HHS Public Access

Author manuscript

Peptides. Author manuscript; available in PMC 2023 October 01.

Published in final edited form as:

Peptides. 2022 October ; 156: 170858. doi:10.1016/j.peptides.2022.170858.

Adding of neurotensin to non-small cell lung cancer cells increases tyrosine phosphorylation of HER3

Terry W. Moody[a,*], **Irene Ramos-Alvarez**[b], **Robert T. Jensen**[b]

[a]Department of Health and Human Services, National Institutes of Health, National Cancer Institute, Center for Cancer Training, Bethesda, MD 20892

[b]National Institute of Diabetes, Digestive and Kidney Disease, Digestive Diseases Branch, 9000 Rockville Pike, Bethesda, MD 20892 U.S.A.

Abstract

Neurotensin (NTS) receptor 1 regulates the growth non-small cell lung cancer (NSCLC) cells. NTS binds with high affinity to NTSR1, leading to increased tyrosine phosphorylation of the EGFR and HER2. Using Calu3, NCI-H358, or NCI-H441 cells, the effects of NTS on HER3 transactivation were investigated. HER3 tyrosine phosphorylation was increased by NTS or neuregulin (NRG1) addition to NSCLC cells. NCI-H358, NCI-H441, and Calu-3 cells have HER3, NTSR1 and neuregulin (NRG)1 protein. NTSR1 regulation of HER3 transactivation was impaired by SR48692 (NTSR1 antagonist) or monoclonal antibody (mAb)3481 (HER3 blocker). Immunoprecipitation experiments indicated that NTS addition to NCI-H441cells resulted in the formation of EGFR/HER3 and HER2/HER3 heterodimers. The ability of NTS to increase HER3 tyrosine phosphorylation was impaired by GM6001 (MMP inhibitor), PP2 (Src inhibitor), Tiron (superoxide scavenger), or N-acetylcysteine (antioxidant). Adding NTS to NSCLC cells increased phosphorylation of ERK, HER3, and AKT. NTS or NRG1 increased colony formation of NSCLC cells which was strongly inhibited by SR48692 and mAb3481. The results indicate that NTSR1 regulates HER3 transactivation in NSCLC cells leading to increased proliferation.

Keywords

neurotensin; HER3; lung cancer; neuregulin 1; phosphorylation; signal transduction

[*]Corresponding author: moodyt@bprb.nci.nih.gov (T.W. Moody).

Declaration of Competing Interest

The authors declare no conflict of interest

Compliance with ethical standards

This manuscript complies with the responsible conduct of research of the NIH.

Credit authorship contribution statement

Terry W. Moody: Conceptualization, Methodology, Software, Data curation, Writing-original draft, Visualization, Investigation. Irene Ramos-Alvarez: Visualization, Software. Robert T Jensen: Supervision, Writing

1. Introduction

Neurotensin (NTS) is a 13 amino acid peptide that functions in a paracrine manner to alter neural activity [Carraway and Leeman 1973; Kitabgi, 2006]. High concentrations of NTS are present in and secreted from lung cancer cells [Wood et al. 1981, Moody et al. 1985). NTS immunoreactivity is present in 60% of lung adenocarcinomas (Alifano et al., 2010). Upon secretion, NTS binds with high affinity to lung cancer cells (Allen et al. 1988). After binding to NTSR1, NTS causes phosphatidylinositol (PI) turnover resulting in elevation of cytosolic Ca^{2+} (Staley et al., 1989) and activation of protein kinase C (Guha et al., 2002). NTS stimulates, whereas SR48692, which is a highly specific antagonist for NTSR1, inhibits the growth of lung cancer cells (Moody et al., 2001). The results indicate that NTS is an autocrine growth factor for lung cancer cells.

NTSR1 regulates the transactivation of receptor tyrosine kinases (RTKs) in lung cancer cells (Moody et al., 2021). RTKs for epidermal growth factor (EGFR), HER2, and HER3 are present in lung cancer cells (Kaufman et al., 2011). The EGFR has an extracellular ligand binding domain for EGF, heparin binding EGF, or transforming growth factor (TGF)α and an intracellular tyrosine kinase domain (Lemon et al., 2014). The EGFR is inhibited by small molecule tyrosine kinase inhibitors (TKI) such as gefitinib or erlotinib which impair the ability of the TK to bind ATP and phosphorylate protein substrates (Roskowski, 2014). In contrast, HER2 has no known ligand binding domain but does have a TK domain. HER2 is phosphorylated when it forms heterodimers with the EGFR or HER3 (Yarden and Pines, 2012). HER2 is blocked by monoclonal antibodies (mAb) such as trastuzumab or TKIs such as lapatinib (Wang 2017). HER3 has an extracellular binding site for neuregulin (NRG1) but has weak tyrosine kinase activity. HER3 is biologically active primarily due to the formation of HER2/HER3 heterodimers (Karachallou, 2017). HER3 activity is inhibited by mAb3481 (Lee et al., 2020). The growth of cancer is inhibited by TKIs and mAbs (Thai et al., 2021) but stimulated by NTS (Evers et al., 2006).

RTK can be transactivated by G protein-coupled receptors (GPCR) such as neurotensin receptor 1 (NTSR1) (Moody et al., 2018). Adding NTS to colon, foregut neuroendocrine, gastric, liver, or prostate cancer cells increases tyrosine phosphorylation of the EGFR (Hassan et al., 2004; Muller et al., 2011; DiFlorio et al., 2013; Wu et al., 2017; Zhou et al., 2015). Adding NTS to lung cancer cells causes PI turnover as well as activation of Src and matrix metalloprotease (MMP) causing the release of TGFα which activates the EGFR (Moody et al., 2014). The phosphorylated EGFR stimulates the MEK/ERK and PI3K/AKT pathways leading to increased cancer proliferation and survival (Evers, 2006). The growth of NSCLC is stimulated by NTS or TGFα but inhibited by SR48692 or gefitinib (Moody et al., 2014). Lung cancer patients with high NTSR1 levels have lower relapse-free survival than patients with low NTSR1 levels (Alifano et al., 2010). Elevated NTS and NTSR1 are present in most of the NSCLC cells lines examined (Ocejo-Garci et al., 2001).

The NTSR1 regulates the formation of EGFR homodimers or EGFR-HER2 heterodimers (Moody et al., 2019). The NTSR1 transactivation of HER2 is inhibited by SR48692 or lapatinib. HER2 is amplified in many NSCLC patients. When JMV449, a potent NTSR1 agonist, is added to NSCLC cells for 72 hours, EGFR, HER2, and HER3 expression

increases (Younes et al., 2014). In contrast, NTS addition to NSCLC cells for 5 minutes, increases tyrosine phosphorylation of the EGFR and HER2 with little effect on total EGFR or HER2 (Moody et al., 2019). The increase in EGFR tyrosine phosphorylation after adding NTS to NSCLC cells is inhibited if the cells are treated with siRNA to NTSR1, indicating NTSR1 is involved (Moody et al., 2014). SR48692 is synergistic with gefitinib or lapatinib at inhibiting the growth of NSCLC cells.

