Inactivation of neuregulin-1 by nitration

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1Division of Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine, University Hospitals of Cleveland, Case Western Reserve University, and 2Departments of Pathobiology and Pulmonary, Allergy and Critical Care Medicine, Cleveland Clinic Foundation, Lerner Research Institute, Cleveland, Ohio

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Nethery DE, Ghosh S, Erzurum SC, Kern JA. Inactivation of neuregulin-1 by nitration. Am J Physiol Lung Cell Mol Physiol 292: L287–L293, 2007. First published September 15, 2006; doi:10.1152/ajplung.00058.2006.—Nitration is a posttranslational modification that can compromise protein function. We hypothesized that nitration of growth factors secreted in the lung may alter their interaction with their respective receptors and modulate the normal growth and differentiation program induced by ligand-receptor interaction. We tested this hypothesis in vitro by nitration of neuregulin-1’s (NRG-1) EGF-like domain and studying the effect on NRG-1’s activity. Nitration of NRG-1’s (nNRG-1) EGF-like domain resulted in an inability to activate its receptor, the human epidermal growth factor receptors 2 and 3 (HER2/HER3) heterodimer, as defined by loss of HER2 tyrosine phosphorylation induced by nNRG-1 in MCF-7 cells. Receptor activation was not restored with increasing nNRG-1 concentration or exposure times. nNRG-1 did not compete with NRG-1 for HER2/HER3 binding in competition assays. In addition, nNRG-1 no longer induced proliferation of the MCF-7 cell line, as MCF-7 cells exposed to nNRG-1 and NRG-1 concurrently had the same proliferation rate as that induced by NRG-1 alone. Thus nitration of NRG-1’s EGF-like domain caused it to lose its ability to bind and activate its receptor with loss of ligand-induced proliferation. Posttranslational nitration of growth factor in states where reactive nitrogen species are increased may be an important means of regulating growth factor receptor effects in the lung.

GROWTH FACTORS AND THEIR RECEPTORS play critical roles in epithelial cell growth and development (26, 33), and their interactions are carefully regulated to control cellular proliferation and differentiation. Previous reports from our laboratory identified neuregulin-1 (NRG-1) and the human epidermal growth factor receptor (HER) family as an important receptor family expressed in the pulmonary epithelium. This receptor family consists of EGFR (HER1), HER2, HER3, and HER4, for which expression has also been identified in breast, intestine, prostate, and cardiac tissue. In the lung, NRG-1 activation of the HER2/HER3 complex stimulates pulmonary epithelial cell proliferation and differentiation (19, 26). Signal transduction is accomplished by NRG-1 binding to HER3, the complex then heterodimerizing with HER2 and activating HER2’s intrinsic tyrosine kinase domain. The resultant phosphorylation of tyrosine residues in HER2 and HER3 cytoplasmic domains initiate intracellular signaling cascades (19, 26, 30). NRG-1’s EGF-like domain over amino acids 177–244 carries all known functional activity of the full-length protein (10, 15, 30). Our recent in vitro studies have shown that in transformed pulmonary epithelial cell lines, the NRG-1/HER2/HER3 interaction is an autocrine process (11), and in vivo, receptor activation plays a role in recovery from lung injury and fibrosis (22). The functional effects of the ligand-receptor interaction are regulated at both a transcriptional level and a posttranscriptional level through spatial segregation of the receptor and ligand (34).

