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Rapid tyrosine phosphorylation of focal adhesion kinase, paxillin, and p130^{Cas} by gastrin in human colon cancer cells

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Abstract

Although the expression of CCK_2 receptors is widely reported in human colorectal cancers, little is known on its role in mediating the proliferative effects of mature amidated gastrin (G17 amide) on colorectal cancers. The purpose of the present study was to determine the effects of G17 amide on tyrosine phosphorylation of focal adhesion kinase (FAK), paxillin, and p130 Crk-associated substrate (p130^{Cas}) in Colo 320 cells, a human colorectal cancer cell line which expresses CCK_2 receptors. By immunoprecipitation and immunoblotting, an increase in tyrosine phosphorylation of FAK (tyrosine-397), paxillin (tyrosine-31), and p130^{Cas} was detected in a time- and dose-dependent manner. Overexpression of CCK_2 receptors in Colo 320 cells (Colo 320 WT) by stable transfection with the human CCK_2 receptor cDNA resulted in an increased tyrosine phosphorylation of FAK, paxillin, and p130^{Cas}. After incubation with 1 μM L-365,260, a specific CCK_2 receptor antagonist, this increase was completely inhibited. Our results demonstrate that in human colon cancer cells, gastrin caused a rapid tyrosine phosphorylation of FAK, paxillin, and p130^{Cas} by activation of CCK_2 receptor. The phosphorylation of these proteins might be important in mediating gastrin effects on proliferation, apoptosis, and metastasis.

Keywords: Tyrosine phosphorylation; Colon cancer; Gastrin; CCK_2 receptor; G protein-coupled receptor; Signal transduction

1. Introduction

Gastrin, a peptide hormone and trophic factor, has long been recognized to stimulate mucosal growth in the upper digestive tract [1,2]. More recent findings suggest that gastrin has proliferative effects in colon cancer as well. Many studies have shown that exogenously administered gastrin stimulates the growth and proliferation of colon cancer cells in culture [3,4], transplanted colon tumors in mice [5], and carcinogen-induced tumors in rats [6]. In addition, antagonism of gastrin effects by antigastrin antisera [7,8], gastrin receptor antagonists [3,9,10], and antisense gastrin RNA [11] inhibited growth of colon cancer cells in culture and *in vivo* in animal models. The proliferative effect of gastrin has been shown to be mediated by the CCK_2 receptor in different cellular models [12,13] including cells transfected with the CCK_2 receptor cDNA [14]. This receptor which has been cloned from different species by several laboratories [15,16] belongs to the family of G protein-coupled receptors which are known to be linked to the phospholipase C/protein kinase C (PLC/PKC) signaling pathway. Gastrin-dependent activation of the CCK_2 receptors has been shown to induce phosphatidyl-inositol bisphosphate (PIP_2) hydrolysis by phopholipase C (PLC) that results in inositol triphosphate (IP_3) and diacylglycerol (DAG) production leading to intracellular Ca^{2+} mobilization and stimulation of PKC [17,18]. Gastrin, like many ligands that bind to G protein-coupled receptors, has also been shown to induce tyrosine kinase activity [19,20].

A rapid increase in the tyrosine phosphorylation of the non-receptor tyrosine kinase FAK and the adaptor proteins p130^{Cas} and paxillin, which localize to focal adhesion plaques, has been identified as a prominent early event in cells stimulated by diverse signaling molecules that regulate cell proliferation, migration, and apoptosis

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Abbreviations: CCK_2R, CCK_2 receptor (formerly CCK-B/gastrin receptor); FAK, focal adhesion kinase; G17 amide, mature amidated gastrin heptadecapeptide; GPCR, G protein-coupled receptor; p130^{Cas}, p130 Crk-associated substrate; PBS, phosphate buffered saline; PAGE, polyacrylamide gel electrophoresis; P-tyr, phosphotyrosine.

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[21,22]. These include CCK and other neuropeptides that act via GPCR [23,24], LPA and other bioactive lipids [25,26], and growth factors such as platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), and epidermal growth factor (EGF) [27–29]. Thus, the tyrosine phosphorylation of these focal adhesion proteins represents a point of convergence in the action of GPCR agonists, growth factors, integrins, and oncogenes [30].

It has been reported that activation of CCK_2 receptors stably transfected into mouse fibroblasts can cause tyrosine phosphorylation of FAK and paxillin [14,31], however, it is still unknown whether gastrin can cause tyrosine phosphorylation of FAK and paxillin in human cells, and especially in human cancer cells. In the present study, we have analyzed whether gastrin induces tyrosine phosphorylation of FAK and paxillin and $p130^{Cas}$ in a human colon cancer cell line, Colo 320, which expresses low levels of CCK_2 receptors. Meanwhile, three different clones of wild type CCK_2 receptor stably transfected Colo 320 cells (Colo 320 WT) were generated to confirm and amplify the effect of gastrin.

2. Materials and methods

Gastrin-17 amide was purchased from Bachem. The gastrin receptor antagonist L-365,260 was kindly provided by ML Laboratories. ^{125}I-CCK-8 was obtained from NEN. Rabbit polyclonal antibodies for FAK and paxillin, protein A agarose were obtained from Santa Cruz Biotechnology, mouse anti-phosphotyrosine monoclonal antibody (PY-20) and anti-$p130^{Cas}$ monoclonal antibody from Transduction Laboratories. The phosphospecific rabbit antibodies to Tyr-397 of anti-FAK and Tyr-31 of paxillin were purchased from BioSource International. Anti-mouse and anti-rabbit secondary antibody, ECL reagents were obtained from Amersham Pharmacia. Nitrocellulose membranes were from Invitrogen GmbH. All other reagents were obtained from Sigma. The rabbit antiserum against CCK_2 receptor was prepared as described by Schmitz *et al.* [32].

2.1. Cell culture

Stock culture of human colon cancer cell lines Colo 320 and SW787 were maintained at 37° in RPMI 1640 medium, supplemented with 10% fetal bovine serum in a humidified atmosphere containing 5% CO_2 and 95% air. For experimental purposes, cells were plated in 35 mm dishes at 1×10^5 cells/dish and grown in RPMI 1640 medium containing 10% fetal bovine serum for 5–7 days.