While high levels of EGFR and HER2 are present in most NSCLC cells, HER3 mRNA is less abundant but high levels are present in Calu-3, NCI-H358, and NCI-H441 cells (Lee et al., 2020). Adding of bombesin or NRG1 to NSCLC cells increases tyrosine phosphorylation of HER3 (Lee et al., 2020). In this communication, the effects of NTS on HER3 were investigated. NTS or NRG1 increased tyrosine phosphorylation of HER3. The NTSR1 regulation of HER transactivation was impaired by SR48692, mAb3481, PP2 (Src inhibitor), GM6001 (MMP inhibitor), or N-acetylcysteine (antioxidant). When NTS is added to NSCLC cells, HER2/HER3 and EGFR/HER3 heterodimers form. When NTS was added to NSCLC cells, ERK and AKT phosphorylation increased leading to the proliferation and survival of NSCLC cells. The growth of NSCLC cells was significantly stimulated by NTS or NRG1 but inhibited by SR48692 or mAb3481. The results indicate that HER3 transactivation is important for NSCLC growth.

2. Materials and Methods

2A. Cell culture

NSCLC cell lines Calu-3 (adenocarcinoma), NCI-H358 (bronchoalveolar carcinoma), and NCI-H441 (adenocarcinoma; American type tissue collection; Rockville, MD) were cultured in Roswell Park Memorial Institute (RPMI)-1640 containing 10% fetal bovine serum (FBS; Invitrogen, Grand Island, NY) with Pen/Strep and Glutamine. These cell lines were chosen because they had high levels of HER3 and NTSR1. Each week the cells were split with Trypsin/EDTA and seeded 1/10 into a new T175 flask. The cells were used when they were in exponential growth phase after incubation at 37°C in 5%CO_2/95% air.

2B. Western blot and immunoprecipitation.

Calu-3, NCI-H358 or NCI-H441 cells were placed in 6 well plates or 10 cm dishes. When the cells were confluent, they were washed with SIT medium (RPMI-1640 containing 3 $\times 10^{-8}$ M sodium selenite, 5 μg/ml bovine insulin and 10 μg/ml apo-transferrin (Sigma-Aldrich, St. Louis, MO)) for 2 hours. The cells were treated with inhibitors such as SR48692 (Tocris Bioscience), mAb3481 (R & D Systems, Minneapolis, MN), PP2, GM6001, PD98059, LY294002, N-acetylcysteine or Tiron (Sigma-Aldrich, St. Louis, MO) for 30 min. Then NTS (Tocris Bioscience) or NRG1 (R & D Systems, Minneapolis, MN) was added for 5 min at 37°C and the plates were rinsed in PBS and treated with 0.5 ml lysis buffer (TBS containing 1% deoxycholate, 1% Triton, 1 mM EDTA, 0.4 mM EGTA, and 0.1% sodium azide; Sigma-Aldrich, St. Louis, MO). The lysate was sonicated, centrifuged and the supernatant assayed for protein using the BCA reagent (Thermo Scientific, Rockford, IL).

For immunoprecipitation, 600 μg of protein was immunoprecipitated with anti-PY (BD Biosciences) and 20 μl of protein A/G Plus agarose (Santa Cruz Biotech, Santa Cruz, CA) for 16 hr at 4°C. The beads were washed in TBS 3 times and treated with loading buffer at 90°C. After centrifugation, the supernatant was loaded onto 15 well 4–20% polyacrylamide gels (In Vitrogen, Frederick, MD). After transfer to nitrocellulose (Biorad, Hercules, CA), the blot was probed with anti-HER3 (CS12708), anti-PY^{1289}-HER3 (CS4791), or anti-$PT^{202}PY^{204}$-ERK (CS9101; Cell Signaling Technologies, Danvers, MA) at a 1:2000 dilution. For the dimerization experiments, 600 μg of protein was incubated with anti-EGFR (CS2232) or anti-HER2 (CS2242) at a 1:200 dilution and the immunoprecipitation performed. After the Western blot and transfer, the blots were probed with anti-PY^{1289}-HER3. For the 6-well plates, 40 μg of protein was loaded onto the gel and the blots probed with anti-PY^{1289}-HER3 (CS4791), anti-HER3 (CS12708), anti-NTSR1 (SC15311), anti-NRG1 (ABT179), anti-$PT^{202}PY^{204}$-ERK (CS9101), anti-ERK (CS9102), anti-PT^{308}AKT (CS9275), anti-AKT (CS9272), or anti-tubulin (CS2148). The blots were washed 2 times with 5% nonfat milk in TBS and 3 times in TBS. The blots were dried and incubated for 4 min in Super Signal West Femto enhanced chemiluminescent detection reagent (Thermo-Fisher Scientific, Rockford, IL). The protein bands were analyzed on a densitometer.

2C. Proliferation

Growth studies were conducted using the clonogenic assay. In the clonogenic assay, the effects of NTS, NRG1, SR48692, and mAb3481 were determined using NCI-H441 cells. The bottom layer contained 3 ml of 0.5% agarose in SIT medium with 5% FBS in 6 well plates. The top layer contained 3 ml of SIT medium in 0.3% agarose, NTS, NRG1, mAb3481, and/or SR48692 with 5×10^4 NCI-H441 cells. After 2 weeks colonies, larger than 50 μm were counted in each well.

2D. Statistics

The statistical analysis was performed using Excel and the mean value + S.D. is indicated. The significance was analyzed by the student's t-test or one-way ANOVA with Bunnett's multiple tests as a posttest. The statistical significance $p < 0.05$, * or $p < 0.01$, ** is indicated.

3. Results

3A. NTS and HER3 tyrosine phosphorylation

The ability of NTS to increase HER3 tyrosine phosphorylation was investigated using NSCLC cells. Adding 0.1 μM NTS to Calu-3, NCI-H358, or NCI-H441 cells increased the tyrosine phosphorylation of HER3 by 163, 195, and 231%, respectively (Fig 1A) but had little effect on total HER3 or tubulin. Figure 1B shows that Calu-3, NCI-H358, and NCI-H441 cells had NTSR1, NRG1, HER3, and tubulin protein. Fig. 1C shows that SR48692 in a concentration-dependent manner inhibited the phosphorylation of HER3 and ERK caused by adding NTS to NCI-H441 cells. The phosphorylation of HER3 and ERK was significantly increased by NTS addition to 292 and 259%, but it was significantly inhibited by 1 μM SR48692 but inhibited totally by 10 μM SR48692 (Fig. 1D). As a control, NTS or SR48692

had little effect on tubulin. The results indicate that NTSR1 regulates HER3 transactivation in NSCLC cells.

3B. NRG1 and HER3 tyrosine phosphorylation

The effects of NRG1 were investigated on NSCLC cells. Fig. 2A shows that adding NRG1 to NCI-H441 cells increases P-HER3 in a dose-dependent manner. There was little stimulation using 0.0001 or 0.001 μg/ml of NRG1, however, there was a large increase in P-HER3 at 0.01 and 0.1 μg/ml NRG1. Fig. 2B shows that 0.01 or 0.1 μg/ml NRG1 increased significantly P-HER3 to 175 and 290% respectively but had little effect on total HER3. Fig 2C shows that mAb3481 blocked the increase in P-HER3 caused by NTS addition. Fig. 2D shows that NTS increased P-HER3 by 274% and 0.1 μg/ml of mAb3481 inhibited significantly P-HER3. Previously, mAb3481 inhibited the increase in P-HER3 caused by adding bombesin or NRG1 to NSCLC cells (Lee et al., 2020). The results indicate that NRG1 or NTS increase phosphorylation of HER3 which is impaired by mAb3481.

