Metalloproteinase-dependent Transforming Growth Factor-α Release Mediates Neurotensin-stimulated MAP Kinase Activation in Human Colonic Epithelial Cells*

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Expression of the neuropeptide neurotensin (NT) and its high affinity receptor (NTR1) is increased during the course of Clostridium difficile toxin A-induced acute colitis, and NTR1 antagonism attenuates the severity of toxin A-induced inflammation. We recently demonstrated in non-transformed human colonic epithelial NCM460 cells that NT treatment caused activation of a Ras-mediated MAP kinase pathway that significantly contributes to NT-induced interleukin-8 (IL-8) secretion. Here we used NCM460 cells, which normally express low levels of NTR1, and NCM460 cells stably transfected with NTR1 to identify the upstream signaling molecules involved in NT-NTR1-mediated MAP kinase activation. We found that inhibition of the epidermal growth factor receptor (EGFR) by either an EGFR neutralizing antibody or by its specific inhibitor AG1478 (0.2 μM) blocked NT-induced MAP kinase activation. Moreover, NT stimulated tyrosine phosphorylation of the EGFR, and pretreatment with a broad spectrum metalloproteinase inhibitor batimastat reduced NT-induced MAP kinase activation. Using neutralizing antibodies against the EGFR ligands EGF, heparin-binding-EGF, transforming growth factor-α (TGFα), or amphiregulin we have shown that only the anti-TGFα antibody significantly decreases NT-induced phosphorylation of EGFR and MAP kinases. Furthermore, inhibition of the EGFR receptor by AG1478 significantly reduced NT-induced IL-8 promoter activity and IL-8 secretion. This is the first report demonstrating that NT binding to NTR1 transactivates the EGFR and that this response is linked to NT-mediated proinflammatory signaling. Our findings indicate that matrix metalloproteinase-mediated release of TGFα and subsequent EGFR transactivation triggers a NT-mediated MAP kinase pathway that leads to IL-8 gene expression in human colonic epithelial cells.

NT-stimulated MAP kinase activation in human colonic epithelial cells may contribute to NT-induced interleukin-8 secretion. Our study also suggests that inhibition of the EGFR pathway by AG1478 may provide a therapeutic strategy to prevent colitis. Further experiments are needed to determine the specific role of EGFR in NT-induced MAP kinase activation.

Neurotensin (NT), a 13-amino acid neuropeptide originally isolated by Carraway and Leeman (1) from bovine hypothala-...
has been shown to mediate MAP kinase activation by numerous G protein-coupled receptors such as endothelin-1, lysophosphatidic acid, thrombin (28), substance P (29), and bradykinin (30), as well as by exposure of target cells to the pathogenic bacterium Helicobacter pylori (31) and to dermatoeectoxic toxin produced by Pasteurella multocida (32). Transactivation of EGFR receptors by several GPCRs involves metalloproteinase-dependent cleavage of either proHB-EGF (33–35), TGFα (36), or amphiregulin (35, 37). We report here that NTR1 engagement leads to EGFR receptor tyrosine phosphorylation, which is responsible for ERK1/2 activation in NT-treated non-transformed human colonic epithelial NCM460 cells. Our results also indicate that the mechanism of NT-induced EGFR receptor transactivation involves metalloproteinase-dependent TGFα release.

MATERIALS AND METHODS

Cell Lines and Reagents—Non-transformed human colonic epithelial cells NCM460 and NTR1-overexpressed NCM460 cells (NCM460-NTR1) have been described previously (20). NT was purchased from Phoenix Pharmaceuticals (Belmont, CA). Cell culture medium M3D for NCM460 and NCM460-NTR1 cells was obtained from INCELL Corp. (San Antonio, TX). Antibodies against total and phosphorylated EGF receptor were from Santa Cruz Biotechnology (Santa Cruz, CA). HB-EGF, TGFα, amphiregulin, and their respective neutralizing antibodies were from R&D Systems (Minneapolis, MN). EGFR and EGFR receptor neutralizing antibody were from Sigma and Upstate Biotechnology (Charlottesville, VA). AG1478 and pertussis toxin were from Calbiochem and batimastat (BB94) from British Biotech (Oxford, UK).