2.2. Cell transfection

Colo 320 cells were stably transfected with the wild type CCK_2 receptor cDNA cloned into the eukaryotic expression vector pCR3.1 (Invitrogen) using the Effectene™ reagent (Qiagen) according to the manufacturer's instructions. Following transfection, cells were seeded at very low density to obtain single cells in individual wells of 96-well plates and further expanded in the presence of 500 μg/mL G418. G418 resistant clones were screened for CCK_2 receptor expression by ^{125}I-CCK-8 radioligand binding, semi-quantitative RT-PCR, and immunoblotting as described below.

2.3. RNA extraction and reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from Colo 320 and Colo 320 WT using the RNeasy mini kit (Qiagen). First strand cDNA was synthesized from 1 μg total RNA using murine moloney leukemia virus (MuMLV) reverse transcriptase and the first strand cDNA synthesis kit from Promega (Promega) in a total volume of 20 μL. One microliter of each product was subjected to PCR amplification with 20 cycles of 95° for 30 s, 58° for 45 s, and 72° for 60 s using primers with following specific primer for the CCK_2 receptor: sense: 5′-AACCAGTGGGGCCTCGTGT-3′; antisense: 5′-GAAGCGCGTGGTGCGAATGGT-3′. The PCR products were visualized in ethidium bromide-stained agarose gels (1%).

2.4. Radioligand binding studies

Colo 320 and Colo 320 WT cells were split into 24-well dishes (5×10^4 cells/well). After 24 hr, competition binding experiments were performed in Hank's balanced salt solution supplemented with 25 mM HEPES (pH 7.3), 0.2% bovine serum albumin and 0.15 mM phenymethysulfonyfluoride. Twenty picomolar ^{125}I-CCK-8 (NEN) and increasing concentrations of unlabeled gastrin-17 amide (0.05–500 nM) were added into the binding buffer. After incubation for 80 min at 37°, cell monolayers were washed three times with Hank's balanced salt solution, hydrolyzed in 1 N NaOH, and bound radioactivity was quantified. All IC_{50} values reported represent data obtained from at least three independent experiments.

2.5. Stimulation and immunoprecipitation

Quiescent cultures of Colo 320 cells grown in 35 mm dishes (1×10^5 cells) were serum starved overnight and then treated with gastrin or CCK_2 receptor antagonist L-365,260. The stimulation was terminated on ice by aspirating the medium and solubilizing the cells in 1 mL of ice-cold RIPA buffer containing 1× PBS, 1% 1 pegal CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 10 mg/mL phenylmethylsulfonyl fluoride, 60 μg/mL aprotinin, 100 mM sodium orthovanadate.

Cell lysates were centrifuged at 10,000 *g* for 10 min. The supernatants were transferred into new micro-centrifuge tubes, and proteins were immunoprecipitated at 4° for at least 4 hr using specific antibodies and protein A

agarose. The precipitates were washed three times with ice-cold PBS buffer and subsequently solubilized in 2× SDS–polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer (128 mM Tris–HCl, pH 7.6, 4.6% SDS, 10% glycerol, 4% β-mercaptoethanol). Samples were boiled and resolved by 10% SDS–PAGE.

2.6. Immunoblotting

Following SDS–PAGE, proteins were transferred to nitrocellulose membranes. For detection of proteins, membranes were blocked using 5% nonfat dried milk in Tris buffer containing 0.1% Tween (TBS-T) and then incubated

