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

Giovanni Mario Pitari
Thomas Jefferson University

Follow this and additional works at: https://jdc.jefferson.edu/petfp

Part of the Medical Pharmacology Commons, and the Pharmacy and Pharmaceutical Sciences Commons

Let us know how access to this document benefits you

Recommended Citation

This Article is brought to you for free and open access by the Jefferson Digital Commons. The Jefferson Digital Commons is a service of Thomas Jefferson University's Center for Teaching and Learning (CTL). The Commons is a showcase for Jefferson books and journals, peer-reviewed scholarly publications, unique historical collections from the University archives, and teaching tools. The Jefferson Digital Commons allows researchers and interested readers anywhere in the world to learn about and keep up to date with Jefferson scholarship. This article has been accepted for inclusion in Department of Pharmacology and Experimental Therapeutics Faculty Papers by an authorized administrator of the Jefferson Digital Commons. For more information, please contact: JeffersonDigitalCommons@jefferson.edu.
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
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

ST
Uroguanylin

8-Br-cGMP
Milrinone
ST Inhibits Intestinal Cell Proliferation
ST Inhibition is Dose- and Time-Dependent

**Graphs:**
- **Left Graph:** 
  - **X-axis:** ST (nM)
  - **Y-axis:** $^3$H-Thymidine Incorporation ($\text{cpm} \times 10^3$)
  - Data points with * indicate significance at 10 nM.
  - Data points with ** indicate significance at 100 nM.

- **Right Graph:**
  - **X-axis:** Time (12 h, 24 h, 48 h)
  - **Y-axis:** $^3$H-Thymidine Incorporation ($\text{cpm} \times 10^3$)
  - Comparison between Control and ST.
ST Delays, But Does Not Arrest, the Cell Cycle

Control

<table>
<thead>
<tr>
<th>Phase</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>48%</td>
</tr>
<tr>
<td>S</td>
<td>33%</td>
</tr>
<tr>
<td>G₂/M</td>
<td>16%</td>
</tr>
<tr>
<td>sub-G₁</td>
<td>3%</td>
</tr>
</tbody>
</table>

ST

<table>
<thead>
<tr>
<th>Phase</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>47%</td>
</tr>
<tr>
<td>S</td>
<td>34%</td>
</tr>
<tr>
<td>G₂/M</td>
<td>16%</td>
</tr>
<tr>
<td>sub-G₁</td>
<td>3%</td>
</tr>
</tbody>
</table>

3H-Thymidine Incorporation (cpm x 10³)

Hours

0 6 12 18 24 30

Control

ST

*
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

Fold Over CTR

[cGMP]i [cAMP]i

*** **

Milrinone
KT5823, RP8pCPT-cGMP
KT5720, Rp-cAMPs

-25 0 25 50 75 100

*** **

0 25 50 75 100

0 10 20 30 40
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

<table>
<thead>
<tr>
<th>Phase</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2/M</td>
<td>16%</td>
</tr>
<tr>
<td>sub-G1</td>
<td>3%</td>
</tr>
<tr>
<td>G1</td>
<td>48%</td>
</tr>
<tr>
<td>S</td>
<td>33%</td>
</tr>
</tbody>
</table>

27 h

ST

<table>
<thead>
<tr>
<th>Phase</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2/M</td>
<td>16%</td>
</tr>
<tr>
<td>sub-G1</td>
<td>3%</td>
</tr>
<tr>
<td>G1</td>
<td>47%</td>
</tr>
<tr>
<td>S</td>
<td>34%</td>
</tr>
</tbody>
</table>

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.
Acknowledgements

Scott A. Waldman
Matthew Di Guglielmo
Stephanie Schulz
Jason Park

Henry Wolfe
Shiva Kazerounian
Inez Ruiz-Stewart

NIH RO1 HL65921, RO1 CA7512, R21 CA7966
Targeted Diagnostics and Therapeutics, Inc.