Western Blot Analysis—Cells were washed twice with ice-cold phosphate-buffered saline and then incubated in radioimmune precipitation assay buffer containing a protease inhibitor mixture (Roche Applied Science) for 10 min. Cell lysates were centrifuged at 10000 × g for 10 min. Equal amounts of cell extracts were separated by SDS-PAGE (10%), and proteins were transferred onto nitrocellulose membranes (Bio-Rad Laboratories) at 400 mA for 2 h at 4 °C. Membranes were blocked in 5% nonfat dried milk in TBST (50 mM Tris, pH 7.5, 0.15 M NaCl, 0.05% Tween 20) and then incubated with primary antibody overnight at 4°C. Membranes were washed with TBST and incubated with horseradish peroxidase-labeled secondary antibodies for 1 h. Peroxidase activity was detected by SuperSignal chemiluminescent substrate (Pierce).

TGFα Measurements—The levels of TGFα in conditioned media were determined by enzyme-linked immunosorbent assay. Briefly, 96-well Nunc immunoplates (Fisher Scientific) were coated with 0.4 μg/ml TGFα capture antibody (R&D Systems) diluted in phosphate-buffered saline overnight at room temperature, washed with phosphate-buffered saline containing 0.1% Tween 20 (PBST) three times, and then incubated with phosphate-buffered saline containing 2% bovine serum albumin for 1 h. After washing once with PBST, each well was incubated with 100 μl of conditioned media or TGFα standard serially diluted in M3D media for 2 h at room temperature. Wells were washed, incubated with 0.5 μg/ml biotin-conjugated anti-TGFα antibodies (R&D Systems) for 60 min, washed three times, and then incubated with streptavidin-horseradish peroxidase (1:2,000 dilution, Amersham Biosciences) for 30 min. Peroxidase activity was detected by TMB peroxidase substrate reagents (KPL Inc., Gaithersburg, MD).

 Luciferase Reporter Assay—A reporter construct containing 1521 bp (nucleotides 1481 to +49) of the IL-8 promoter region of the human IL-8 gene was used to measure transcription of the IL-8 gene as described previously (20). To determine the IL-8 promoter activity in response to NT, cells were seeded in 12-well plates (0.2 × 10⁶ cells/well) overnight and transiently transfected using Effectene transfection reagent (Qiagen) with IL-8 promoter luciferase constructs or a control luciferase construct pRL-TK (Promega) or other DNA constructs as indicated. Transfected cells were serum-starved for 24 h and then treated with the indicated pharmacological inhibitors followed by exposure to NT for 4 h. Firefly and Renilla luciferase activities in cell extracts were measured using the dual-luciferase reporter assay system (Promega, Madison, WI). The relative luciferase activity was then calculated by normalizing IL-8 promoter-driven firefly luciferase activity to control Renilla luciferase activity. Data from all experiments are presented as the relative luciferase activity (mean ± S.E.) from at least two independent sets of experiments, each with triplicate measurements.

Statistical Analyses—Results were expressed as means ± S.E. Data were analyzed using the SIGMA-STAT™ professional statistics software program (Jandel Scientific Software, San Rafael, CA). Analyses of variance with the protected t test were used for intergroup comparison.

RESULTS

NT Induces Tyrosine Phosphorylation of the EGFR Receptor—To determine whether NT can activate the EGFR receptor, NCM460 and NCM460-NTR1 cells were treated with NT (10⁻⁷ M) for various times. Equal amounts of cell lysates were subjected to Western blot analysis using the polyclonal antibodies against phosphorylated EGFR receptor and total EGFR receptor. The results show that NT induced phosphorylation of the EGFR receptor with a maximal level at 2 min after stimulation (Fig. 1A). To examine whether NT induces phosphorylation of the
EGF receptor in a dose-dependent manner, quiescent NCM460 cells were treated with NT (10^{-10} to 10^{-6} M) for 2 min. Equal amounts of cell proteins were used to measure EGF receptor phosphorylation as described above. The data show that NT activates phosphorylation in a concentration-dependent manner with a maximal stimulation at a concentration of 10^{-6} M (Fig. 2A). However, concentrations of 10^{-8} and 10^{-9} M were also able to induce this response (Fig. 1B).

NT is known to bind to three receptors including the high affinity GPCR, NTR1, and two low affinity receptors (one GPCR and another intracellular sortilin) (16). We have previously shown that NCM460 cells express high affinity receptor NTR1, which mediates NT-induced ERK1/2 activation (20). To further confirm that NT-induced EGFR phosphorylation is also mediated by NTR1, NCM460 cells were pretreated with a specific NTR1 antagonist SB48692 (1 μM) and then treated with NT (10^{-7} M) for 2 min. The data show that the NTR1 antagonist almost completely inhibited NT-induced EGF receptor phosphorylation, indicating that this receptor mediates NT-induced EGF receptor activation in these cells (Fig. 1C).