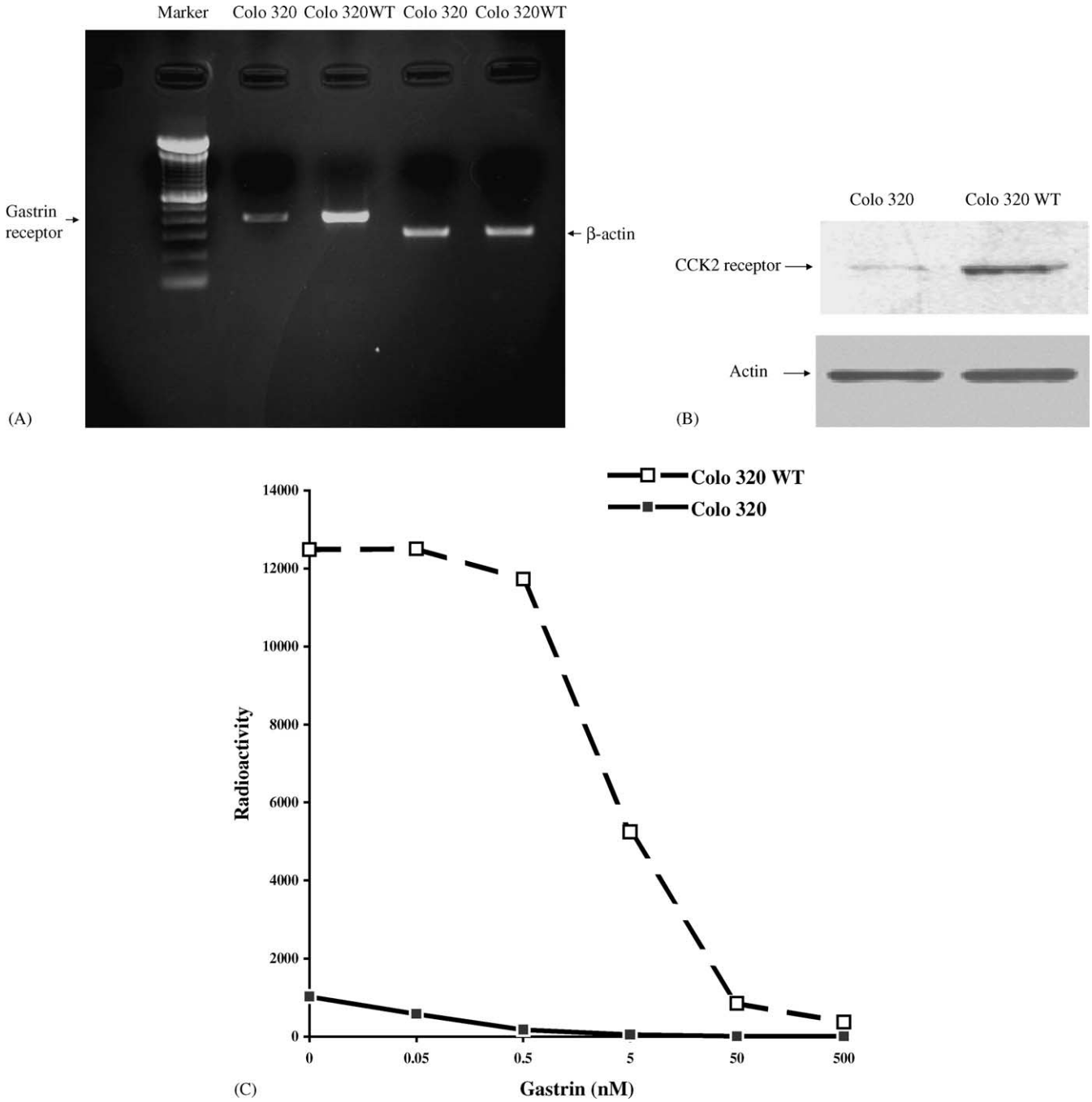
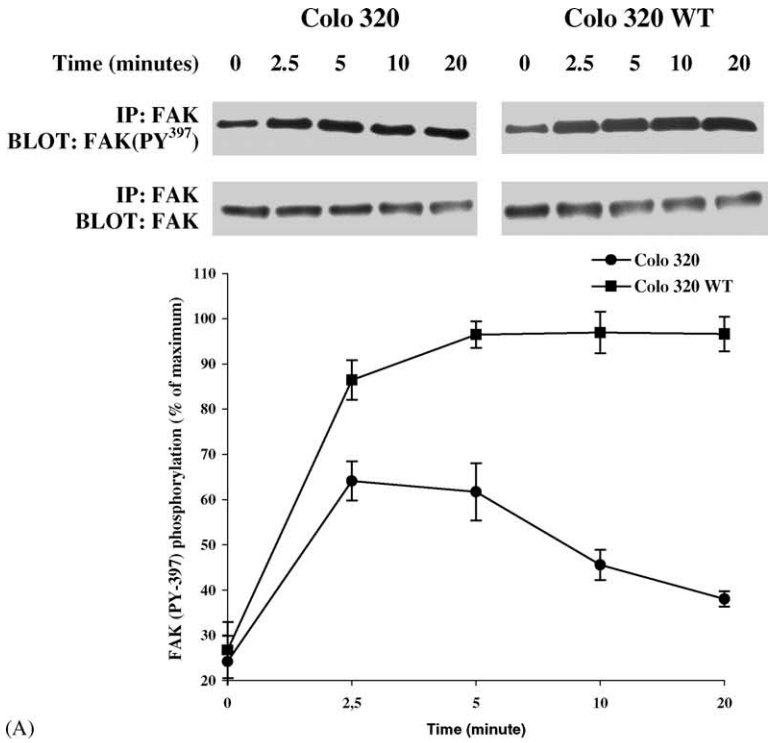


Fig. 1. The expression and receptor binding characteristics of CCK_2 receptor in Colo 320 and Colo 320 WT cells. (A) RT-PCR from Colo 320 and Colo 320 WT cells extracted RNA showing DNA amplified sequences with the expected size of 418 bp for gastrin receptor. The amplication of β-actin with the size of 318 bp was employed to show the equal amount of RNA. (B) Immunoblotting from Colo 320 and Colo 320 WT cells extracted protein showing CCK_2 receptor. Blotting result of β-actin was used to show the equal loading. (C) Radioligand binding studies in Colo 320 and Colo 320 WT cells. Competition binding experiments were performed in Hank's balanced salt solution supplemented with 25 mM HEPES (pH 7.3), 0.2% bovine serum albumin and 0.15 mM phenymethysulfonyfluoride. Twenty picomolar ^{125}I-CCK-8 (NEN) and increasing concentrations of unlabeled G17 amide (0.05–500 nM) were added into medium. After incubation for 80 min at 37°, cell monolayers were washed three times with Hank's balanced salt solution, hydrolyzed in 1 N NaOH, and bound radioactivity was quantified. Binding studies were performed three times in duplicates.