3C. ErbB heterodimers

To become biologically active, members of the ErbB family dimerize. Due to weak tyrosine kinase activity, HER3 must form heterodimers and not homodimers to become biologically active (Wang et al, 2017). Fig. 3A shows that when NCI-H441 cells are treated with NTS in a dose-dependent manner and then immunoprecipitated with anti-HER2, PY^{1289}-HER3 increased. There was little increase in P-HER3 using 0.010 μM NTS but a significant increase at 0.1 or 1 μM NTS to 256 and 268%, respectively (Fig 3B). Similarly, when NCI-H441 cells are immunoprecipitated with EGFR, NTS increases PY^{1289}-HER3 using 0.1 or 1 μM NTS (Fig.3A). Fig 3B shows that 0.1 or 1 μM NTS increased PY^{1289}-HER3-EGFR heterodimers to 186 and 210%, respectively. The results indicate that NTS addition to NSCLC cells results in the formation of more HER3-HER2 heterodimers than HER3-EGFR heterodimers.

3D. Inhibitors of transactivation

Inhibitors of HER3 transactivation were investigated. Fig. 4A shows that PP2 (Src inhibitor) and GM6001 (MMP inhibitor) reduce the ability of NTS to increase HER3 and ERK phosphorylation. When NTS is added to NCI-H441 cells P-HER3 increased to 264%, and the increase was inhibited significantly by PP2 or GM6001 (Fig. 4B). N-acetylcysteine or Tiron inhibit the ability of NTS to increase HER3 tyrosine phosphorylation (Fig. 4C). When NTS is added to NCI-H441 cells P-HER3 increased significantly to 293% which was impaired by N-acetyl cysteine (NAC) or Tiron (Tir). The results indicate that generation of reactive oxygen species (ROS) are essential for NTSR1 to regulate HER3 tyrosine phosphorylation. Previously, NAc, Tiron, PP2, or GM6001 impaired the ability of NTS to increase tyrosine phosphorylation of the EGFR and HER2 in NSCLC cells (Moody et al., 2021).

3E. Signal Transduction and Proliferation

HER3 can phosphorylate protein substrates such as PI3K and Grb2, leading to activation of AKT and ERK, respectively (Wang et al., 2017). Figure 5A shows that the MEK inhibitor

PD98059 inhibited the ability of NTS to increase ERK phosphorylation, but not HER3 or AKT phosphorylation. Also, the PI3K inhibitor LY294002 inhibited the ability of NTS to increase AKT phosphorylation but had little effect on HER3 or ERK phosphorylation. Fig. 5B shows that adding NTS to NCI-H441 cells increased P-HER3, P-ERK, and P-AKT to 232%, 260%, and 301%, respectively. LY294002 inhibited significantly P-AKT but had little effect on P-HER3 or P-ERK. PD98059 inhibited P-ERK significantly but not P-HER3 or P-AKT. These results indicate that ERK and AKT are downstream from HER3.

The effects of SR48692, mAb3481, NRG1 and NTS were investigated on NSCLC proliferation. In the clonogenic assay, 1 μM SR48692 or 0.1 μg/ml mAb3481 significantly reduced colony number by 45% and 26%, respectively. There were fewer NCI-H441 colonies when SR48692 and mAb3481 were added together relative to either agent alone. In contrast, 0.01 μg/ml NRG1 or 0.1 μM NTS significantly increased colony number by 49% and 55%, respectively. mAb3481 inhibited the ability of NRG1 or NTS to increase colony number. SR48692 inhibited the ability of NTS to increase colony number. In summary, NTS and NRG1 increased NSCLC proliferation whereas mAb3481 and SR48692 reduced proliferation.

4. Discussion

HER3 contains 1342 amino acids with an extracellular domain of 643 amino acids, a 21 amino acid transmembrane (TM) domain, and an intracellular domain of 678 amino acids containing amino acids (Sithanandam and Anderson, 2008). The extracellular domain of HER3 binds NRG1 with high affinity, however, the intracellular domain of HER3 has low TK activity. When HER3 is expressed in lung adenocarcinoma tumors, patients have poor survival (Sitanandam and Anderson, 2008). HER3 gene amplifications have been detected in tumors of lung cancer patients (Reinmuth et al., 2000 et al.). HER3 can be tyrosine phosphorylated when it forms heterodimers with the EGFR or HER2 (Mota et al., 2017). Fig. 3 shows that 5 min after the addition of NTS to NSCLC cells, the tyrosine phosphorylation of HER3 is increased due to the formation of EGFR/HER3 and HER2/HER3 heterodimers. Previously, the expression of P-EGFR, P-HER2 and P-HER3 was elevated significantly in certain breast cancer cells (Dupouy et al., 2014).

By Western blot, NSCLC cell lines Calu-3, NCI-H358, and NCI-H441 expressed HER3, NTSR1, and NRG1 (Fig. 1). Previously, NTSR1 but not NTSR2 mRNA was present in 10 NSCLC cell lines including NCI-H358 and NCI-441 (Moody et al., 2019). Figure 1 shows that adding NTS to NSCLC cells increased tyrosine phosphorylation of HER3 which was blocked by SR48692. Also, mAb3481 impaired the ability of NTS to increase HER3 tyrosine phosphorylation (Fig. 2). The results indicate that NTSR1 regulates the transactivation of HER3 in NSCLC cells.

The mechanism by which NTSR1 caused HER3 transactivation was investigated. PP2 and GM6001 inhibited the ability of NTS to increase HER3 tyrosine phosphorylation indicating that Src and MMP are essential (Fig. 4). When NTS was added to prostate cancer cells tyrosine phosphorylation of Stat 5b, Src, and the EGFR increased (Amorino et al., 2007). When NTS was added to A549 cells tyrosine phosphorylation of c-Src, β-catenin, PYK-2,

and the EGFR increased (Moody et al., 2014). The results indicate that NTS causes the phosphorylation of numerous proteins leading to increased proliferation of NSCLC cells. Figure 4 shows that NAc and Tiron inhibited the ability of NTS to increase HER3 tyrosine phosphorylation indicating that ROS are essential.

HER3 can be phosphorylated at Y^{1054}, Y^{1197}, Y^{1199}, Y^{1222}, Y^{1260}, Y^{1262}, Y^{1276}, Y^{1289}, and Y^{1328}. The phosphate can be transferred to protein substrates such as p85, Grb2, and SHC. P85 is the regulatory subunit of PI3K which facilitates phosphorylation of AKT and increased cellular survival. Grb2 and SHC are adaptor proteins which facilitate activation of RAS leading to the phosphorylation of Raf, MEK, and ERK. The MEK inhibitor PD98059 inhibits the ability of NTS to increase P-ERK but not P-HER3 (Fig. 5). Phosphorylated ERK can enter the nucleus leading to increased cellular proliferation. The PI3K inhibitor LY294002 inhibits the phosphorylation of AKT but not HER3. PI3K may increase the survival of NSCLC cells.

