collect the immunocomplexes using protein A agarose IP reagent for 1 hour at

THEORETICAL RESULTS

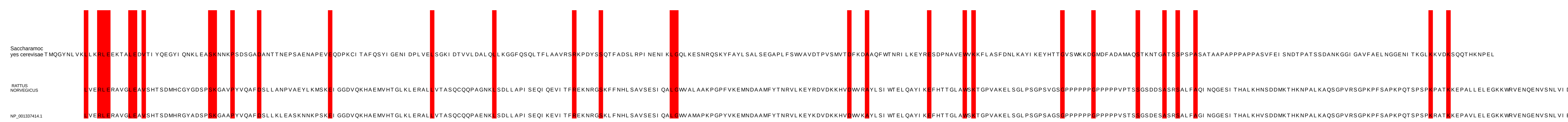

Figure 1: This figure shows that the amino acid sequences of human and rat CAP1 are similar to the amino acid sequence of *Saccharomyces Cerevisiae*. They all contain the AC-binding domain between the residues 1 and 36. The residues present in all three sequences are highlighted in red.

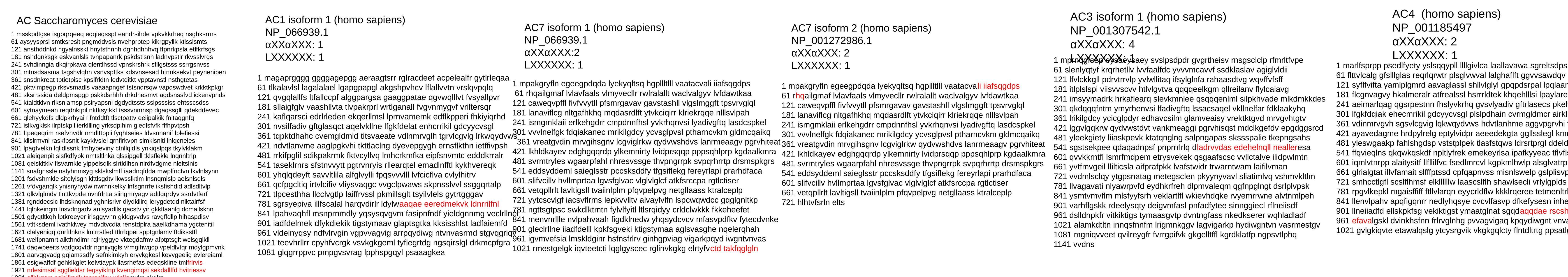

Figure 2: Based on the AC sequences above, AC3 is shown to have the highest number of motifs (αXXαXXX and LXXXXXX) for CAP1, indicating that it should have the highest affinity for CAP-1. The CAP1-binding domain is highlighted in red. L: lysine; X: any amino acid; α: any hydrophobic amino acid.

EXPERIMENTAL RESULTS

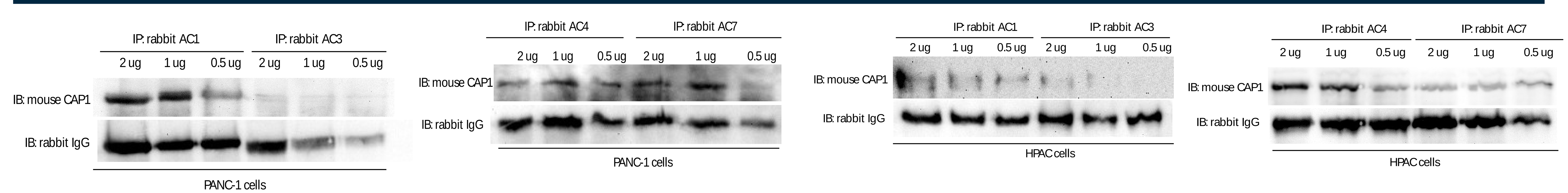

Figure 3: The sequential co-immunoprecipitation experiments were done using both HPAC cells and the PANC-1 cells. The figure indicates that CAP1 does interact with all 4 isoforms of AC in both cells lines; however, in both cells lines, CAP1 interacts most with AC1 and AC4 compared to the others. A rabbit IgG antibody was used as a loading control.

CONCLUSION

- Based on the theoretical data, the amino acid sequences of human and rat CAP1 are similar to the amino acid sequence of *Saccharomyces Cerevisiae*. They all contain the AC-binding domain between the residues 1 and 36.
- Based on the theoretical data, AC3 has the highest affinity for CAP1 since it has the higher number of repetitive motifs for CAP1 compared to all the other isoforms.
- Based on the experimental data, AC1 and AC4 have the highest affinity for CAP1 in PANC1 cells. In HPAC, AC4 had the highest affinity for CAP1.
- We can therefore conclude that AC4 has the overall highest affinity for CAP1, and this goes against our hypothesis that AC3 had the highest affinity for CAP1 ... binding domain of AC is correct.

CLINICAL IMPLICATIONS

A better understanding of the mechanism by which CAP1 interacts with AC isoforms can help develop treatment that will suppress the metastases and improve the overall survival rates of pancreatic cancer.

REFERENCES

[1] Siegel R. L, Miller K. D, Jemal A. Cancer Statistics, 2018. CA Cancer J Clin 2018; 68; 7-30.

[2] Wu, J., et al., *Recurrent GNAS mutations define an unexpected pathway for pancreatic cyst development.* Sci Transl Med, 2011. **3**(92): p. 92ra66.

[3] Sabbatini ME. Transmembrane Adenylyl Cyclases. In: The Pancreapedia Exocrine Pancreas Knowledge Base 2013. University of Michigan Library. (DOI: 10.3998/panc.2013.8).

[4] Sabbatini ME, Gorelick F., Glaser, S., Adenylyl cyclases in the digestive system. Cellular signaling, 26 (2014) 1173-1181.

[5] Quinn SN, Graves SH, Dains-McGahee C, Friedman EM, Hassan H, Witkowski P, Sabbatini ME. Adenylyl cyclase 3/adenylyl cyclase-associated protein 1 (CAP1) complex mediates the anti-migratory effect of forskolin in pancreatic cancer cells. Mol Carcinog. 2017;56(4):1344-1360.

[6] Ono, S., The role of cyclase-associated protein in regulating actin filament dynamics- more than a monomer-sequestration factor. J Cell Sci, 2013. 126(Pt 15): p 3249-58.

[7] Hubberstey, A.V. and E.P. Mottillo, Cyclase-associated proteins: CAPacity for linking signal transduction and actin polymerization. FASEB J, 2002. **16**(6): p. 487-99.

[8] Nishida, Y., Shima, F., Sen, H., Tanaka, Y., Yanagihara, C., Yamawaki-Kataoka, Y., Kataoka, T. (1998). Coiled-coil Interaction of N-terminal 36 Residues of Cyclase-associated Protein with Adenylyl Cyclase Is Sufficient for Its Function in Saccharomyces cerevisiae Ras Pathway. Journal of Biological Chemistry, 273(43), 28019-28024.

ACKNOWLEDGEMENTS

- Department of Biological Sciences, Augusta University.
- Augusta University Pamplin College of Arts, Humanities, and Social Sciences.
- Augusta University CURS Program.