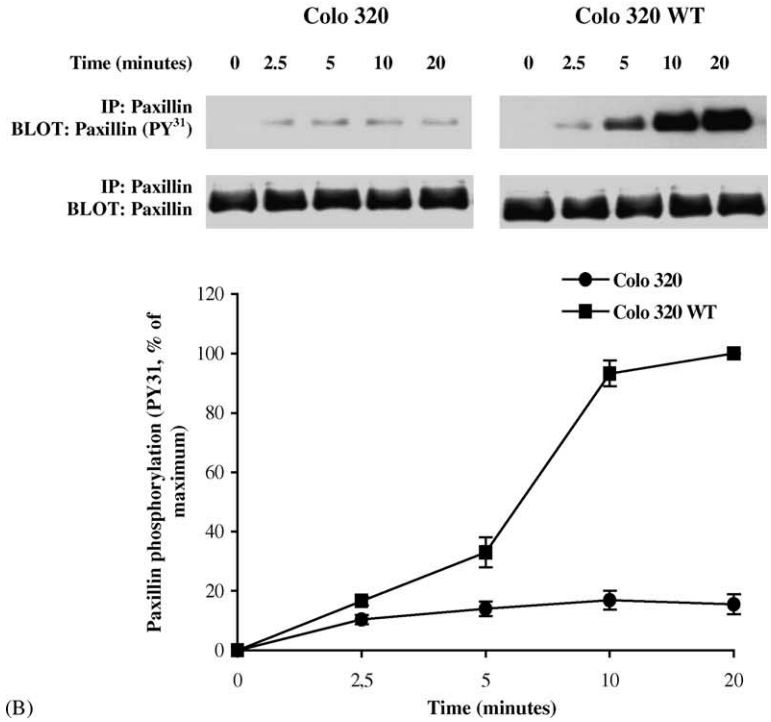


Fig. 2. Time-course of G17 amide stimulation of FAK, paxillin, and $p130^{Cas}$ phosphorylation in Colo 320 and Colo 320 WT cells. Colo 320 and Colo 320 WT cells are treated for various times as indicated with 10 nM G17 amide and then lysed. Whole cell lysates were immunoprecipitated by anti-FAK, paxillin, and phosphotyrosine polyclonal or monoclonal antibody and immunoblotted with anti-phospho-FAK (Tyr-397), FAK; phospho-paxillin, paxillin, and $p130^{Cas}$. The blotting results of FAK and paxillin were used to show equal loading. These results are representative of at least three independent experiments. Quantification of FAK phosphorylation at Tyr-397, paxillin phosphorylation at Tyr-31, and $p130^{Cas}$ phosphorylation was performed by densitometry. Values shown are the mean ± SD of at least three independent experiments and are expressed as the percentage of the maximal increase in FAK, paxillin and $p130^{Cas}$ phosphorylation value. The results for Colo 320 WT cells were obtained from three different clones.

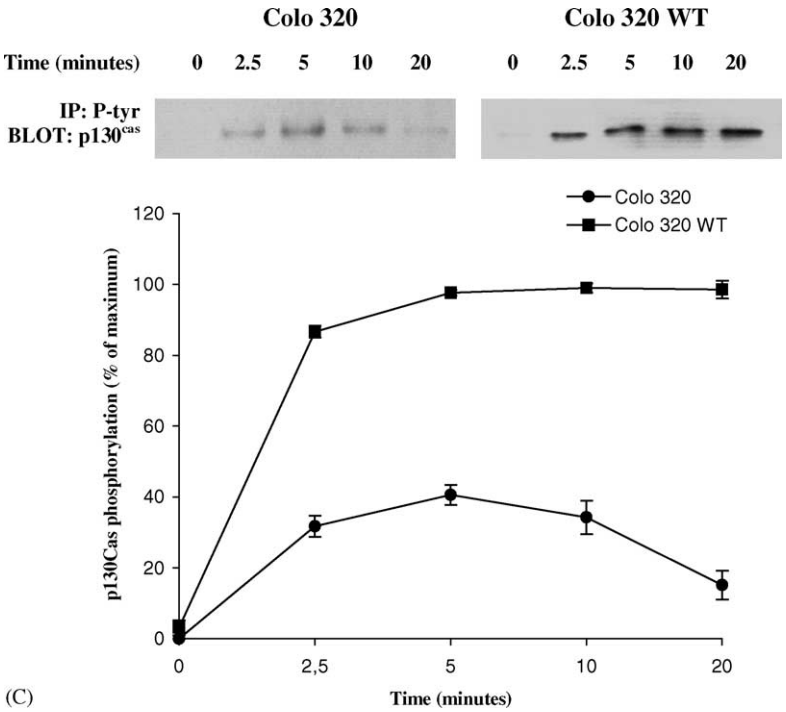


Fig. 2. (*Continued*).

at 4° overnight with specific antibodies diluted in TBS-T containing 5% non-fat milk. Bound primary antibodies to immunoreactive bands were visualized by enhanced chemoluminescence (ECL) detection with horseradish peroxidase conjugated anti-rabbit or anti-mouse antibodies.

2.7. Densitometry

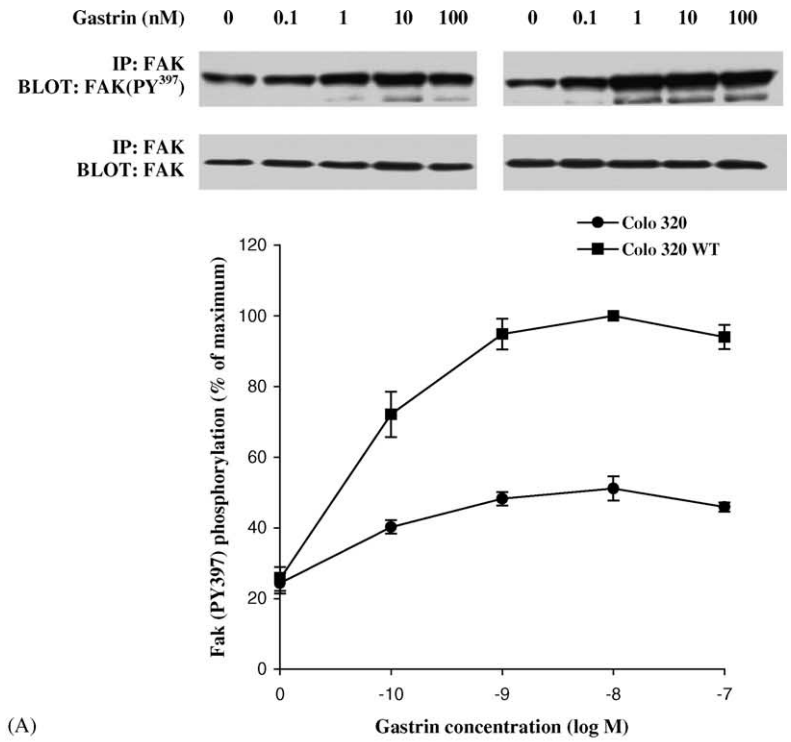
Autoluminograms were scanned using the BioDocAnalysis system (Biometra GmbH) and the software for quantity was provided by Biometra.

3. Results

The transfection efficiencies in Colo 320 and Colo 320 WT cells were evaluated by RT-PCR, immunoblotting, and radioligand binding assay. Although none of these methods is capable to discriminate between endogenous and transfected CCK_2 receptor expression, our results of RT-PCR and immunoblotting showed that Colo 320 cells expressed low levels of CCK_2 receptor mRNA and protein. Stable transfection with CCK_2 receptor cDNA led to a 4-fold overexpression of the CCK_2 receptor at the protein and message level (Fig. 1A and B). As shown in Fig. 2C, Colo 320 WT cells displayed significantly higher numbers of ^{125}I-CCK-8 binding sites. The IC_{50} values calculated for Colo 320 and Colo 320 WT cells were 0.19 ± 0.1 nM and 0.9 ± 0.1 nM, respectively, and not statistically significantly different. These results suggest that overexpression of CCK_2 receptors only increased the amount of CCK_2 receptor at the cell surface without altering the binding characteristics of the receptor protein.

