STRUCTURAL, KINETIC AND FUNCTIONAL PROPERTIES OF CAP1/AC COMPLEXES

Simran Mehrotra, Maria Eugenia Sabbatini, PhD

Department of Biological Sciences, Augusta University

INTRODUCTION

Cancer is a disease where the body's cells divide continuously and without control, as well as spread into surrounding tissues causing harm. Of all the different cancers pancreatic cancer is one of the major unsolved health problems **(1).** An activation of the transmembrane adenylyl cyclase 3 (AC3) by forskolin has shown to inhibit migration of pancreatic cancer cells by regulating actin filament dynamics **(2).** However, the mechanism for the inhibitory effect of AC3 is not completely understood. Therefore, our goal was to identify proteins interacting with AC isoforms.

Adenylyl cyclases associated protein -1 (CAP1) is a scaffolding protein that regulates actin filament formation, cell migration and invasion **(3).** CAP1 binds to G-actin and inhibits a spontaneous actin polymerization **(4).** CAP1 has been conserved between yeasts and mammals during evolution **(5).** Like Crv2/CAP **(6),** human CAP1 has an adenylyl cyclase-binding domain in its structure **(7).**

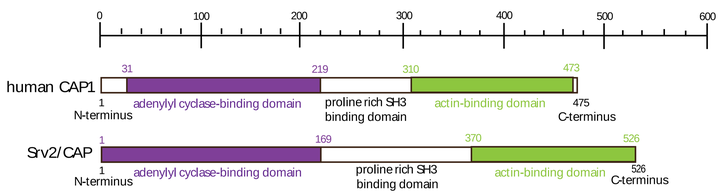

Figure 1: Schematic representation of the structure of the human CAP1 and yeast Saccharomyces cerevisiae Srv2/CAP. The number of amino acid residues is reported on the side of each structure. Domains are represented in color. CAP structure is conserved between yeasts and mammals during evolution. Both human CAP1 and yeast Srv2/CAP have three domains: adenylyl-cyclase binding domain, proline rich SH3 binding domain and actin-binding domain.

HYPOTHESIS AND OBJECTIVE

Hypothesis: Because CAP1 has an AC-binding domain in its structure, we hypothesize that a number of transmembrane AC isoforms interact with CAP1 to facilitate CAP1's ability to sequester G-actin, and thereby, inhibit cell migration.

Objective: Determine in which extent CAP1 interacts with a number of transmembrane AC isoforms and study the impact of these interactions on the invasive behavior of pancreatic cancer cells.

MATERIALS AND METHODS

- **Cell lines:** human pancreatic cancer HPAC and PANC-1 were purchased from American Type Culture Collection (ATCC).
- **Co-immunoprecipitation**:
  - Stimulate PANC-1 and HPAC cells with forskolin (20 uM) for 12 min.
  - Wash the cells once with PBS.
  - Lyse the cells with an immunoprecipitation buffer (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 aproptin, 1mM sodium vanadate, 25 mM sodium fluoride, 1mM dithiothreitol).
  - Rotate lysate overnight with antibodies against AC isoforms.
  - Collect CAP1-AC complex using protein G agarose IP reagent for 1 h at 4°C.
  - Wash immunocomplex three times with the lysis buffer.
  - Perform Western-blotting using rabbit anti-CAP1 antibody.

RESULTS

CAP1 interacts with a number of transmembrane AC isoforms in pancreatic cancer cells.