Posttranslational modification through cell-based nitrating reactions may be another mechanism to regulate growth factor function in biological systems. Recently, a number of proteins have been found to be modified by such processes and associated with reduced function. In cardiac tissue, nitration of contractile proteins, especially α-actinin, by the oxidant peroxynitrite results in contractile dysfunction (4). In asthma, nitration inactivates manganese superoxide dismutase (MnSOD), an important antioxidant enzyme, resulting in impaired antioxidant defenses and increased apoptosis in airway epithelial cells (6). Insulin undergoes nitration following reaction with peroxynitrite (5), altering binding to its receptor without affecting its biological function (5). In the lung, NRG-1 and inducible nitric oxide synthase (iNOS) are both localized to the airway epithelium’s apical surface (24, 34), raising the possibility of nitric oxide-induced nitration of NRG-1. Importantly, tyrosines in NRG-1’s EGF-like domain are critical for its binding to the HER2/HER3 heterodimer (17). Thus nitration of these residues may affect NRG-1 function and significantly alter receptor signaling.

In the present study, we hypothesized that nitration of secreted growth factors in the lung may alter their interaction with their respective receptors and modulate proliferation induced by ligand-receptor interaction. We tested this hypothesis in vitro and in vivo by nitrating NRG-1’s EGF-like domain and assessing the functional characteristics of nitrated NRG-1 (nNRG-1).

MATERIALS AND METHODS

Cell culture. The cell lines MCF-7 and A549 (human breast and lung adenocarcinoma, respectively) were purchased from American Type Culture Collection (Manassas, VA) and maintained in DMEM/Ham’s F-12 (1:1) medium supplemented with penicillin (100 IU/ml), streptomycin (100 μg/ml), l-glutamine (0.292 mg/ml), and 10% heat-inactivated fetal bovine serum (FBS; all from Mediatech, Herndon, VA) at 37°C in a humidified 5% CO2 atmosphere.

NRG-1 nitration. Recombinant NRG-1177–244 (a gift from Genentech, San Francisco, CA) or bovine serum albumin (BSA; Sigma Chemical, St. Louis, MO), in the amount of 2.5 μM, was nitrated by reaction with 12 mM tetranitromethane (TNM; Sigma Chemical) at room temperature. After 30 min, the nitration reaction was stopped by 10.220.32.247 on June 24, 2017 http://ajplung.physiology.org/ Downloaded from http://ajplung.physiology.org/ by 10.220.32.247 on June 24, 2017

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with 2-mercaptoethanol (5% final concentration). All samples were dialyzed against a 3-kDa molecular weight cut-off membrane to remove TNM and 2-mercaptoethanol before use (Microcon YM-3; Millipore, Bedford, MA). Nitrification was confirmed by Western blotting-treated NRG with antinitrotyrosine (1:5,000 in 2% BSA overnight at 4°C; Upstate Biotechnology, Charlotte, VA).

Western blot analysis and immunoprecipitation. Cells were lysed in cold lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100) containing protease inhibitors (50 μg/ml aprotinin, 10 μg/ml leupeptin, 50 μg/ml pepstatin, 0.4 mM EDTA, 0.4 mM sodium vanadate, 10 mM sodium fluoride, and 10 mM sodium pyrophosphate), transferred to Eppendorf tubes, and centrifuged to pellet cell debris. Protein concentrations were determined by the Bradford method (Bio-Rad, Hercules, CA). Equal protein amounts of lysate were added to Laemmli sample buffer and heated (100°C) for 5 min. Proteins were separated by electrophoresis on 7.5% or 10% SDS-PAGE gels (Bio-Rad) and electroblotted onto polyvinylidene difluoride membranes. Nonspecific binding was blocked by incubating blots in 5% nonfat dry milk or 3% BSA in PBS/Tween 20 overnight at 4°C. Membranes were probed with NRG-1 polyclonal antibody (C20), phosphorylated HER2 polyclonal antibody (Tyr1248), HER2 polyclonal antibody (C18) (all from Santa Cruz Biotechnology, Santa Cruz, CA), or FLAG polyclonal antibody (Affinity Bioreagents, Golden, CO). Specific protein bands were visualized by enhanced chemiluminescence detection (Amersham, Piscataway, NJ) following incubation with secondary antibodies conjugated to horseradish peroxidase (1:5,000 dilution, Santa Cruz Biotechnology). Autoradiographs were digitized and the relative amounts of individual proteins quantified by densitometry using NIH Image software. SDS-PAGE gels were silver-stained for total protein using Plus-One Silver Stain Kit (Amersham Biosciences) following the manufacturer’s recommendations. Following development, gels were visualized and digitized (Mustek 1200 III EP scanner, Mustek, Irvine, CA).