To investigate whether the tyrosine kinase FAK can serve as a substrate for gastrin-stimulated tyrosine phosphorylation in human colon cancer cells, Colo 320 and Colo 320 WT cells were serum starved overnight and subsequently incubated with 10 nM G17 amide for 0, 2.5, 5, 10 and 20 min. The lysates of the gastrin-treated cells were immunoprecipitated with an anti-FAK polyclonal antibody (C-20) and the immunoprecipitates were analyzed by immunoblotting with a phosphospecific polyclonal rabbit antibodies directed against Tyr-397 of FAK. Figure 2A shows that G17 amide caused a rapid increase in tyrosine phosphorylation of FAK in Colo 320 cells with a maximum (1.7-fold increase) at 2.5 min followed by a gradual decrease after 5 min. In Colo 320 WT cells, G17 amide also induced a rapid but much more striking increase of FAK phosphorylation with a maximum (4-fold increase) at 5 min that was maintained for at least 5 hr (data not shown).

To determine whether gastrin also promotes tyrosine phosphorylation of the cytoskeleton-associated protein paxillin, cell lysates were immunoprecipitated with a polyclonal anti-paxillin antibody followed by immunoblotting with phosphospecific polyclonal rabbit antibodies to the Tyr-31 site of paxillin. Similar to its effect on FAK, G17 amide caused a striking increase in the amount of phosphorylated paxillin (Tyr-31) immunoreactivity in anti-paxillin immunoprecipitates of Colo 320 cells, which migrated as a broad, diffuse band at a M_r 72–87 kDa. The maximum was reached at 5 min and maintained for at least 20 min



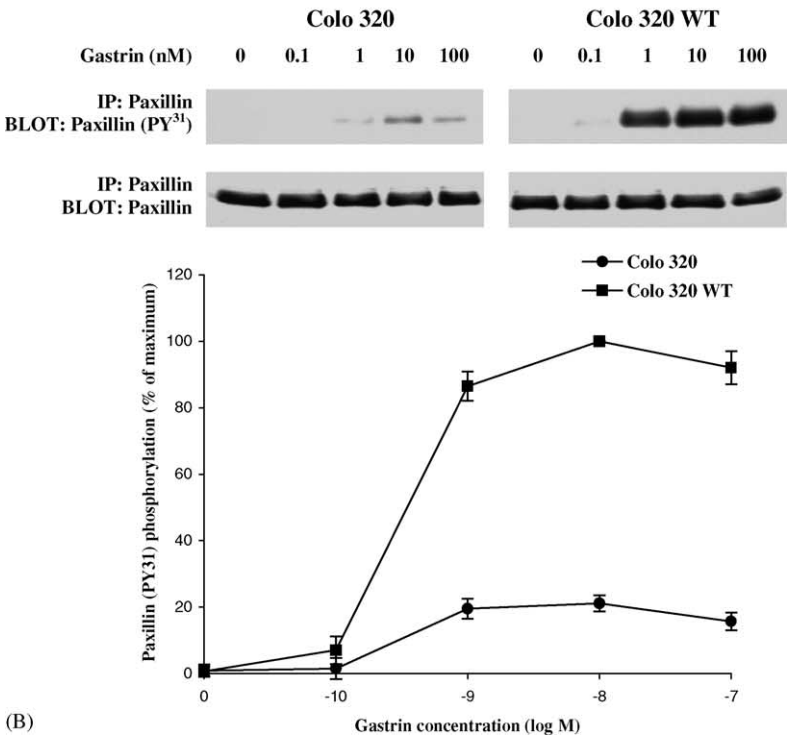


Fig. 3. Concentration-dependence of G17 amide stimulation of FAK (A), paxillin (B), and $p130^{Cas}$ (C) tyrosine phosphorylation in Colo 320 and Colo 320 WT cells. Colo 320 and Colo 320 WT cells are treated with various concentrations of gastrin-17 as indicated for 5 min and then lysed. FAK, paxillin, and $p130^{Cas}$ tyrosine phosphorylation were determined by immunoprecipitation and immunoblotting as described in the legend to Fig. 2. The results were from experiments with no addition or with various concentrations of gastrin-17. Values shown are the mean ± SD of at least three independent experiments and are expressed as the percentage of the maximal increase in FAK, paxillin and $p130^{Cas}$ phosphorylation. The results for Colo 320 WT cells were obtained from three different clones.

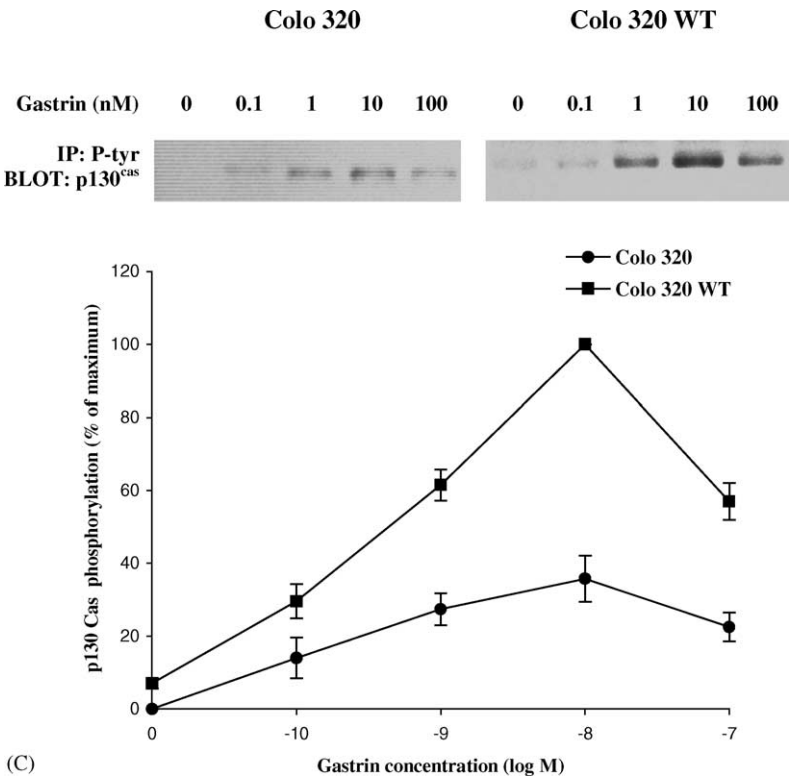


Fig. 3. (*Continued*).