It was previously shown that NT-induced ERK1/2 activation was partially inhibited by pretreatment with pertussis toxin (PTX), which selectively blocks the Goα subfamily of heterotrimeric G proteins in NTR1-transfected Chinese hamster ovary cells (25). To examine whether Goα G proteins are also involved in NT-induced ERK1/2 activation in NCM460 cells, NCM460 or NCM460-NTR1 cells were pretreated with PTX (100 ng/ml) overnight and then stimulated with NT (10^{-7} M) or lysophosphatidic acid (LPA, 25 μM) for 5 min. LPA was used as a positive control because it is known to mediate ERK1/2 activation primarily through PTX-sensitive Goα proteins in several cell types (38, 39). Our results indicate that LPA-pretreated ERK1/2 activation was almost completely inhibited by PTX (Fig. 1D). However, PTX pretreatment had no effect on NT-induced ERK1/2 activation in both NCM460 and NCM460-NTR1 cells (Fig. 1D), indicating that the Goα proteins are not required in this NT response.

Inhibition of EGFR Activation Blocks NT-induced ERK Phosphorylation—Although it is known that EGF receptor activation triggers the Ras-Raf-1-MEK-ERK cascade, whether this pathway is involved in NT-induced ERK1/2 activation remains to be determined. Two separate approaches were used to examine this possibility. First, quiescent cells were pretreated with different concentrations of AG1478, a specific pharmacologic inhibitor of the EGF receptor, for 30 min and stimulated with NT (10^{-7} M) for 10 min. Equal amounts of cell lysates were subjected to Western blot analysis using a monoclonal antibody directed against dual phospho-ERK1/2. To confirm equal protein loading, a polyclonal antibody against total ERK1/2 was also used. We found that low concentrations of AG1478 (up to 0.1 μM) almost completely inhibited NT-induced ERK1/2 activation (Fig. 2A). Next, we used an EGF receptor neutralizing antibody to further examine EGF receptor involvement in NT-induced ERK1/2 activation. First, we confirmed that phosphorylation of the EGF receptor in response to NT (10^{-7} M, 2 min), EGF (10 ng/ml, 2 min), or TGFGα (10 ng/ml, 2 min) was blocked by pretreatment with the EGF receptor neutralizing antibody (20 μg/ml) but not by equal amounts of a control antibody (Fig. 2B). To determine whether this neutralizing antibody inhibits NT-induced ERK1/2 activation, cells were pretreated for 30 min with two different doses of the EGF receptor neutralizing antibody or a control antibody and stimulated with NT (10^{-7} M) for 10 min. The results showed that pretreatment with the EGF receptor neutralizing antibody significantly reduced NT-induced ERK1/2 activation in both parental NCM460 cells and NCM-NTR1 cells (Fig. 2C). Taken together, these findings indicate that both extracellular ligand binding and intracellular kinase activity of the EGF receptor are required for NT-induced ERK1/2 activation.

TGFA Release Is Involved in NT-induced ERK Activation—Our EGF receptor neutralization experiments described above (Fig. 2) also suggested that NT-induced ERK1/2 activation involves an extracellular ligand of the EGF receptor. It is known that the EGF receptor can be bound and activated by four different ligands: EGF, HB-EGF, TGFA, and amphiereglin. To identify the specific EGF receptor ligand(s) involved in NTR1 signaling, we pretreated cells for 30 min with neutralizing antibodies against EGF (20 μg/ml), HB-EGF (20 μg/ml), amphiereglin (20 μg/ml), or TGFA (10 and 20 μg/ml) or their respective control antibodies at the same concentration followed by stimulation with NT (10^{-7} M, 10 min) EGF (10 ng/ml, 5 min), HB-EGF (10 ng/ml, 5 min), or TGFA (10 ng/ml, 5 min). Our results indicate that pretreatment with neutralizing antibodies against either EGF, HB-EGF, or amphiereglin had no effect on NT-induced ERK1/2 activation (Fig. 3A), whereas TGFA antibody pretreatment significantly reduced NT-induced ERK1/2 activation (Fig. 3B). Next we examined whether NT stimulates TGFA release. Quiescent cells were treated with NT (10^{-7} M) for the indicated times, and equal amounts of conditioned media were used to measure TGFA concentrations by enzyme-linked immunosorbent assay. The results showed that in parental NCM460 cells, NT significantly increased the release of TGFA into the extracellular space 2 min after NT treatment (Fig. 4A). However, no significant increase in TGFA levels was noted after 5 and 10 min of NT exposure (Fig. 4A). In transfected NCM460-NTR1 cells, which express higher levels of NTR1, NT also induced TGFA release 2 min after NT administration to levels comparable with the parental NCM460 cells (Fig. 4B). However, in contrast to the parental cells, TGFA levels in the conditioned media continued to rise up to 10 min after NT exposure (Fig. 4B). Taken together, our results demonstrate that NT-induced TGFA release is at least in part, responsible for NT-induced ERK1/2 activation.