When NTS is added to pancreatic cancer cells, ERK and PKD are activated (Ehlers et al., 2000; Guha et al., 2002; Kisfalvi et al., 2005). Adding NTS to A549 lung cancer cells increased the phosphorylation of β-catenin leading to an increased expression of NTSR1 (Souaze et al., 2006). When NTS is added to colon cancer cells, epithelial-to-mesenchymal transitions increased by activating the Wnt/ β-catenin pathway. When NTS is added to colon cancer cells, NFκB and Akt are activated increasing cellular survival (Zhao et al., 2003).

NRG1 is present in NSCLC cells (Fig. 1). NRG1 has an IG-like domain, an EGF-like domain, a juxtamembrane domain, a TM domain, and a cytoplasmic tail. NRG1 is a membrane- anchored glycoprotein with an extracellular EGF component (Laskin et al., 2020). Due to alternative processing, 6 different proteins of NRG1 have been identified as well as 31 isoforms. Most NRG1 isoforms are membrane-anchored with an extracellular EGF domain (Laskin et al., 2020). Proteolytic processing by ADAM-17 leads to the release of bioactive NRG1 which can activate HER3 (Mota et al., 2017). Also, gene fusions occur with the NRG1 gene driving NSCLC proliferation (Jung et al., 2015) by increasing expression of HER3 (Isozadi et al., 2016).

Preliminary data indicate that addition of NTS to NCI-H441 cells increases the secretion of NRG1 into the cellular media (T. Moody, unpublished). Treatment of NSCLC cells with JMV449 increased but SR48692 decreased secretion of NRG1 from the certain lung cancer cells (Younes et al., 2014).

The 13 amino acid NTS is derived from a 170 amino acid proproNTS by cellular enzymes, however, proNTS or long-fragment NTS (LF-NTS) is present in NSCLC specimens (Wu et al., 2019). LF-NTS, which is biologically active and present in patient sera, is neutralized by mAb LF-NTS. Mab LF-NTS impairs the ability of LF-NTS to activate NTSR1. Mab LF-NTS reduces P-EGFR, P-HER2, P-HER3, P-ERK, P-AKT, P-Src, P-c-jun in adenocarcinoma cells reducing proliferation. Traditionally, adenocarcinoma patients are treated with chemotherapy (cisplatin plus pemedtrexed), however drug resistance develops. mAb LF-NTS restores sensitivity of adenocarcinoma cells to cisplatin. The results indicate that mAb LF-NTS may be therapeutic for NSCLC patients.

Numerous mAbs have been elicited against HER3 including patritumab which inhibits ligand binding and dimerization. A Phase I clinical trial was conducted in patients with advanced NSCLC in combination with erlotinib (EGFR tyrosine kinase inhibitor) and patritumab combined with erlotinib was well tolerated in Japanese patients with advanced NSCLC (Shimizu et al., 2017). Seribantumab is another mAb that prevents NRG1 binding to HER3 and phase II clinical trials were conducted with erlotinib in NSCLC patients (Sequist et al., 2019). Additional trials are being conducted with seribantumab and docetaxel in NSCLC patients with high NRG expression (Mishra et al., 2018).

SR48692 inhibited the proliferation of NCI-H441, NCI-H358 and Calu-3 cells with IC_{50} values of 3, 1.3, and 2 μM. Table I shows that NTS or NRG1 increased NCI-H441 colony number by 55 and 49%, respectively. The increase in colony number by NRG1 was impaired by mAb3481. SR48692 or mAb3481 reversed the increase in colony number caused by NTS. The results suggest that NTS and NRG1 may be important growth factors in NSCLC.

5. Summary

HER3 tyrosine phosphorylation is increased significantly when NTS is added to NSCLC cells. The increase in HER3 transactivation regulated by NTSR1 is impaired by Src inhibitors, MMP inhibitors, and reactive oxygen species. Because NTS increases HER3 phosphorylation and NSCLC proliferation, SR48692 may be useful agent to reduce NSCLC growth.

Funding

This research is supported by the NIH intramural program of NIDDK and the NCI.

References

[1]. Alifano M, Souaze F, Dupouy S, Camilleri-Broet S, Younes M, Ahmed-Zaid SM et al. Neurotensin receptor 1 determines the outcome of non-small cell lung cancer. Clin. Cancer Res 2010; 16: 4401–10. [PubMed: 20810387]

[2]. Allen AE, Carney DN, Moody TW. Neurotensin binds with high affinity to small cell lung cancer cells. Peptides 1988; 9: 57–61. [PubMed: 2856809]

[3]. Amorino GP, Deeble PL, Parsons SL. Neurotensin stimulates mitogenesis of prostate cancer cells through a novel c-Src/Stat 5b pathway. Oncogene 2007; 26: 745–756. [PubMed: 16862179]

[4]. Carraway R, Leeman SE. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalamus. J. Biol. Chem 1973; 248: 6854–61. [PubMed: 4745447]

[5]. Di Florio A, Sancho V, Moreno P, Delle Fave G, Jensen RT. Gastrointestinal hormones simulate growth of foregut neuroendocrine tumors by transactivating the EGF receptor. Biochem. Biophys. Acta 2013; 1833: 573–82. [PubMed: 23220008]

[6]. Dupouy S, Doan VK, Wu Z, Mourra N, Liu J, De Wever O. et al. Activation of EGFR, HER2 and HER3 by neurotensin/neurotensin receptor 1 renders breast tumors aggressive yet highly responsive to lapatinib and metformin in mice. Oncotarget 2014; 5: 8235–51. [PubMed: 25249538]

[7]. Ehlers RA, Zhang Y, Hellmich MR, Evers BM. Neurotensin-mediated activation of the MAPK pathways and AP-1 binding in the human pancreatic cancer cell line MIA PaCa-2. Biochem. Biophys. Res. Comm 2000; 269: 704–708. [PubMed: 10720480]

[8]. Evers BM. Neurotensin and growth of normal and neoplastic tissues. Peptides 2006; 27: 2424–2433. [PubMed: 16904238]

[9]. Guha S, Rey O, Rozengurt E. Neurotensin induces protein kinase C-dependent protein kinase D activation and DNA synthesis in human pancreatic carcinoma cell line PANC-1. Cancer Res. 2002; 62: 1632–40. [PubMed: 11912133]

[10]. Hassan S, Dobner PR, Carraway RE. Involvement of MAP kinase, PI-3-kinase and EGF-receptor in the stimulatory effect of neurotensin on DNA synthesis in PC3 cells. Regul. Peptides 2004; 120: 155–166.

[11]. Isozaki H, Ichihara E, Takigawa N, Ohashi K, Ociii N, Yasugi M et al. Non-small cell lung cancer cells acquire resistance to the ALK inhibitor alectinib by activating alternative receptor tyrosine kinases. Cancer Res. 2016; 76: 1506–1516. [PubMed: 26719536]

[12]. Jung Y, Yong S, Kim P, Lee HY, Jung Y, Keum J et al. , Fusion gene is a novel oncogenic driver of non-small-cell lung adenocarcinoma. J. Thorac. Oncol 2015; 10: 1107–1111. [PubMed: 26134228]

[13]. Karachallou N, Lazzari C, Verlicchi A, Sosa AE, Rosell R HER3 as a therapeutic target in cancer. Biodrugs 2017; 31: 63–73., [PubMed: 28000159]

[14]. Kaufman J, Horn L. Carbone D. Chapter 74. Molecular biology of lung cancer. In: DeVita V Jr, Lawrence TS, Rosenberg SA, editors. Cancer: principles and practice of oncology. Philadelphia: Lippincott, Williams & Wilkens; 2011, p. 789–98.