(Fig. 2B). Overexpression of CCK_2 receptors significantly increased the effect of gastrin on paxillin phosphorylation (Fig. 2B), and the elevated phosphorylation persisted even after 5 hr (data not shown).

Focal adhesion protein $p130^{Cas}$ is a potential target for FAK and functions as adaptor protein in signal transduction. To detect the effect of gastrin on phosphorylation of $p130^{Cas}$, cell lysates were immunoprecipitated by anti-phosphotyroine antibody and immunoblotted with an anti-$p130^{Cas}$ antibody. In Colo 320 cells, an elevated tyrosine phosphorylation of $p130^{Cas}$ was detected in anti-phosphotyroine immunoprecipitates. Figure 2C shows weak signals at a M_r of 130 kDa which appeared at 2, 5, 10 min, and disappeared after 20 min. In Colo 320 WT cells, prominent signals at a M_r of 130 kDa were detected at 2.5 min which remained stable for more than 20 min. As we immunoprecipitated with an anti-phosphotyroine antibody and Western blotted with an antibody directed against $p130^{Cas}$, we cannot ultimately rule out that there is some enrichment of tyrosine phosphorylated complexes including $p130^{Cas}$. However, our data clearly indicate that $p130^{Cas}$ phosphorylation is under the regulatory control of gastrin in our model.

The effect of G17 amide on tyrosine phosphorylation of FAK, paxillin and $p130^{Cas}$ was concentration-dependent and the concentration-dependence was biphasic (Fig. 3). From 0 to 10 nM, the effect of G17 amide on tyrosine phosphorylation of FAK, paxillin, and $p130^{Cas}$ gradually increased and the maximal effect occurred at 10 nM G17 amide both in Colo 320 and Colo 320 WT cells. A further increase of the G17 amide concentration resulted in a decrease of the maximal tyrosine phosphorylation of FAK, paxillin and $p130^{Cas}$.

To further characterize the effect of gastrin on tyrosine phosphorylation of FAK, paxillin and $p130^{Cas}$, serum starved Colo 320 and Colo 320 WT cells were pre-incubated with or without the specific CCK_2 receptor antagonist L-365,260 for 30 min, followed by a treatment with different concentrations of G17 amide (0.1, 10 nM) for 2.5 and 5 min, respectively. Cell lysates were immunoprecipitated and immunoblotted with phosphorylation state-specific antibodies as described above. As shown in Fig. 3, 1 μM L-365,260 alone had no effect on tyrosine phosphorylation of FAK, paxillin, and $p130^{Cas}$, but significantly inhibited FAK (Fig. 4A, Colo 320 lanes 4, 6; Colo 320 WT lanes 4, 6), paxillin (Fig. 4B, Colo 320 lanes 4, 6; Colo 320 WT lanes 4, 6) and $p130^{Cas}$ (Fig. 4C Colo 320 lanes 4, 6; Colo 320 WT lanes 4, 6) tyrosine phosphorylation in response to G17 amide.

To exclude the possibility that the vector itself mediated the G17 amide-stimulated phosphorylation of FAK, paxillin, and $p130^{Cas}$, Colo 320 cells were transfected temporarily by pCR3.1 vector and then pre-incubated with or without various concentrations of G17 amide. Cell lysates were immunoprecipitated and immunoblotted with phosphorylation state-specific antibodies as described above. The vector pCR3.1 did not induce any phosphorylation of FAK, paxillin, and $p130^{Cas}$ in Colo 320 cells. In pCR3.1 vector transfected Colo 320 cells,

G17 amide stimulated the same content of increase on phosphorylation of FAK, paxillin, and $p130^{Cas}$ as those without transfection.

In the present study, the effect of G17 amide on phosphorylation of FAK, paxillin, and $p130^{Cas}$ was also examined in a colorectal cancer cell line—SW787, in which no expression of CCK_2 receptor can be detected by RT-PCR or immunoblotting. Our results showed that G17 amide did not affect the phosphorylation of FAK, paxillin, and $p130^{Cas}$ in SW787 cells.

4. Discussion

Tyrosine phosphorylations are believed to play a role in the regulation of cellular growth induced by multiple receptors, such as tyrosine kinase receptors, receptor coupled to cytosolic tyrosine kinase (growth hormone and cytokine receptors) as well as G protein-coupled receptors.

In the present study, we demonstrate for the first time that G17 amide causes time- and concentration-dependent tyrosine phosphorylation of FAK (tyrosine-397), paxillin (tyrosine-31), and $p130^{Cas}$ in human colon cancer cells (Colo 320). Furthermore, we show that overexpression of the CCK_2 receptor can enhance the effect of G17 amide on phosphorylation of FAK, paxillin, and $p130^{Cas}$. Our observations that (i) a specific CCK_2 receptor inhibitor blocks the effect of G17 amide and that (ii) G17 amide does not affect the phosphorylation of FAK, paxillin, and $p130^{Cas}$ in a CCK_2 negative cell line suggest that CCK_2 receptor mediates gastrin-induced phosphorylation of FAK, paxillin, and $p130^{Cas}$.

The cellular function caused by the tyrosine phosphorylation of FAK, paxillin and $p130^{Cas}$ in human colon cancer cells has not been addressed in this study. In other cell lines, FAK, paxillin and $p130^{Cas}$ were reported to function as regulator of cell motility [33,34], which is related to invasion and metastasis of human cancer.