For immunoprecipitation studies, cell medium was collected following stimulation with cytokines (see protocol below) and incubated with 2 μg of appropriate antibody [FLAG or nitrotyrosine (NT)] overnight at 4°C with mixing. The antibody-protein complexes were incubated with protein A beads for 2 h at 4°C and recovered by centrifugation. The beads were washed three times in PBS and resuspended in Laemmli sample buffer. Western blot analysis was conducted as described above.

Cell stimulation with NRG-1. For receptor studies, MCF-7 cells were plated in six-well culture plates (200,000 cells/well) in DMEM/Ham’s F-12 medium containing 10% FBS and allowed to adhere for 24 h. Medium was then changed to serum-free DMEM/Ham’s F-12, and cells were incubated for an additional 24 h. Cells were stimulated with various concentrations of NRG-1 or nNRG-1 in serum-free medium, alone or in combination, for various times depending on the particular experiment being conducted.

For proliferation studies, MCF-7 cells were plated in 96-well cell culture plates (2,000 cells/well) and allowed to adhere in DMEM/Ham’s F-12 medium containing 10% FBS and allowed to adhere for 24 h. Medium was then changed to serum-free DMEM/Ham’s F-12 containing 1% BSA overnight at 4°C and recovered by centrifugation. The beads were washed three times in PBS and resuspended in Laemmli sample buffer. Western blot analysis was conducted as described above.

NGF-1-FLAG construction. NRG-1-FLAG was constructed from a full-length NRG-1 cDNA template using PCR techniques to epitope tag the carboxy terminus with a FLAG sequence (forward primer 5’-GTCGACGGCGGAGATGTCGCCAGCGCAAGAGA-3’, reverse primer 5’-GTCGACATTATTTTTGCTGTGCAGTCTTGGTAGTCTCACGCAATAGGTC-3’). PCR products were gel-purified on a 1% agarose/Tris-ace-tate-EDTA gel. DNA bands were extracted using a QiAquick Gel Extraction Kit (Qiagen, Valencia, CA) and ligated into pcDNA3 (Invitrogen, Carlsbad, CA). Escherichia coli (HB101 competent cells, Invitrogen) were transformed and selected for ampicillin resistance. Isolated colonies were analyzed by Xhol digestion. Clones that yielded restriction fragments of the proper size were expanded, and pcDNA3.NRG-1-FLAG plasmid was isolated using the QIAfilter Plasmid Maxi Kit (Qiagen).

Cytokine stimulation. A549 cells were transfected with pcDNA3.NRG-1-FLAG using Lipofectamine (Invitrogen) following the manufacturer’s protocol. Transfected cells were stimulated with a cytokine mixture consisting of 0.5 ng/ml IL-1β, 10 ng/ml TNF-α, and 10,000 IU/ml IFN-γ in serum-free medium with or without polyethylene glycol superoxide dismutase (PEG-SOD; 2,000 U/A). Unstimulated control cells were maintained in serum-free medium without cytokine exposure. After 72 h, conditioned medium was collected and concentrated against a membrane with a 30-kDa cut-off (Amicon Centriprep-30, Millipore). The concentrated, conditioned media was subjected to immunoprecipitation using FLAG or NT antibodies and Western blotted for FLAG or NT.