[15]. Kisfalvi K, Guha S, Rozengurt E. Neurotensin and EGF induce synergistic stimulation of DNA synthesis by increasing the duration of ERK signaling to ductal pancreatic cancer cells. J. Cell. Physiol 2005; 202: 880–90. [PubMed: 15389644]

[16]. Kitabgi P Functional domains of the subtype 1 neurotensin receptor (NTS1). Peptides 2006; 27: 2461–8, [PubMed: 16901586]

[17]. Laskin J, Liu SV, Tolba K, Heining C, Schlenk RF, Cheema P et al. NRG1 fushion-driven tumors: Biology, detection, and the therapeutic role of afatinib and other ErbB-targeting agents. Ann. Oncol 2020; Dec31:1693–1703.

[18]. Lee L, Ramos-Alvarez I, Moody TW, Mantey SA, Jensen RT. Neuropeptide bombesin receptor activation stimulates growth of lung cancer cells through HER3 with a MAPK-dependent mechanism. Biochem. Biophys. Acta Mol. Cell Res 2020; Apr; 1867(4):118625.

[19]. Lemmon MA, Schlessinger J, Ferguson KM. The EGFR family: Not so prototypical receptor tyrosine kinase. Cold Spring Harbor Perspect. Biol. 2014; 6: a020768. Doi: 10.1016.

[20]. Mishra R, Patel H, Alanazi S, Yuan L, Garrett JT. HER3 signaling and targeted therapy in cancer. Oncology Reviews 2018; 12: 355: 45–62.

[21]. Moody TW, Carney DN, Korman LY, Gazdar AF, Minna JD. Neurotensin is produced and secreted by classic small cell lung cancer cells. Life Sci. 1985; 36: 1727–1732. [PubMed: 2984506]

[22]. Moody TW, Chan DC, Mantey SA, Moreno P, Jensen RT. SR48692 inhibits non-small cell lung cancer proliferation in an EGFR receptor-dependent manner. Life Sci. 2014; 100: 25–34. [PubMed: 24496038]

[23]. Moody TW, Chiles J, Casibang M, Moody E, Chan D, Davis TP. SR48692 is a neurotensin receptor antagonist which inhibits the growth of small cell lung cancer cells. Peptides 2001; 22: 109–115. [PubMed: 11179604]

[24]. Moody TW, Lee L, Ramos-Alvarez I, Jensen RT. Neurotensin receptors regulate transactivation of the EGFR and Her2 in a reactive oxygen species-dependent manner. Eur. J. Pharmacol 2019; 865: 172735. Doi: 10.1016/j.ejphar.2019.172735. Epub 2019 Oct. 12. [PubMed: 31614143]

[25]. Moody TW, Ramos-Alvarez I, Jensen RT. Neuropeptide G Protein-coupled receptors as oncotargets. Front. Endocrinol (Lausanne) 2018; 9,345.

[26]. Moody TW, Ramos-Alvarez, I, Jensen RT. Bombesin, endothelin, neurotensin and pituitary adenylate cyclase activating polypeptide cause tyrosine phosphorylation of receptor tyrosine kinases. Peptides 2021; 137. 170480. [PubMed: 33385499]

[27]. Mota JM, Collier KA, Costa RLB, Taxter T, Kalyan A, Leite CA et al. , A comprehensive review of heregulins, HER3 and HER4 as potential therapeutic targets in cancer. Oncotarget 2017; 8: 89284. [PubMed: 29179520]

[28]. Muller KM, Tveteraas IH, Aasrum M, Odegard J, Dawood M, Dajani O et al. Role of protein kinase C and epidermal growth factor receptor signaling in growth stimulation by neurotensin

in colon carcinoma cells. BMC Cancer 2011; 11: 421. doi: 10.1186/1471-2407.11- [PubMed: 21961726]

[29]. Ocejo-Garci M, Ahmed SI, Coulson JM, Woll PJ. Use of RT-PCR to detect expression of neuropeptides and their receptors in lung cancer. Lung Cancer. 2001; 33:1–9. [PubMed: 11429190]

[30]. Reinmuth N, Brandt B, Kunze WP, Junker K, Thomas M, Achatzy R et al. , Ploidy, expression of erbB1, ErbB2, p53 and amplification of erbB1, erbB2 and erbB3 in non- small cell lung cancer. Eur J Respir J 2000 16: 991–996.

[31]. Roskoski R Jr ErbB/HER protein-tyrosine kinases: Structures and small molecule inhibitors. Pharmacological Research 2014; 87: 42–59. [PubMed: 24928736]

[32]. Sequist LV, Gray JE, Harb WA, Lopex-Chavez A, Doebele RC, Modano MR et al. Randomized phase II trial of seribantumab in combination with erlotinib in patient with EGFR wild-type non-small cell lung cancer. Oncologist 2019; Aug 24: 1095–1102.

[33]. Shimizu T, Yonesaka K, Hayashi H, Iwasa T, Haratani K, Yamada H et al. Phase 1 study of new formulation of patritumab (U3–1287) process 2, a fully human anti-HER3 monoclonal antibody in combination with erlotinib in Japanese patients with advanced non-small cell lung cancer. Cancer Chemother. Pharmacol 2017; 79: 489–495. [PubMed: 28144730]

[34]. Sithanandam G, Anderson LM. The ERBB3 receptor in cancer and gene therapy. Cancer Gene Ther. 2008; 15: 413–448. [PubMed: 18404164]

[35]. Souaze F, Viardot-Foucault V, Roullet N, You-Miou-Leong M, Gompei A, Bruyneel E, et al. Neurotensin receptor 1 gene activation by the Tcf/β-catenin pathway is an early event in human colonic adenomas. Carcinogenesis 2006; 27: 708–16. [PubMed: 16299383]

[36]. Staley J, Fiskum G, Davis TP, Moody TW. Neurotensin elevates cytosolic calcium in small cell lung cancer cells. Peptides 1989; 10: 121–7. [PubMed: 2501767]

[37]. Thai AA, Solomon BJ, Sequist LV, Gainor JG, Heist RS. Lung Cancer. Lancet 2021; 398: 535–54.doi.org/10.1016/S0140-6736(21)00312-3 [PubMed: 34273294]

[38]. Wang Z ErbB receptors and cancer. In Methods in Molecular Biology, 2017; 1652: 3–35.

[39]. Wood SM, Wood JR, Ghatei MA, Lee YC, O'Shaughnessy D, Bloom SR. Bombesin, somatostatin and neurotensin-like immunoreactivity in bronchial carcinoma. J. Clin. Endocrinol 1981; 53: 1310–2.