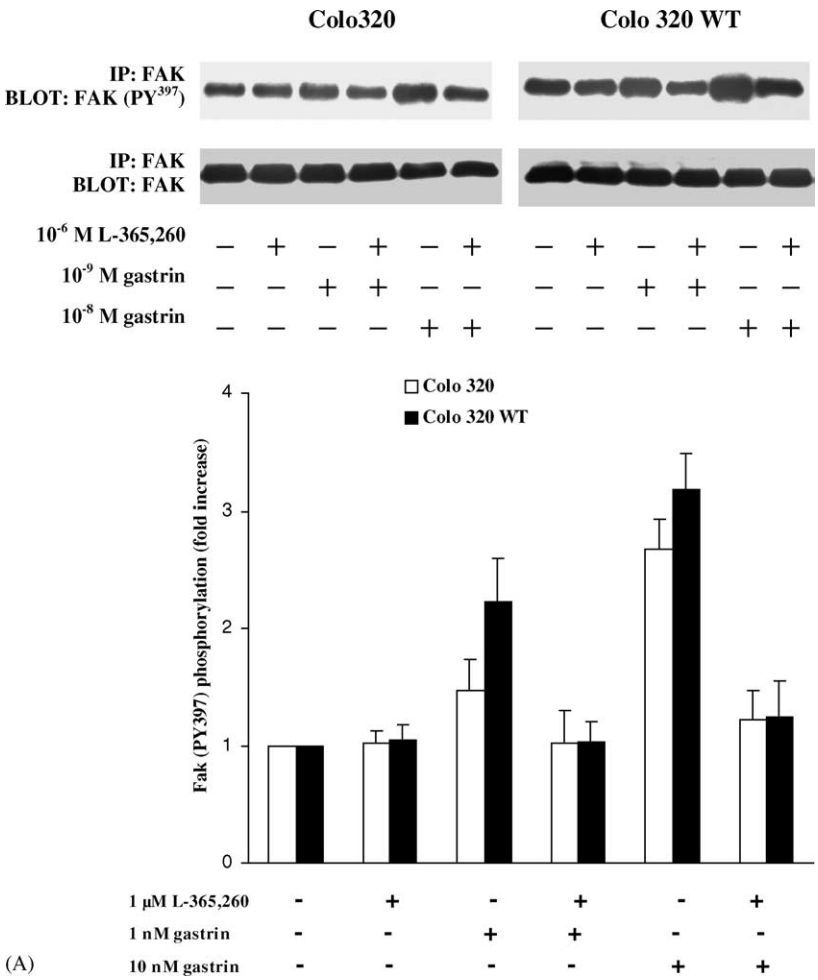


Fig. 4. Effect of the gastrin/CCK_2 receptor antagonist, L-365,260, on G17 amide stimulated FAK, paxillin, and $p130^{Cas}$ tyrosine phosphorylation in Colo 320 and Colo 320 WT cells. Colo 320 and Colo 320 WT cells were treated with various concentrations of G17 amide as indicated for 5 min with or without preincubation with gastrin/CCK_2 receptor antagonist, L-365,260 for 30 min and then lysed. FAK, paxillin, and $p130^{Cas}$ tyrosine phosphorylation were determined by immunoprecipitation and immunoblotting as described in Section 2. Values shown are the mean ± SD of at least three independent experiments and are expressed as the fold of the increase in FAK, paxillin and $p130^{Cas}$ phosphorylation. The results for Colo 320 WT cells were obtained from three different clones.

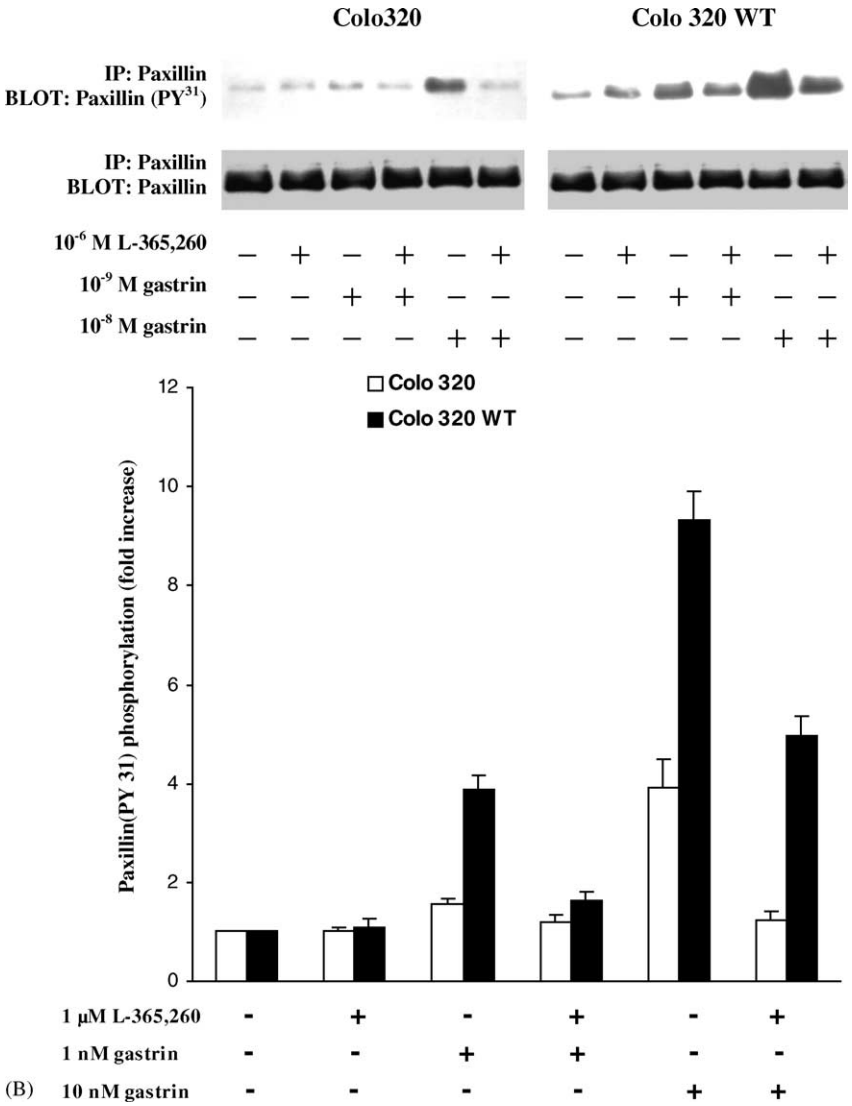


Fig. 4. (*Continued*)

Phosphorylation of FAK at the site tyrosine-397 can enhance cell motility by association with the SH2/SH3-adaptor protein Crk, inducing $p130^{Cas}$ phosphorylation and formation of $p130^{Cas}$/Crk [35,36]. Therefore, FAK/Src-dependent activation of the $p130^{Cas}$/Crk signaling complex is a central feature of both matrix and growth factor-induced cell migration. Meanwhile, some investigators also show that phosphorylation of the cytoskeletal protein paxillin at the tyrosine-31 site increases cell motility by formation of paxillin/Crk complexes [37].

