

# CCK<sub>2</sub> receptor expression transforms non-tumorigenic human NCM356 colonic epithelial cells into tumor forming cells

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Expression of gastrin and cholecystokinin 2 (CCK<sub>2</sub>) receptor splice variants (CCK<sub>2</sub>R and CCK<sub>214svR</sub>) are upregulated in human colonic adenomas where they are thought to contribute to tumor growth and progression. To determine the effects of ectopic CCK<sub>2</sub> receptor variant expression on colonic epithelial cell growth *in vitro* and *in vivo*, we employed the non-tumorigenic colonic epithelial cell line, NCM356. Receptor expression was induced using a retroviral expression vector containing cDNAs for either CCK<sub>214svR</sub> or CCK<sub>2</sub>R. RT-PCR and intracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>) imaging of RIE/CCK<sub>2</sub>R cells treated with conditioned media (CM) from NCM356 revealed that NCM356 cells express gastrin mRNA and secrete endogenous, biologically active peptide. NCM356 cells expressing either CCK<sub>2</sub>R or CCK<sub>214svR</sub> (71 and 81 fmol/mg, respectively) grew faster *in vitro*, and exhibited an increase in basal levels of phosphorylated ERK (pERK), compared with vector. CCK<sub>2</sub> receptor selective antagonist, YM022, partially inhibited the growth of both receptor-expressing NCM356 cells, but not the control cells. Inhibitors of mitogen activated protein kinase pathway (MEK/ERK) or protein kinase C (PKC) isozymes partially inhibited the elevated levels of basal pERK and *in vitro* growth of receptor-expressing cells. Vector-NCM356 cells did not form tumors in nude mice, whereas, either CCK<sub>2</sub> receptor-expressing cells formed large tumors. Autocrine activation CCK<sub>2</sub> receptor variants are sufficient to increase *in vitro* growth and tumorigenicity of non-transformed NCM356 colon epithelial cells through a pathway involving PKC and the MEK/ERK axis. These findings support the hypothesis that expression of gastrin and its receptors in human colonic adenomas contributes to tumor growth and progression.

**Key words:** gastrin, CCK<sub>2</sub> receptors, colorectal tumorigenesis, NCM356 cells

**Abbreviations:** A: adherent; APC: adenomatous polyposis coli; BK: bradykinin; CCK<sub>2</sub>: cholecystokinin 2; CM: conditioned media; DMSO: dimethyl sulfoxide; FBS: fetal bovine serum; G17: Gastrin 1-17; Gly-G: glycine-extended gastrin; H&E: hematoxylin and eosin; IRES: internal ribosome entry site; JNK: Jun N-terminal kinase; LTR: long terminal repeat; MAPK: mitogen-activated protein kinase; MLV: murine leukemia retrovirus; MCS: multiple cloning site; NA: non-adherent; PCR: polymerase chain reaction; pERK: phosphorylated ERK; PKC: protein kinase C; RIE: rat intestinal epithelial

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The adenoma- to carcinoma-multistage sequence of colorectal cancer development is characterized by specific histopathologic criteria as well as defined genetic mutations, which result in the activation of oncogenes (e.g., *K-ras*) and inactivation of tumor suppressors [e.g., adenomatous polyposis coli (APC) and p53].<sup>1</sup> It is now well recognized that the key initiating events that underlie most cases of colorectal tumorigenesis, whether familial or sporadic, are the mutations within the Wnt/APC/ $\beta$ -catenin signaling pathway.<sup>2</sup> However, increasing evidence also indicate that epigenetic changes in DNA and/or chromatin structure, causing aberrant mRNA splicing and/or inappropriate expression of normal genes, can interact with genetic mutations to contribute to the development of the malignant phenotype.<sup>3</sup> Aberrant expression of the gastrin/cholecystokinin 2 (CCK<sub>2</sub>) receptor, gastrin and its biosynthetic precursors, in a majority of pre-malignant adenomatous polyps strongly implicate a relevant role for this signaling axis in the adenoma-carcinoma sequence.<sup>4-6</sup>

Carboxyl-terminus-amidated gastrin (i.e., mature gastrin) is produced from the cleavage and post-translational processing of a preprohormone protein. Although both gastrin precursors and mature gastrin are prevalent during the early stages of malignant transformation, as well as in established