[40]. Wu Z, Fournel L, Stadler N, Liu J, Boullier A, Hoyeau N et al. Modulation of lung cancer cell plasticity and heterogeneity with restoration of cisplatin sensitivity by neurotensin antibody. Cancer Letters 2019; 444: 147–161. [PubMed: 30583074]

[41]. Wu Z, Galmiche A, Liu J, Stadler N, Wendum D, Segal-Bendirdjian E et al. Neurotensin regulation induces overexpression and activation of EGFR in HCC and restores response to erlotinib and sorafenib. Cancer Lett. 2017; 388: 73–84. [PubMed: 27914862]

[42]. Yarden Y, Pines G. The ERBB network: At last, cancer therapy meets system biology. Nature Reviews/Cancer 2012; 12: 553–63. [PubMed: 22785351]

[43]. Younes M, Wu Z, Dupouy S, Lupo AM, Mourra N, Takahashi T et al. Neurotensin (NTS) and its receptor (NTSR1) causes EGFR, HER2 and HER3 over-expression and their autocrine/paracrine activation in lung tumors, confirming responsiveness to erlotinib. Oncotarget 2014; 5: 8252–8269. [PubMed: 25249545]

[44]. Zhao D, Khunt-Moore S, Zeng H, Wu JS, Moyer MP, Pothoulakis, C. Neurotensin stimulates IL-8 expression in human colonic epithelial cells through Rho GTPase-mediated NF-kappa B pathways. Am. J. Physiol. Cell Physiol 2003; 284: C1397–1404. [PubMed: 12584113]

[45]. Zhou Z, Xie J, Cai Y, Yang S, Chen Y, Wu HR. The significance of NTR1 expression and its correlation with β-catenin and EGFR in gastric cancer. Diagn. Pathol 2015; 100: 173 doi:10.1186/s13000-015-356-3.

Highlights

- The increase in tyrosine phosphorylation of HER3 caused by NTS is blocked by SR48692.
- The NTSR1 regulation of HER3 transactivation requires Src, MMP, and ROS.
- The ability of NRG1 to increase HER3 tyrosine phosphorylation is blocked by mAb3481.
- NTS addition to lung cancer cells increases the phosphorylation of ERK and AKT.
- NTS or NRG1 increase but SR48692 or mAb3481 inhibit NSCLC growth.

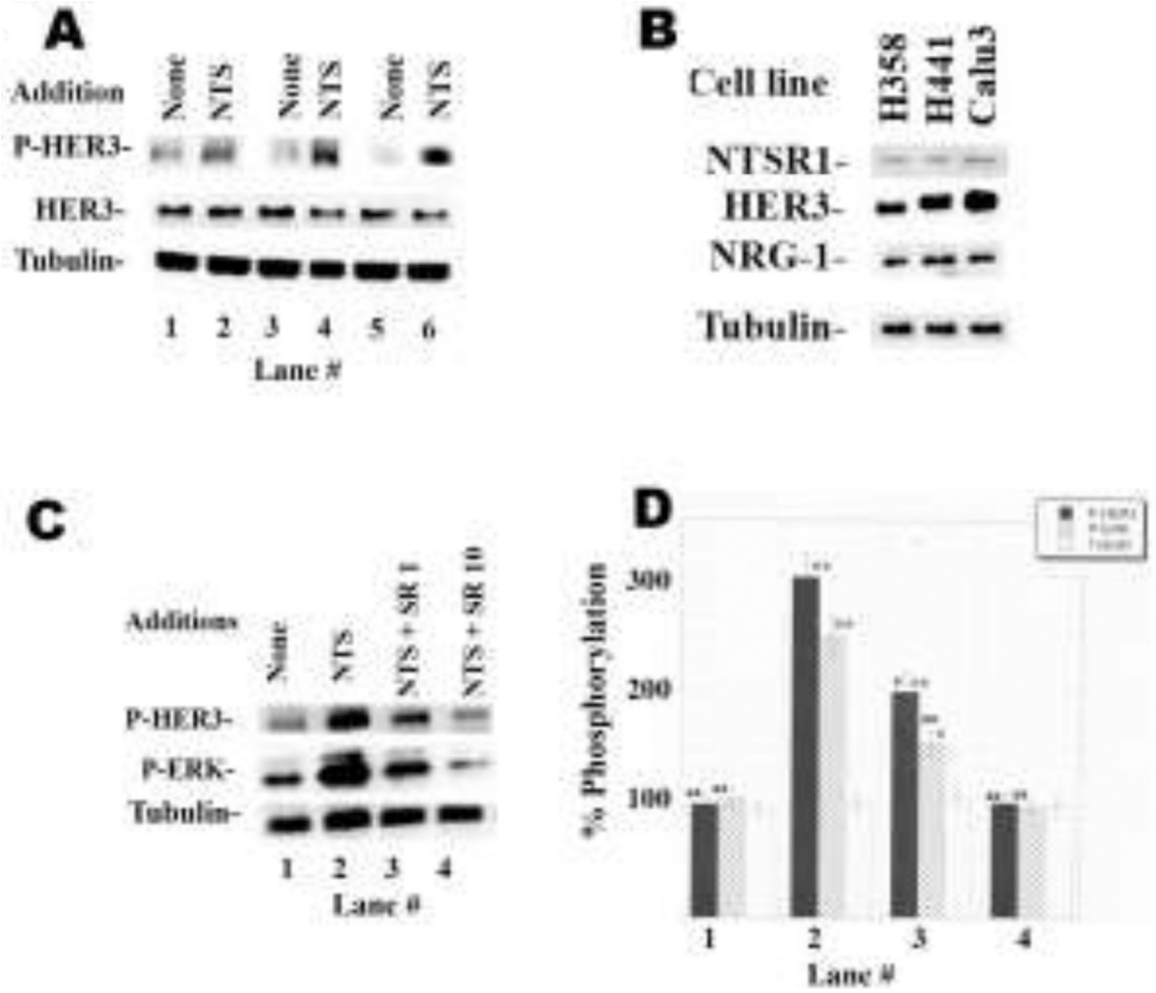

Figure 1.

NTS and P-HER3. (A) NTS (0.1 μM) was added to Calu-3 cells (Lane 2), NCI-H358 cells (Lane 4) and H441 cells (Lane 6) for 5 min and the phosphorylation of HER3 determined. (B) Western blot of NTSR1, NRG-1, HER3 and tubulin in NCI-H358, NCI-H441 and Calu-3 cells. (C) The ability of 1 or 10 μM SR48692 to inhibit phosphorylation of HER3 or ERK was determined after adding NTS to NCI-H441 cells. (D) The mean value + S.D. of 3 determinations to inhibit phosphorylation caused by NTS was determined; $p < 0.05$, *, $p < 0.01$, ** relative to no additions; $p < 0.05$, ^{a}; $p < 0.01$, ^{aa} relative to NTS by ANOVA. NTS or SR48692 had little effect on tubulin.