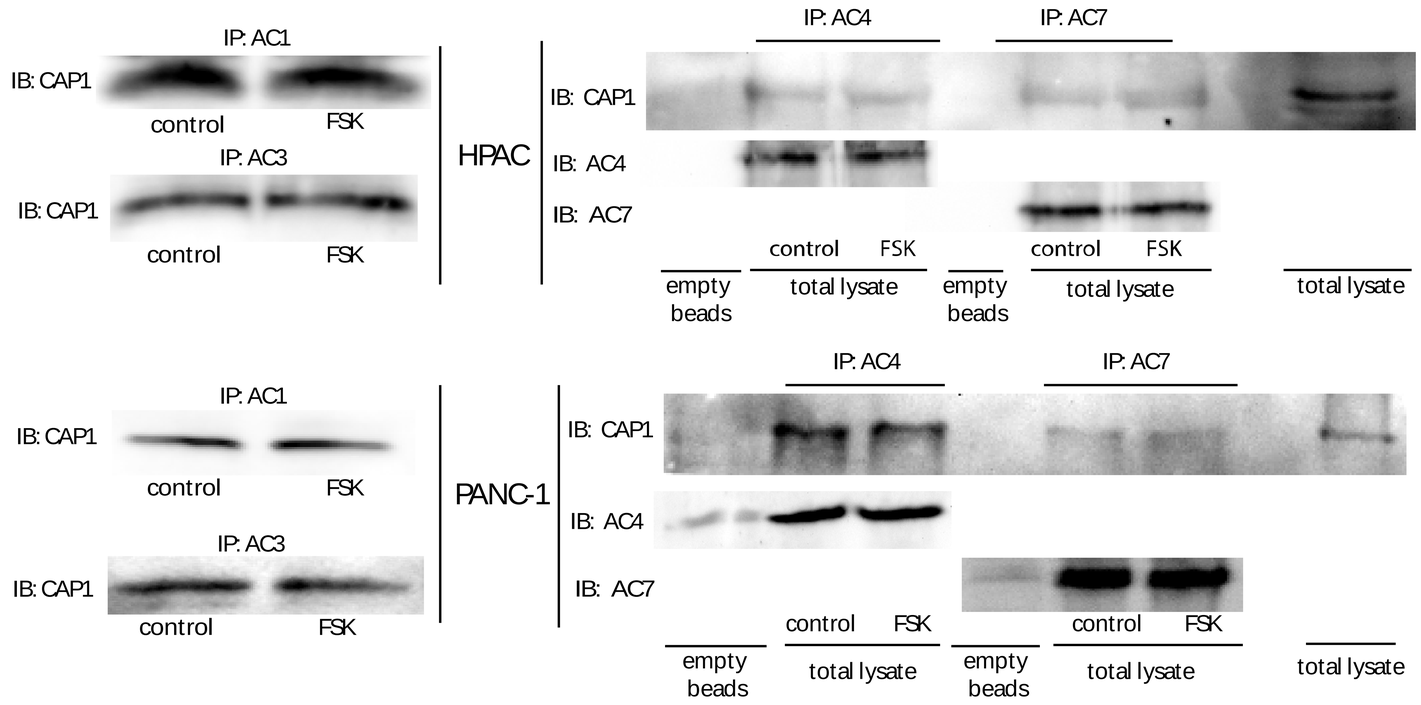

Figure 1: HPAC and PANC-1 cells were stimulated with forskolin (20 uM) for 12 min. Co-immunoprecipitation experiments were performed using rabbit anti-AC1, anti-AC3, anti-AC4 and anti-AC7 antibodies. Representative immunoblots for CAP1 show that a number of transmembrane AC isoforms interact with CAP1. Comparable immunoprecipitation of AC4 and AC7 was analyzed by Western-blotting using anti-AC4 and anti-AC7 antibodies, respectively. FSK: forskolin. (n: 3 experiments).

CONCLUSION

- All studied transmembrane AC isoforms physically interact with CAP1 in non-stimulated and forskolin-stimulated conditions.
- Because nothing is known about the affinity of CAP1 for each AC isoform, sequential co-immunoprecipitation studies will be carried out.
- To address whether CAP1 expressed in pancreatic cancer cell is sufficient to interact with all studied transmembrane AC isoforms, quantitative immunoblotting will be employed to evaluate the stoichiometric ratio of these proteins.

CLINICAL IMPLICATIONS

A better understanding of the mechanism of 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) What Is Cancer? (2015, February 9). Retrieved March 05, 2017, from https://www.cancer.gov/about-cancer/understanding/what-is-cancer#types-of-cancer.

(2) Quinn, S., Graves, S., Dains-McGahee, C., Friedman, E., Hassan, H., Witkowski, P., Sabbatini, M.E. Adenylyl cyclase 3/adenylyl cyclase-associated protein 1 (CAP1) complex mediates the anti-migratory effect of forskolin in pancreatic cancer cells. Mol Carcinog. 2016 Nov 27. doi: 10.1002/mc.22598.

(3) 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.

(4) 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.

(5) Yu, G., J. Swiston, and D. Young, *Comparison of human CAP and CAP2, homologs of the yeast adenylyl cyclase-associated proteins.* J Cell Sci, 1994. **107 (Pt 6)**: p. 1671-8.

(6) Field, J., et al., *Cloning and characterization of CAP, the S. cerevisiae gene encoding the 70 kd adenylyl cyclase-associated protein.* Cell, 1990. **61**(2): p. 319-27.

(7) Lee, S., et al., *Adenylyl cyclase-associated protein 1 is a receptor for human resistin and mediates inflammatory actions of human monocytes.* Cell Metab, 2014. **19**(3): p. 484-97.

ACKNOWLEDGEMENTS

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