Metalloproteinase Activity Is Required for NT-induced TGFA Release and Activation of the EGF Receptor and ERK—Because several G protein-coupled receptors activate the EGF receptor, it was unknown whether metalloproteinase-mediated cleavage of precursors of the EGF receptor ligands, we examined whether metalloproteinase activity is required for NT-induced tyrosine phosphorylation of the EGF receptor. Cells were pretreated with AG1478 or batimastat, a broad spectrum metalloproteinase inhibitor, for 30 min and stimulated with NT (10^{-7} M) for 2 min. Equal amounts of cell proteins were used to determine tyrosine phosphorylation as described under “Materials and Methods.” The data show that pretreatment with AG1478 or batimastat blocked NT-induced activation of the EGF receptor (Fig. 5A). Cells were also pretreated with batimastat for 30 min and treated with NT for 10 min, and equal amounts of cell proteins were subjected to Western blot analysis using a dual phospho-ERK-specific antibody. Our results demonstrate that batimastat significantly reduced NT-induced ERK activation (Fig. 5B).

To further examine whether the effect of the MMP inhibitor on NT-induced activation of the EGF receptor and ERK is because of inhibition of TGFA release, cells were pretreated with batimastat for 30 min and treated with NT for 10 min. Equal volumes of the conditioned media were used to determine TGFA concentration by enzyme-linked immunosorbent assay. The results indicate that pretreatment with batimastat significantly inhibited NT-induced TGFA release in the NCM460 (Fig. 6A) and NCM460-NTR1 cells (Fig. 6B) to similar levels. Our results also show that in the parental NCM460 cells
basal levels of TGFα are higher than those in the transfected NCM460 cells (Fig. 6). Because TGFα levels were measured as pg/ml of cell supernatant, this difference may reflect a higher cell number used in the experiments with NCM460 cells versus transfected NCM460-NTR1 cells.

Effects of Batimastat and AG1478 on NT-induced IL-8 Promoter Activity—Previously we showed that inhibition of ERK1/2 activation reduced NT-induced IL-8 promoter activity (20). To further examine whether NT-induced activation of MMPs and the EGF receptor is involved in NT-induced IL-8 gene expression, cells were first transiently transfected with an IL-8 promoter-luciferase reporter construct together with an internal control plasmid. Cells were then rendered quiescent, pretreated with batimastat or AG1478 for 30 min, and then treated with NT for 4 h. Equal amounts of cell extracts were used to measure luciferase activity as described under “Materials and Methods,” and IL-8 promoter activity was normalized to equal control luciferase activity. Our results showed that pretreatment with AG1478 or batimastat significantly reduced NT-induced IL-8 promoter activity (Fig. 7).

DISCUSSION

It is well established that NT stimulates MAP kinase phosphorylation and activation in several cell types, including non-transformed and transformed human colonic epithelial cells (20, 25), as well as in pancreatic carcinoma cell lines (25, 26). MAP kinase activation also plays an important role in NT-induced proinflammatory cytokine gene expression in human...
colonic epithelial cells (20) and cell proliferation in the pancreatic carcinoma cell line, PANC-1 (26). However, the molecular mechanisms by which NT activates MAP kinase appear to be different in different cell types. In NCM460 cells, NT-induced MAP kinase activation is dependent on the small GTPase, Ras (20), whereas in PANC-1 cells, MAP kinase activation is mediated by protein kinase C (26). In this current study, we demonstrate that NT-induced MAP kinase activation depends on metalloproteinase-mediated TGFα release and subsequent transactivation of the EGF receptor. To our knowledge, this is the first report demonstrating that a metalloproteinase-dependent EGF receptor transactivation signaling event is involved in MAP kinase activation in response to an intestinal neuropeptide.