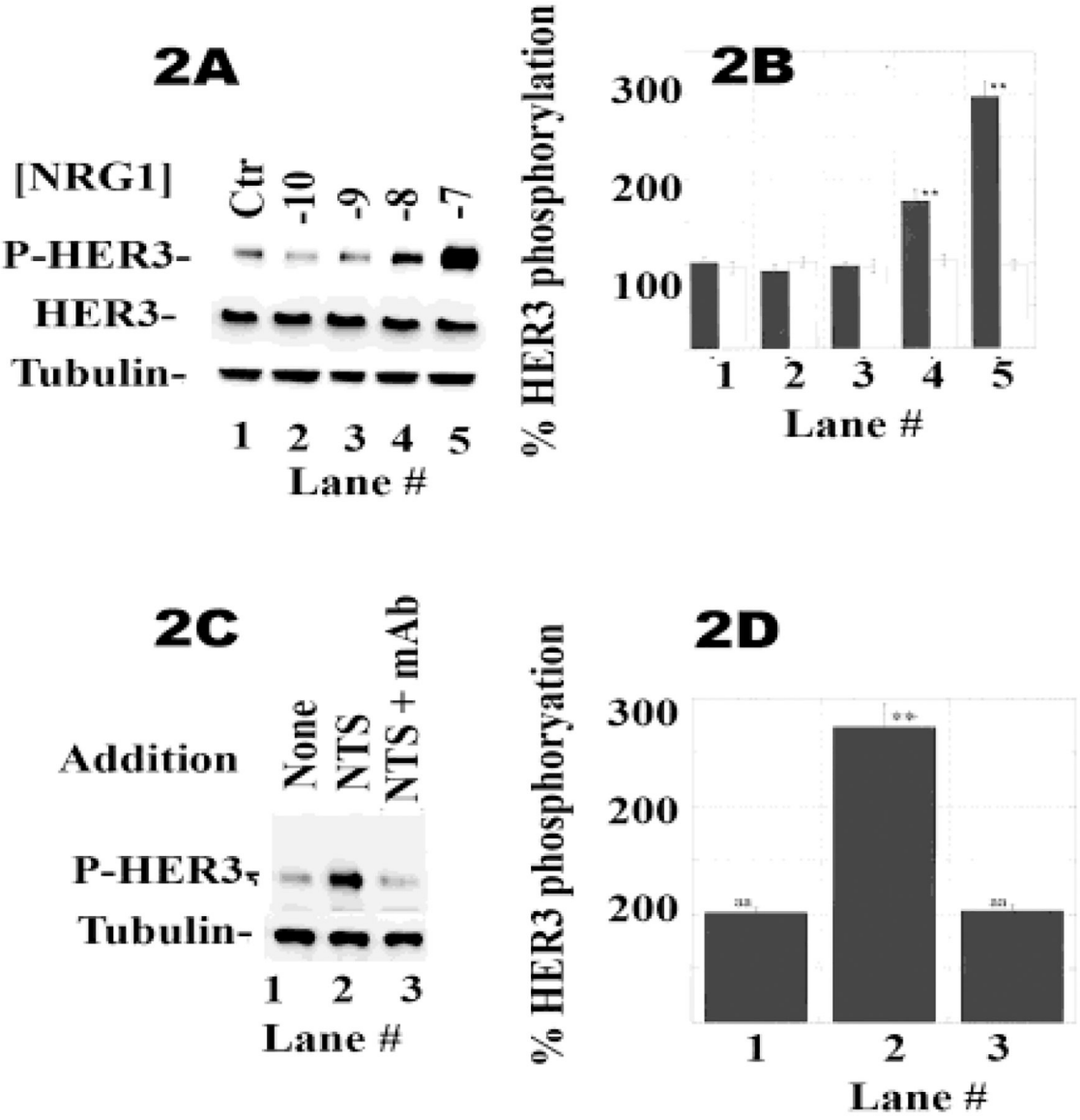

Figure 2.

NRG1 and HER3 phosphorylation. (A) NCI-H441 cells are treated with 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} g/ml NRG1 and the P-HER3 determined. (B) The mean value + S.D. of 3 determinations is indicated; $p < 0.01$, ** relative to no additions by ANOVA. (C) The ability of mAb3481 (0.1 μg/ml) to inhibit the increase in phosphorylation caused by adding 0.1 μM NTS to NCI-H441 cells was determined. (D) The mean value + S.D. of 3 determinations is indicated; $p < 0.01$, ** relative to no additions; $p < 0.01$, ^{aa} relative to NTS. NTS or mAb3481 has little effect on tubulin.

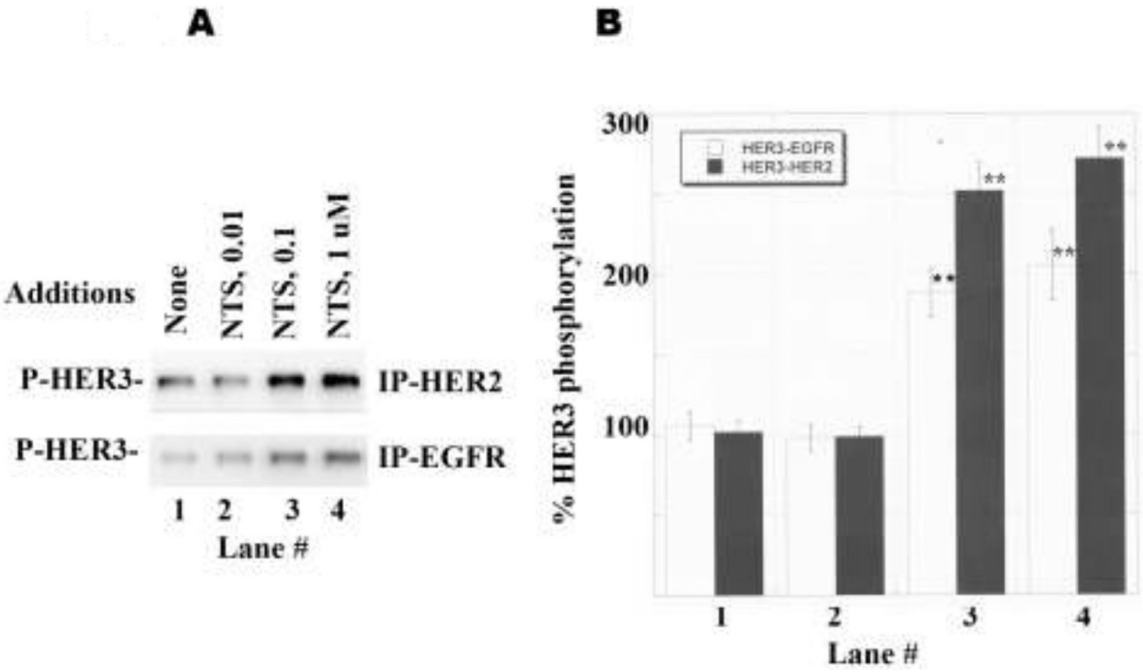

Figure 3.

HER3 dimerization. NCI-H441 cells were treated with varying doses of NTS. The cell extracts were immunoprecipitated with anti-HER2 or anti-EGFR (A) A Western blot was performed and the resulting nitrocellulose was probed with anti-PY^{1289}-HER3. (B) Densitometry performed on the 180 KDal bands. The mean value + S.D. of 3 determinations is indicated; $p < 0.01$ ** relative to control by ANOVA.

