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

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Guanylyl Cyclase Family

Diagram showing the structure of pGC and sGC with various domains labeled.
The E. coli Heat-Stable Enterotoxin (ST) Binds GC-C
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?*
Cell Lines: T84, Caco-2, SW480

Pro-Proliferative Agents: FBS, L-Glutamine

KT5823
RP8pCPT-cGMP
KT5720
Rp-cAMPS

8-Br-cGMP
Milrinone

ST
Uroguanylin
ST Inhibits Intestinal Cell Proliferation

![Graphs showing the effect of ST on cell proliferation and protein content in T84, Caco-2, and SW480 cells.](image)

- **Increase in Cell Number (%)**
  - T84: Control, ST
  - Caco-2: Control, ST
  - SW480: Control, ST

- **Increase in Protein Content (%)**
  - T84: Control, ST
  - Caco-2: Control, ST
  - SW480: Control, ST

- **% of Control FBS-Stimulated ³H-Thymidine Incorporation**
  - T84: Control, ST
  - Caco-2: Control, ST
  - SW480: Control, ST

- **T84 induced to proliferate by L-Gln**

Legend:
- Control (white bars)
- ST (red bars)

Significance Levels:
- *: p < 0.05
- **: p < 0.01
- ***: p < 0.001
ST Inhibition is Dose- and Time-Dependent

\[ 3^H\text{-Thymidine Incorporation (cpm x 10^3)} \]

- **Control**
- **ST**

**Graph:**
- **Y-axis:** 3H-Thymidine Incorporation (cpm x 10^3)
- **X-axis:** ST (nM)

**Statistical Symbols:**
- *: p < 0.05
- **: p < 0.01

**Time Points:**
- 12 h
- 24 h
- 48 h
ST Delays, But Does Not Arrest, the Cell Cycle

Control

- G1: 48%
- S: 33%
- G2/M: 16%
- Sub-G1: 3%

ST

- G1: 47%
- S: 34%
- G2/M: 16%
- Sub-G1: 3%

* * * * *

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

Hours

Control

ST
GC-C Agonists Do Not Induce Apoptosis or Necrosis

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

** p<0.01

Legend:
- CTR
- ST
- URO
- TACS

DNA Content

Log FITC

bp

1500 1000 500 300 200

bp

PBS URO ST IBMX URO+IBMX ST+IBMX DMSO U937 DNA
ST Cell Signaling Pathway for the Inhibition of Proliferation
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 \([\text{cGMP}]_i\)
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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