4 Discussion

Many colorectal cancers exhibit a proliferative response upon stimulation with exogenous or endogenous gastrin (3). The epidemiological evidence that the gastrointestinal peptide hormone gastrin plays a role in the development of colorectal cancers has aroused considerable interest over the past several years. Recent reports have demonstrated that hypergastrinemia induced by Helicobacter pylori infection is often associated with increased COX-2 expression in gastric and colorectal tissues, and overexpression has been observed in the pathogenesis of colon cancer formation (21, 38, 86). Although increased expression of COX-2 in human and rodent intestinal tumors has been widely observed, the mechanisms that regulate the expression of COX-2 in colonic tumors are not completely understood. The purpose of the present investigation was therefore to further elucidate the molecular mechanisms which control the overexpression of COX-2 in human colon carcinoma cells and nontumorigenic cells with special emphasis on a recently cloned novel cDNA encoding a splice variant CCK-B/gastrin receptor retaining intron 4 which was proposed to exhibit augmented growth-promoting effects in response to gastrin and to exert ligand-independent activity. To study the structure-function relationship of the CCK-BRi4sv receptor, tumorigenic human colorectal cancer cells and nontumorigenic COS-7 cells were transfected with the cDNAs encoding the wild type or splice variant CCK-BR. In this model, the CCK-BRi4sv potently mediated the transactivation of the COX-2 promoter through a proximal promoter element located between -203 bp to -68 bp from the transcription initiation site using multiple signaling pathways acting synergistically.

Gastrin has been previously shown to be a potent stimulator of COX-2 expression in intestinal epithelial cells through CCK-BR-mediated activation of multiple protein kinase pathways. Upregulation of COX-2 expression induced by gastrin was postulated to represent a hormone-dependent carcinogenic step, especially in colon (20). Moreover, recent research suggests that the CCK-BRi4sv stimulates cell growth in a constitutive and gastrin-17-independent manner (22). In the present study, induction of COX-2 expression was explored at the mRNA level using real time RT-PCR. Results show that COX-2 mRNA expression started to increase after 2 hours following stimulation with gastrin-17 and continued to increase at least for 12 hours and a very obvious peak appeared at 10 hr in Colo320SV cells with a 50-fold increase compared to the control. Prostaglandins derived from COX-2 are thought to play an important role in intestinal
carcinogenesis, although the precise mechanism(s) by which they act is not yet understood. Understanding the pathways that control the expression of COX-2 and exploring the signaling mechanisms involved in the regulation of COX-2 expression may lead to a better understanding of its dysregulation in colorectal carcinomas (59, 64). In the present study, a time-dependent increase of PGE\textsubscript{2} secretion in Colo320 cells transfected with the CCK-BRi4sv was observed after stimulation with gastrin-17 providing evidence that the gene expression was also induced at the protein level. Transcriptional regulation of COX-2 has been evaluated in multiple cell lines. Recent studies have indicated that expression of COX-2 in human colorectal carcinoma cells is regulated at the transcriptional, post-transcriptional, and protein level. Moreover, a number of signaling pathways are involved in the regulation of COX-2 expression in colorectal carcinoma cells. The regulation of COX-2 expression is linked to the MAPK pathway, oncogenic signaling pathways (such as Akt/PKB and Rho B) and the Wnt signaling transduction pathway and can cooperate with the ras signal transduction pathway. The MAPK pathways, ERK1/2, JNK, p38\textsuperscript{MAPK}, play a central role in the regulation of COX-2 expression in response to a variety of extracellular stimuli (1, 65). In the present investigation, the signaling cascade utilized by gastrin-17 was explored by Western blotting using antibodies directed against phosphorylation state-specific intracellular protein kinases of the MAPK pathways. In Colo320SV cells, phosphorylated p44/42\textsuperscript{MAPK}, phosphorylated p38\textsuperscript{MAPK} and phosphorylated JNK were significantly increased at 2.5, 5 and 2.5 minutes after gastrin treatment and remained stable for at least 20 minutes. These data suggest that gastrin can induce a time-dependent increase of the protein phosphorylation through the MAPK pathway in human colon cancer cells transfected with the CCK-BRi4sv.

Previous studies have reported that gastrin and nonsteroidal anti-inflammatory drugs possess opposing effects on cell proliferation. Gastrin has long been recognized as a mitogenic factor that stimulates the growth of preexisting tumours of gastrointestinal origin (12, 67). Interruption of the effects of gastrin as a potential target in the treatment of colorectal cancer using several different approaches such as the gastrin/CCK-B receptor antagonists proglumide and benzotript have been assessed (81, 83). Moreover, some results of these studies demonstrate that COX-2 might represent one of the downstream targets of gastrin and that selective COX-2 inhibition is capable of reversing the trophical properties of gastrin and presumably prevent growth of
colorectal cancer cell induced by hypergastrinaemia (89). In the current investigation, the effects of the selective inhibitors of ERK1/2 (PD98059), p38MAPK (SB202190) and JNK (dnJNK) were explored using a sensitive COX-2/luciferase reporter gene assay as functional read-out. The results demonstrate that COX-2 transcription is significantly inhibited following treatment with these inhibitors which act synergistically. Taken together, these data demonstrate that gastrin can induce the COX-2 promoter in human colon cancer cells transfected with the CCK-BRi4sv and that MAPK activation is required for this transactivation.

Deletions of the 5’ flanking region of the COX-2 promoter revealed a dramatic decrease of promoter activity when the 5’ flanking region was shortened from -230 bp to -68 bp indicating that critical regulatory DNA elements are located within this segment. The -230 bp to -68 bp spanning region of the promoter contains binding motifs for several transcription factors including AP2, SP1 and NF-κB. We therefore hypothesize that one of these transcription factors might be involved in the signal transduction activated by the CCK-BRi4sv receptor. Future studies using electromobility shift assays with labeled double-stranded oligonucleotide segments of this region will be required to ultimately identify the predominant transcription factor which binds to a given recognition motif upon gastrin-driven transactivation of the promoter.

To rule out activation of COX-2 transcription through low level endogenous CCK-B/gastrin receptor expression, our findings were confirmed in COS-7 cells which have no detectable endogenous CCK-B/gastrin receptor message and which have been proven to ideally serve this purpose (60). Although slightly different in magnitude, gastrin also was able to transactivate the COX-2 promoter in COS-7SV cells through previously described signaling pathways.

In summary, the findings of the current investigation indicate that 1) the CCK-BRi4sv transactivates the COX-2 promoter following stimulation with gastrin in two cell lines in vitro and 2) that multiple signaling pathways are required for full activation of the COX-2 promoter by gastrin. 3) The CCK-BRi4sv has neither proven to be superpotent when compared to the wild type CCK-BR nor to be constitutively active with respect to the transcriptional regulation of the COX-2 gene. However, the CCK-BRi4sv receptor is capable to mediate transactivation of the COX-2 promoter and is thus likely to confer oncogenic properties to colon cancer and nontumorigenic cells.