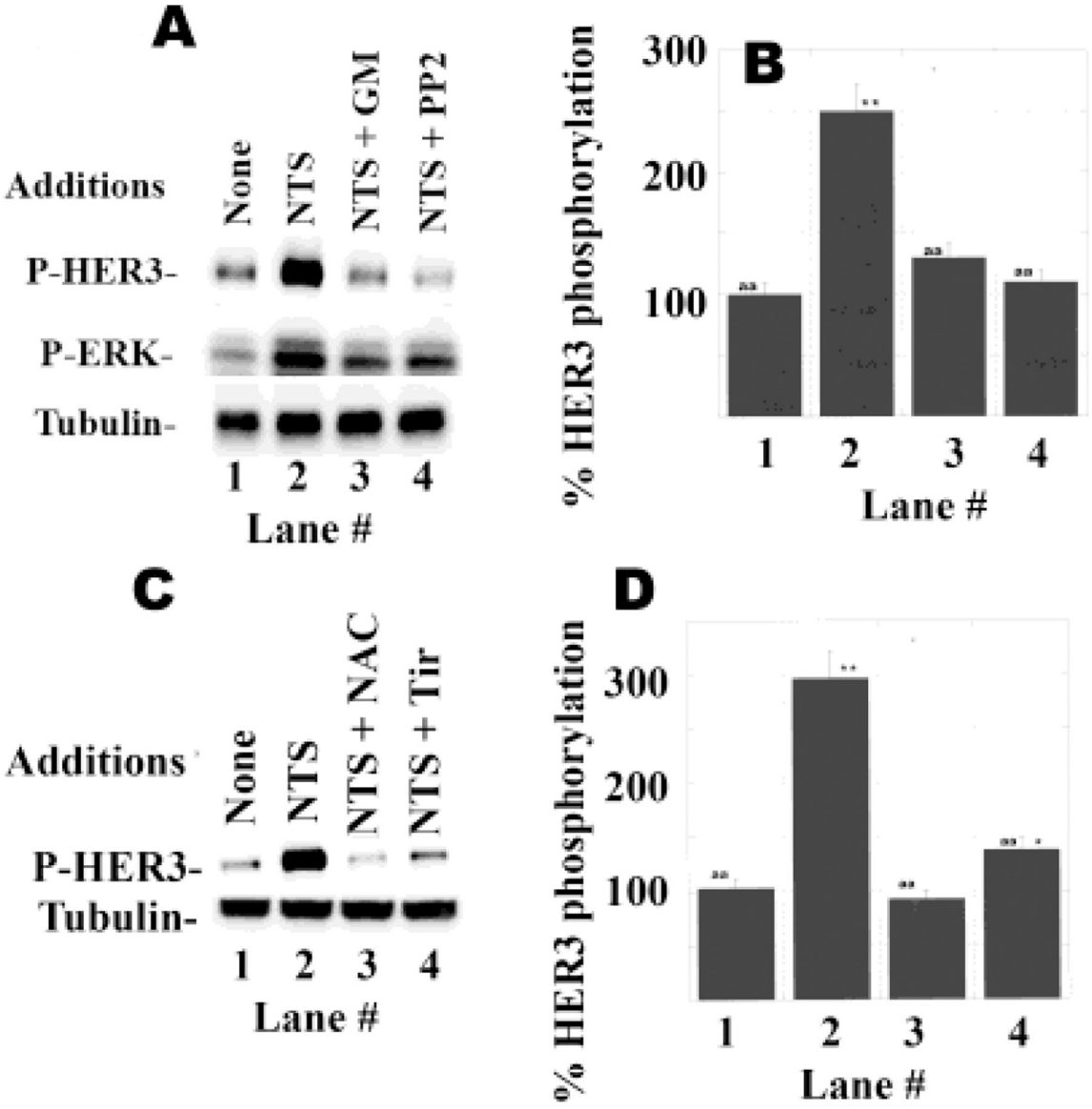

Figure 4.

Inhibitors of transactivation. (A) The ability of 10 μM GM6001 or PP2 to inhibit the increase HER3 tyrosine phosphorylation caused by adding 0.1 μM NTS to NCI-H441 cells was determined. (B) The mean value + S.D. of 3 determinations is indicated; $p < 0.01$ relative to control **; $p < 0.01$ relative to 0.1 μM NTS ^{aa}; by ANOVA. (C) The ability of 10 mM NAC or 5 mM Tiron to inhibit the NTS increase in HER3 tyrosine phosphorylation was investigated. (D) The mean value + S.D. of 3 determinations in indicated; $p < 0.01$ relative to control, **; $p < 0.01$ relative to NTS, ^{aa} by ANOVA.

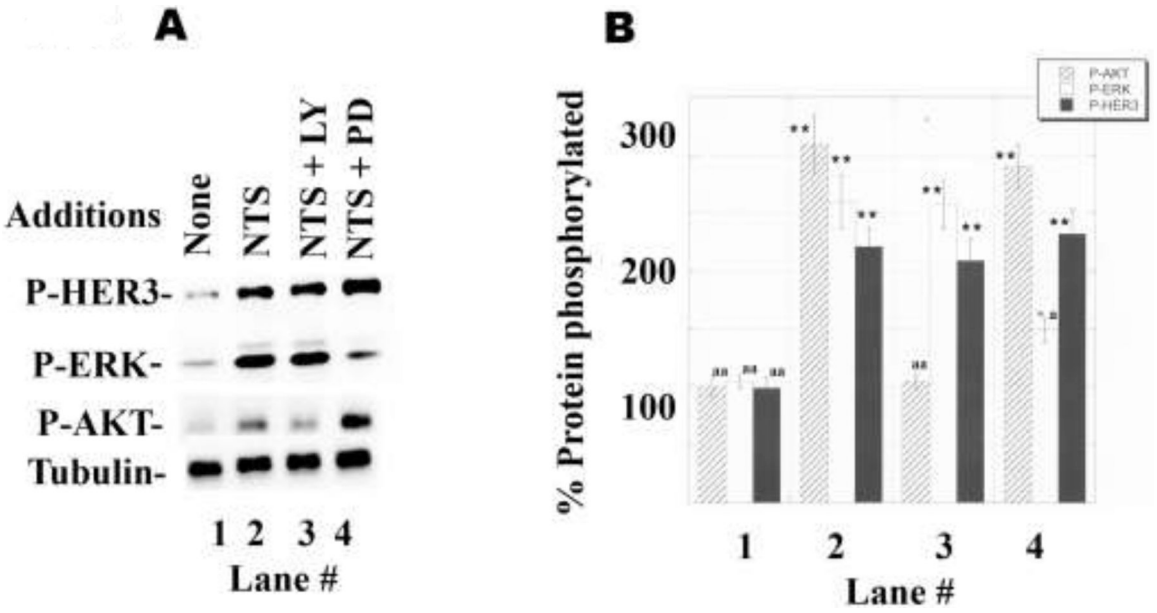

Figure 5.

Effect of PI3K and MEK inhibitors. (A). The ability of 10 μM LY294002 or PD98059 to inhibit the increase phosphorylation of HER3, AKT and ERK caused by adding 0.1 μM NTS to NCI-H441 cells was determined. (B) The mean value + S.D. of 3 determinations is indicated; $p < 0.05$, *; $p < 0.01$, ** relative to control; $p < 0.05$, ^{a}; $p < 0.01$, ^{aa} relative to NTS by ANOVA.

Table. I.

Clonogenic assay

Addition	Basal	+ NTS (0.1 μM)
None	53 + 6	82 + 9 **
SR48692, 1 μM	29 + 5 *	62 + 6
mAb 3481 0.1 μg/ml	39 + 5 *	68 + 9
SR + mAb	20 + 4 **	52 + 4
NRG1, 0.01 μg/ml	79 + 8 *	n.d.
NRG1 + mAb	57 + 6	n.d.

The mean value + S.D. of 3 determinations is indicated using NCI-H441 cells; $p < 0.05$, *; $p < 0.01$, ** by ANOVA relative to no additions; n.d, not determined.