FAK has also been proposed to function as a positive regulator of cell growth. Overexpression of FAK can modestly enhance DNA synthesis in fibroblasts after the cells have been starved and stimulated with serum [38,39]. Conversely, mutants of FAK can function in a dominant negative fashion to inhibit progression through the cell cycle following serum starvation [38,40]. There is some evidence that these effects correlate with expression of cyclin D1 and the cyclin-dependent kinase inhibitor p21 [38]. Furthermore, these FAK-mediated cell cycle effects are dependent on phosphorylation of tyrosine-397 and are believed to require Src and/or PI3-kinase binding to this site. In addition, FAK is predicted to mediate integrin-dependent regulation of the ERK family of MAP kinases through several pathways [41], and these pathways are suggested to act synergistically with mitogenic signaling pathways to regulate cell growth [42,43]. However, the mechanism of FAK function in controlling cell cycle progression has not been completely elucidated yet.

Several studies demonstrate a role for phosphorylation of FAK in promotion of cell survival. FAK has been shown to play an important role in the cell survival of anchorage-dependent cells [44]. Proteolytic cleavage of FAK by caspase-3 has been reported during growth factor deprivation-induced apoptosis in human umbilical vein endothelial cells [45], which implies an association between FAK and apoptosis. FAK was also found to be tyrosine-phosphorylated

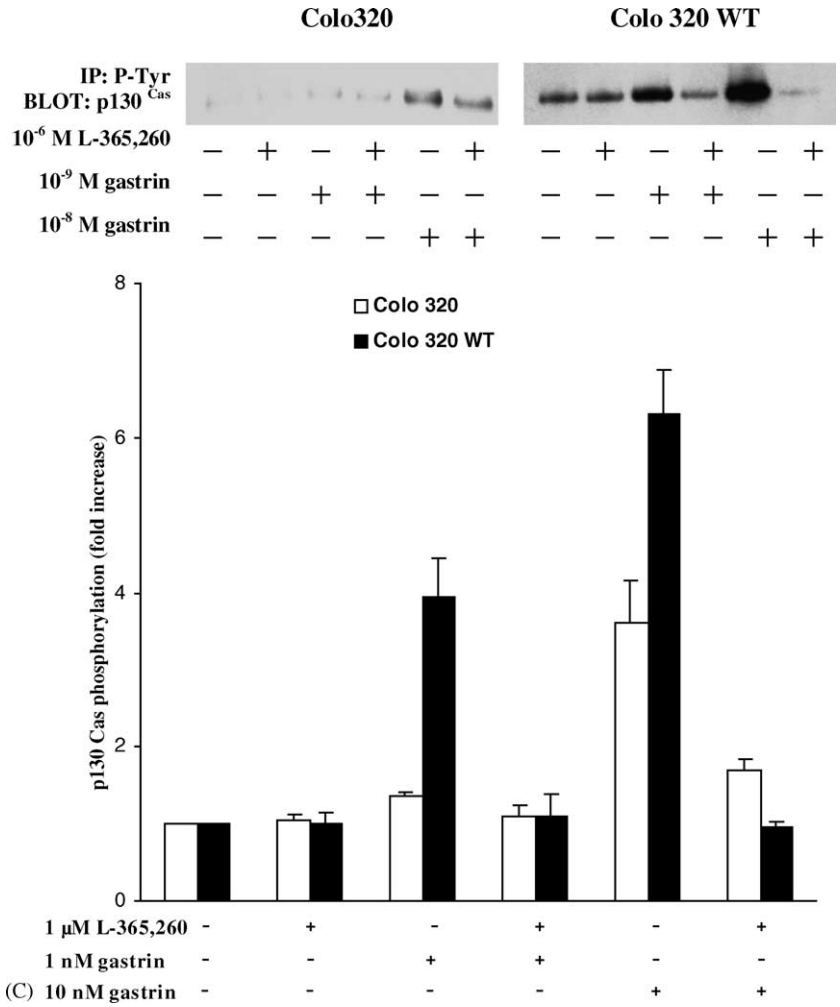


Fig. 4. (*Continued*).

by oxidative stress before apoptosis occurred [46]. Furthermore, protein kinase B/Akt, which has been implicated in the pathway of survival signal, was serine-phosphorylated following tyrosine phosphorylation of FAK. FAK phosphorylation at tyrosine-397 is required to prevent apoptosis, because tyrosine-397 is an autophosphorylation site and a high-affinity binding site for Src homology 2 domains of Src family kinases [47]. PI3-kinase and phospholipase C also interact with this site [48].

In conclusion, our results clearly demonstrate that activation of CCK_2 receptors by gastrin induces phosphorylation of the non-receptor tyrosine kinase FAK, the cytoskeletal protein paxillin, and the adaptor protein $p130^{Cas}$ in human colon cancer cells. These findings indicate that FAK, paxillin, and $p130^{Cas}$ could serve as pharmacological downstream targets of gastrin-associated growth mediated by CCK_2 receptors during metastasis, proliferation and apoptosis in colon cancer cells. These findings also suggest a new approach to explore the potential role of gastrin and its receptor in human colon cancer. An important task in the future will be to elucidate the interactions between FAK, paxillin, and $p130^{Cas}$, to close the gap between G protein binding and FAK phosphorylation and to define the cross-talks and synergistic interactions between the tyrosine and serine/threonine phosphorylation cascades in the promotion of biologic responses stimulated by gastrin.

Acknowledgments

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