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 adenyl 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 Gq/11 (6), human CAP1 has an adenylyl-cyclase-binding domain in its structure (7).

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 μM) for 12 min.
  - Wash the cells once with PBS.
  - Lys 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 μg/mL leupeptin, 10 μg/mL aprotinin, 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.

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


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