Guanylyl Cyclase C (GC-C) Inhibits Human Colon Carcinoma Cell Growth

Giovanni Mario Pitari
Thomas Jefferson University, gmpitari@gmail.com

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GUANYLYL CYCLASE C (GC-C) INHIBITS HUMAN COLON CARCINOMA CELL GROWTH

Giovanni Mario Pitari

Division of Clinical Pharmacology
Department of Medicine
Thomas Jefferson University
Philadelphia, PA 19107
Guanylyl Cyclase Family

Diagram showing the structure of pGC and sGC, highlighting domains such as Amino Terminal, Extracellular Domain, Transmembrane Domain, Juxtamembrane Domain, Kinase Homology Domain, Hinge Region, Guanylyl Cyclase Catalytic Domain, and Carboxyl Terminal.
The E. coli Heat-Stable Enterotoxin (ST) Binds GC-C

N T F Y C C E L C C N P A C A G C Y
GC-C is Localized to Intestinal Epithelial Cells

GC-C Signaling Cascade
Does GC-C Mediate More Than Fluid Transport in Intestine?

• Does GC-C regulate intestinal epithelial cell proliferation?

• What are the molecular mechanisms by which GC-C regulates intestinal cell proliferation?
Protocol Design & Materials

Cell Lines:
T84, Caco-2, SW480

Pro-Proliferative Agents:
FBS, L-Glutamine

KT5823
RP8pCPT-cGMP

KT5720
Rp-cAMPs

ST
Uroguanylin

8-Br-cGMP

Milrinone
ST Inhibits Intestinal Cell Proliferation

- Increase in Cell Number (%)
- Increase in Protein Content (%)
- % of Control FBS-
- Stimulated
- ^3H-Thymidine Incorporation

T84, Caco-2, SW480, T84

- T84 induced to proliferate by L-Gln

- Control
- ST
ST Inhibition is Dose- and Time-Dependent

**3H-Thymidine Incorporation (cpm x 10^3)**

- **ST (nM):** 0, 1, 10, 10^2, 10^3
- **Control** vs **ST** bars for 12 h, 24 h, 48 h
ST Delays, But Does Not Arrest, the Cell Cycle

Control

ST


G_2/M 16%

sub-G_1 3%

G_1 48%

S 33%

G_2/M 16%

sub-G_1 3%

G_1 47%

S 34%

Control

ST


3H-Thymidine Incorporation (cpm x 10^3)

Hours
GC-C Agonists Do Not Induce Apoptosis or Necrosis

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ST (1 μM)</th>
<th>Uro (1 μM)</th>
<th>TACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Apoptosis</td>
<td>7.4 ± 0.5</td>
<td>9.1 ± 1.2</td>
<td>6.9 ± 0.9</td>
<td>75.3 ± 2.1**</td>
</tr>
</tbody>
</table>

** p<0.01
ST Cell Signaling Pathway for the Inhibition of Proliferation

- **GLN-stimulated Thymidine Incorporation (%)**
  - CTR
  - TJU
  - ST
  - URO
  - 8-Br-cGMP
  - ZAP
  - ST + ZAP

- **Fold Over CTR**
  - [cGMP]_i
  - [cAMP]_i

- **Significant Differences**
  - CTR vs. ST: ***
  - ST vs. ZAP: **
  - ST + ZAP vs. CTR: ***

- **Compounds**
  - Milrinone
  - KT5823, RP8pCPT-cGMP
  - KT5720, Rp-cAMPs
Summary

• GC-C activation inhibits colon carcinoma cell proliferation in vitro
• Inhibition of proliferation results from a prolongation of the cell cycle, not cell death
• The cytostatic effect of ST is mediated by an increase in [cGMP]i
ST-Dependent Cytostasis Does Not Reflect Arrest, but Retardation, of the Cell Cycle

Control

- G₂/M: 16%
- sub-G₁: 3%
- S: 33%
- G₁: 48%
- 27 h

ST

- G₂/M: 16%
- sub-G₁: 3%
- S: 34%
- G₁: 47%
- 37 h
Implications of GC-C Regulation of Proliferation

- Endogenous GC-C ligands (guanylin and uroguanylin) may represent cell cycle regulators.
- Along the crypt-to-villus axis, GC-C may regulate the transition of intestinal epithelial cells from proliferative to differentiated states.
- GC-C agonists may be utilized as novel cytostatic agents for the prevention and treatment of colorectal cancer.
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