Previous studies with GPCRs that transactivate EGF receptors indicate that the EGF receptor signaling pathway is linked to Ras activation (40, 41). Our finding that NTR1 coupling leads to transactivation of EGF receptors in colonic epithelial cells is consistent with our previous results demonstrating that NTR1 stimulation leads to activation of Ras (20). Our present findings demonstrating that EGF receptor transactivation is responsible for NT-induced MAP kinase activation in NCM460 cells is also consistent with results indicating that Ras activation is required for NT-induced MAP kinase stimulation in this cell type (20). However, our results are different from the report by Guha et al. (26) showing that in pancreatic carcinoma PANC-1 cells, NT does not cause EGF receptor phosphorylation and that pharmacologic inhibition of the EGF receptor activation has no effect on NT-induced MAP kinase activation. Instead, NT-induced MAP kinase activation in PANC-1 cells is blocked by protein kinase C inhibitors (26). Several reasons may explain these different NT-related signaling responses. For example, it is likely that NT may use different pathways to activate MAP kinase in the non-transformed human colonic epithelial cells used in our study and the transformed pancreatic carcinoma cell lines used by Guha et al. (26). Moreover, PANC-1 cells, like other pancreatic carcinoma cell lines, express a constitutively active K-Ras mutant (26), whereas NCM460 colonic epithelial cells are non-transformed (42). Another possibility is the different activation of upstream signaling molecules in NCM460 cells and PANC-1 cells. Nevertheless, detailed information on different pathways used by NT to activate MAP kinase-related pathways requires further investigation.

Our results (Fig. 1D) show that pretreatment with pertussis toxin has no effect on NT-induced ERK activation in NCM460 cells, indicating that the G<sub>i/o</sub> subfamily of G proteins is not involved in this NT-EGFR-ERK pathway in this cell type. Previous studies suggested that NTR1 may be coupled to the heterotrimeric G proteins G<sub>i</sub> and G<sub>q</sub>, but not G<sub>s</sub>. NT stimulates calcium mobilization and causes protein kinase C activation presumably via the G<sub>i</sub> subfamily of G proteins, although direct evidence for G<sub>i</sub> involvement thus far is not evident (16, 26). NT can also activate G<sub>i</sub> proteins that mediate its inhibitory effect on prostaglandin E1-stimulated cyclic AMP production in neuroblastoma N1E115 cells (43). Although NT enhances cAMP accumulation induced by forskolin, prostaglandin, and cholera toxin, known activators of adenylyl cyclase in PC3 cells, this response involves Ca<sup>2+</sup>-dependent adenylyl cyclase(s) rather than stimulatory G proteins (G<sub>s</sub>) (44). We have previously
shown that NTR1 stimulation in NCM460 cells causes calcium release, suggesting that Gq might mediate NT signaling (20). At this time there is no implication of the involvement of other G proteins in NT signaling in NCM460 cells. Because G proteins including Gs (45), Gi (28, 40), Gq/11 (46, 47), and G13 (48) have been shown to mediate transactivation of the EGF receptor, further studies are needed to identify which G protein(s) mediates EGF receptor transactivation and to determine the signaling molecules involved in this NT response.

Several pieces of evidence indicate that NT-induced MAP kinase activation involves a metalloproteinase-dependent release of TGFα. First, NT is able to rapidly induce TGFα release from human colonic epithelial NCM460 cells and NCM460-NTR1 cells, although the kinetics in these two cells were different. In NCM460 cells, NT-induced TGFα release is consistent with the kinetics of NT-induced EGFR phosphorylation, both of which reached a maximal level 2 min after treatment. Similarly, in NCM460-NTR1 cells, TGFα release in response to NT increased to similar levels to that in NCM460 cells after 2 min (Fig. 4B), at which time the highest EGFR phosphorylation activity was also noted (Fig. 1A). However, 5 and 10 min after NT exposure TGFα levels continued to increase (Fig. 4B) although the levels of EGFR phosphorylation decreased (Fig. 1A).

