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

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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?
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 (%)
- T84 induced to proliferate by L-Gln

- % of Control FBS-Stimulated
- ³H-Thymidine Incorporation

<table>
<thead>
<tr>
<th>Cell Line</th>
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<th>Increase in Protein Content (%)</th>
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<tbody>
<tr>
<td>T84</td>
<td>125</td>
<td>125</td>
</tr>
<tr>
<td>Caco-2</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>SW480</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>T84</td>
<td>25</td>
<td>25</td>
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- T84 induced to proliferate by L-Gln

- % of Control FBS-Stimulated
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ST Inhibition is Dose- and Time-Dependent

\[ \text{ST (nM)} \]

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

\[ \text{Control} \quad \text{ST} \]

\[ \text{12 h} \quad \text{24 h} \quad \text{48 h} \]
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)**

- Control
- ST

![Graph showing thymidine incorporation over time](image-url)
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>
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<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>
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** p<0.01
ST Cell Signaling Pathway for the Inhibition of Proliferation

GLN-stimulated Thymidine Incorporation (%)

-25 0 25 50 75 100
CTR TJU ST URO 8-Br-cGMP ZAP

Fold Over CTR
0 10 20 30 40
[cGMP]i [cAMP]i

Control ST

*** ** **

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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

- G\_2/M: 16%
- sub-G\_1: 3%
- S: 33%
- G\_1: 48%
- Time: 27 h

ST

- G\_2/M: 16%
- sub-G\_1: 3%
- S: 34%
- G\_1: 47%
- Time: 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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