The Lack of AC1 Impairs the Inhibitory Effects of cAMP on Cell Migration and Proliferation in Pancreatic Cancer

Sierra Quinn
Brown Bag--February 6, 2015
Georgia Regents University
Pancreatic Cancer

- 2015 Statistics
  - 48,960 people will be diagnosed
  - 40,560 people will die

- There was an increase in 2.45% rates of estimated deaths

- Shown little improvement in survival over the past 40 years.

- Risk factor: alcohol + smoking

Siegel et al., CA CANCER J CLIN 2015
Early detection can lead to prognostic biomarkers and therapeutic targets.

Mutations in the oncogene GNAS were found in pancreatic cancer lesions.

**General Objective:**

find out which AC isoform is up-regulated in pancreatic cancer and implicated in cell proliferation, migration and invasion.
Adenylyl Cyclase

- Adenylyl cyclase (AC) is an enzyme that induces the conversion of ATP to cAMP and pyrophosphate.
- Large transmembrane protein
- 9 different transmembrane isoforms
Objective 1

- Study which transmembrane AC isoforms are expressed in pancreatic cancer.
Procedures

- **RT-PCR**
  - To determine which isoforms are expressed in human pancreatic cancer cell lines and human pancreatic tissues.

- **Western Blot**
  - To confirm the presence of the isoforms
Results - RT-PCR

Cell lines

HPAC

PANC-1

PDEC

human pancreatic tissues

tumor tissue

adjacent non-tumor tissue
Results - Western Blotting

HPAC cell lysate

PANC-1 cell lysate
AC1 and AC3 isoforms are highly expressed in pancreatic tumor tissue

Real-time quantitative PCR for AC isoforms expression in the adjacent tissue versus tumor tissue

![Graph showing the expression levels of AC1 and AC3 isoforms in normal and tumor tissue.](image-url)
AC₁ and AC₃ isoforms are highly present in pancreatic tumor tissue

*Western-blotting of AC isoforms in the adjacent tissue versus tumor tissue*
Human Pancreatic Adenocarcinoma

http://www.pathologyoutlines.com/topic/pancreassuperpagetumor.html
Cyclic AMP (cAMP)

- is a second messenger generated from adenylyl cyclase activation.
- is implicated in the differentiation of several malignant cell types.
- inhibits cell migration in pancreatic cancer cells.
- has controversial effects on cell proliferation in cancer cells.
Objective 2

To determine the effect that AC1 and AC3 has in cell proliferation, migration and invasion in HPAC cells.
**AC/cAMP pathway**

- **Vasoactive Intestinal polypeptide (VIP)**: VPAC1/2R
  - Secretin: Secretin R
  - EGF: EGFR
  - Somatostatin: ss2R
  - Forskolin (FSK)

**Key Components**:
- ATP
- cAMP
- ACs

**References**: Sabbatini et al., Cell Signal 2015
Only Forskolin increases cAMP levels in HPAC cells
Procedures

Vibrant MTT cell proliferation assay kit
CytoSelect TM 24-well cell migration and invasion assay
siRNA AC1 and siRNA AC3 (100 nM) (Santa Cruz and Ambion)
siRNA AC₁ and siRNA AC₃ knock out AC₁ and AC₃ expression, respectively.
The lack of AC1 or AC3 decreases forskolin-stimulated cAMP formation.
The lack of AC1 impairs the inhibitory effect of Forskolin on cell proliferation.
The lack of AC1 impairs the inhibitory effect of forskolin on cell migration
AC/cAMP pathway is not involved in cell invasion

![Graph showing OD 560 for control and FSK treatments. The graph indicates that the OD 560 for FSK treatment is higher than the control, with a sample size of n=4.]
Conclusions

• Several AC isoforms are expressed in human pancreatic cells:
  ▪ AC1, AC3, AC6, AC7 and AC9 were found in HPAC, PANC-1 and PDEC cell lines.
  ▪ AC1, AC3, AC5, AC6, AC7 and AC9 were found in human pancreatic cancer and adjacent tissues.

• Both AC1 and AC3 transcript levels were highly expressed in human pancreatic cancer tissues than in the adjacent non-tumor tissues.

• AC1 mediates the inhibitory effect of cAMP in cell proliferation and cell migration.

• AC/cAMP pathway is not involved in cell invasion of pancreatic cancer cells.
Acknowledgements

- Dr. Sabbatini
- Kay Bathala, Lucy Chavez, Emily Holcomb, Tison Garnett, Terrill Flakes
- American Physiological Society (APS)
- Department of Biological Sciences
- Center for Undergraduate Research
- Tumor Bank - Cancer Center - Georgia Regents University
- Piotr Witkowski, MD PhD from University of Chicago
- Baoan Ji, MD, PhD from Mayo Clinic, Rochester, MN
Questions are guaranteed in life; Answers aren't.

http://engineerblogs.org/2012/06/open-ended-questions-2/