Relative caspase 3 mRNA expression

Relative FAS mRNA expression

hGAS/+ WT  hGAS/+ CCK2R-/- CCK2R-/-

hGAS/+ WT  hGAS/+ CCK2R-/- CCK2R-/-

*  ***  ***  ***

*  ***  ***  ***
Relative cyclinD1 mRNA expression

hGAS/+ WT hGAS/+ CCK2R/-

* * *

hGAS/+ CCK2R/- CCK2R/-
DCAMKL1 positive cells/fields (x600)

- hGAS\(^{+/+}\)
- WT

Unirradiated
Irradiated

*
A | hGAS\(^{+/+}\) | WT | hGAS\(^{+/+}\)/CCK2R\(^{-/-}\) | CCK2R\(^{-/-}\) 
---|---|---|---|---
Colon | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) | ![Image](image4.png)
Tumor | ![Image](image5.png) | ![Image](image6.png) | ![Image](image7.png) | ![Image](image8.png)

B | ![Graph](image9.png)
---|---
hGAS\(^{+/+}\) | WT | hGAS\(^{+/+}\)/CCK2R\(^{-/-}\) | CCK2R\(^{-/-}\) 

C | ![Graph](image10.png)
---|---
hGAS\(^{+/+}\) | WT | hGAS\(^{+/+}\)/CCK2R\(^{-/-}\) | CCK2R\(^{-/-}\) 

LgR5 positive cells/ x600
ACF number/colon

hGAS/+ hGAS/+ hGAS/+ YM022 CCK2R-/-
Supplemental Figure 1. Immunofluorescence study of murine CCK2R (red), E-cadherin (green) and DAPI (blue) in the colonic crypt of wild type (A) and CCK2R\textsuperscript{-/-} (B) mice (x800). The arrows indicate the locations of CCK2R expressing cells.

Supplemental Figure 2. Progastrin overexpression inhibits caspase 3 and FAS genes expression in the murine colonic mucosa. Quantitative RT-PCR analysis of caspase 3 (A) and FAS (B) genes mRNA levels in the murine colon (n=4/each group). Expression levels were normalized to GAPDH expression levels. All values represent the mean ± SD. (*P<0.05; **P<0.01, ***P<0.001).

Supplemental Figure 3. Progastrin overexpression promotes the upregulation of cyclin D1 in the murine colonic mucosa. Quantitative RT-PCR analysis of cyclin D1 mRNA levels in the murine colon (n=4/each group) was analyzed. Expression levels were normalized to GAPDH expression levels. All values represent the mean ± SD. (*P<0.05).

Supplemental Figure 4. Progastrin overexpression promotes the survival of DCAMKL1 expressing cells after radiation. The DCAMKL1 positive cells in hGAS\textsuperscript{+/+} and WT mice with or without irradiation (8Gy) were counted under high power light microscope (x600) (n=3/each group). All values represent the mean ± SD (*P<0.05).

Supplemental Figure 5. Progastrin overexpression promotes the expansion of LgR 5 positive colonic crypt cells in a CCK2R-dependent manner. (A) Immunohistochemistry for LgR5 in colonic mucosa (upper panels) and colon tumors (lower panels) in mice. The arrows indicate the locations of LgR5 expressing cells. Representative results from four different groups are shown (x600). The average percentage of LgR5 positive cells in the colonic crypts (B), and tumors (C) observed under high power field (x600) in each of the four groups of mice (n=4/each group are shown). All values represent the mean ± SD. (*P<0.05; **P<0.01, ***P<0.001).

Supplemental Figure 6. CCK2R antagonist (YM022) inhibits progastrin-dependent colonic ACF formation. Mice were treated with AOM (10 mg/kg) once a week for two weeks (n=8/each group) with simultaneous intraperitoneal injection of 1 mg/kg YM022 three times per week, and the mice were sacrificed two weeks after the last AOM injection. The whole colons were removed, fixed with 70% ethanol overnight and stained with methylene blue. The ACFs were analyzed under Nikon TE2000 microscope (magnification, x100). All values represent the mean ± SD. (**P<0.01).