We do not have a ready explanation for this inconsistent response. One possibility is that following its activation, EGFR is internalized rapidly (49, 50) and as a result TGFα can no longer bind to EGFR. However other possibilities may also explain this response. For example, we have previously shown that high levels of cell surface expression of NTR1 in NCM460-NTR1 cells are required for NT-induced NF-κB activation and interleukin-8 expression (20). This implies that an unknown signal, activated by NT in NCM460-NTR1 overexpressing cells following NT exposure, might in turn cause desensitization of tyrosine-phosphorylated and non-tyrosine-phospho-
Fig. 7. Effects of AG1478 and batimatostat on NT-induced IL-8 promoter activity. NCM460-NTR1 cells were transiently transfected with an IL-8 luciferase reporter construct together with an internal control plasmid. The transfected cells were serum-starved, pretreated with dimethyl sulfoxide (DMSO), AG1478 (AG, 0.5 µM), or batimatostat (Bat, 3 µg/ml) for 30 min and stimulated with NT (10−7 M) for 4 h. Luciferase activity was determined as described under “Materials and Methods.” The results are representative of three individual experiments, each with triplicate determinations. *, p < 0.05; #, p < 0.001.

Our second piece of evidence for involvement of TGFα in NT-induced ERK activation is that a TGFα neutralizing antibody significantly reduced this NT response. Lastly, our results show that inhibition of MMP activity attenuates NT-induced TGFα release, EGFR phosphorylation, and ERK activation. TGFα is one of the three known EGFR ligands that require cleavage of their precursors for their maturation and subsequent activation (37). Interestingly, using specific neutralizing antibodies against HB-EGF and amphiregulin, we were not able to show participation of these molecules in NT-induced MAP kinase activation. It is not clear why NT stimulates maturation of TGFα rather than HB-EGF and amphiregulin in human colonic epithelial cells. Selective activation of one of the EGFR receptor ligands appears to be stimulus- as well as cell type-specific. For example, Prenzel et al. (33) found that in PC3 prostate carcinoma cells EGFR receptor transactivation in response to LPA, endothelin, thrombin, bombesin, or carbacol requires metalloproteinase-mediated cleavage of pro-HB-EGF. However, in squamous carcinoma SCC-9 cells Gschwind et al. (37) show that LPA- and carbacol-induced EGFR receptor transactivation requires only proamphiregulin cleavage, although these cells express both HB-EGF and TGFα. This selectivity does not appear to be due to specificity of the upstream metalloproteinases for a particular EGFR receptor ligand. For example, the tumour necrosis factor-α converting enzyme, TACE, which selectively cleaves proamphiregulin in SCC-9 cells (37), was also able to cleave HB-EGF and TGFα (35, 51). Thus, there is a possibility that NT-induced TGFα cleavage is also cell type-specific, and the identity of the specific metalloproteinase(s) involved in NT-EGFR receptor transactivation remains to be investigated.

The EGFR receptor transactivation-MAP kinase-associated pathway participates in several diverse cellular responses. In addition to its major role in promoting cell proliferation and tissue healing following injury (52), this pathway is also involved in expression of proinflammatory cytokines such as IL-8. For example, inhibition of the MMP-HB-EGF-EGFR receptor-MAP kinase pathway significantly reduces H. pylori-induced IL-8 gene expression in human gastric epithelial cells (31, 53). Consistent with these observations, our results also show that inhibition of EGFR receptor activation attenuates NT-induced IL-8 gene transcription. Previous results indicate that the nuclear factor-κB plays a critical role in the regulation of IL-8 gene expression (20, 54, 55). However, the effect of the EGFR receptor-mediated MAP kinase pathway in IL-8 gene expression does not always involve NF-κB activation. Our group has previously shown that inhibition of MAP kinase activation does not affect NF-κB DNA binding activity in response to H. pylori (56) and NT (20). Instead, the effect of the MAP kinase pathway in IL-8 gene expression may be mediated through increased transcriptional activity of AP-1, one of three transcriptional factors important for IL-8 gene transcription (22, 57, 58).

In summary, our study demonstrates that NT via NTR1 can stimulate metalloproteinase-mediated TGFα cleavage, which in turn stimulates activation of the EGFR receptor and MAP kinase in non-transformed human colonic epithelial cells. We also present evidence linking EGFR receptor transactivation to increased IL-8 promoter activity in response to NT. Thus, EGFR receptor transactivation may represent a key molecular event participating in NT-mediated cancer growth and inflammation.

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