Mass spectroscopy. The Mass Spectroscopy Core Laboratory at The Cleveland Clinic Foundation conducted mass spectroscopy analyses. Proteins separated by SDS-PAGE and stained with Coomassie blue were cut from the gel, destained, reduced, and alkylated in 10 mM dithiothreitol and 50 mM iodoacetamide in 0.1 M ammonium bicarbonate at room temperature. Protein was then dehydrated, rehydrated, washed in 0.1 M ammonium bicarbonate, and dehydrated again. Trypsin (20 ng/μl in 50 mM ammonium bicarbonate) was added, driven into the gel pieces by rehydration, and digested overnight at room temperature. The digested peptides were extracted, taken to dryness, and reconstituted in 1% acetic acid. Samples were analyzed using a liquid chromatography-tandem mass spectroscopy (LC-MS) system with reversed-phase HPLC columns packed with Phenomenex Jupiter C18. Full-scan mass spectra were acquired from each digest to determine peptide molecular weights. Product ion spectra were acquired to determine amino acid sequence in successive instrument scans.

Statistics. An unpaired t-test was used to compare single variables across groups to determine statistical differences. All data are presented as means ± SD. A P value of <0.05 was taken to indicate statistical significance.

RESULTS

NRG-1 is nitrated in vitro. To determine the effect of nitration on NRG-1, NRG-1’s EGF-like domain (amino acids 177–244) was exposed to the nitrating reagent TNM. NRG-1’s EGF-like domain carries all the biological activity of the full-length molecule. Therefore, its integrity is critical to ligand function. Four tyrosine residues are present in this domain and could serve as targets for nitrification, thus altering ligand function. After TNM exposure, TNM-treated NRG-1 and untreated NRG-1 controls were separated by gel electrophoresis and Western blotted for NT. As shown in Fig. 1A, NT was identified in TNM-treated NRG-1, but not in NRG-1 that was not exposed to TNM.

Nitrification clearly altered NRG-1, first seen with a loss of NRG-1 immunoreactivity. When TNM-treated and untreated NRG-1 were Western blotted using an antibody directed against NRG-1’s EGF-like domain (Fig. 1B), untreated NRG-1 was easily identified as a 7,500-Da band (Fig. 1B, lane 1). However, nNRG-1 was not recognized by the antibody (Fig. 1B, lane 2).

To examine whether loss of immunoreactivity was the result of nonspecific protein degradation, TNM-treated and untreated

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NRG-1 were subjected to gel electrophoresis followed by silver staining for total protein content. Figure 1 shows that the nitration reaction did not degrade NRG-1 to explain the loss of NRG-1 immunoreactivity. The total amount of NRG-1 present after TNM exposure was similar to NRG-1 carried through the same reaction and purification process but not exposed to TNM. In addition, no other peptide bands were present to suggest proteolysis.

Mass spectroscopy was performed to compare the structure of nNRG-1 to NRG-1 and confirm nitration. No amino acids were nitrated in NRG-1. TNM exposure, however, resulted in nitration of three of the four tyrosine residues present in NRG-1’s EGF-like domain. Tyr208 was always nitrated. Tyr224 and Tyr230 were found nitrated together, and Tyr224 was never nitrated in the absence of Tyr230 nitration. Tyr244 was not nitrated.

Nitration reduces NRG-1’s ability to activate its receptor. To examine the effect of nitration on NRG-1’s functional ability, MCF-7 cells were used to determine nNRG-1’s capacity to activate the HER2/HER3 complex. The MCF-7 cell line is derived from a human breast adenocarcinoma, expresses HER2 and HER3, and responds to exogenous NRG-1 with activation of HER2’s kinase domain. Cells were cultured in serum-free medium for 24 h and then exposed to 10 nM NRG-1 or nNRG-1. Figure 2A shows that stimulation of MCF-7 cells with 10 nM NRG-1 activated the HER2 as evidenced by a 21.2 ± 2.05-fold increase in phosphotyrosine content. However, nNRG-1’s ability to induce HER2 phosphorylation was significantly reduced. nNRG-1 induced a 7.03 ± 1.86-fold increase in HER2 phosphorylation, a 66.8% ± 0.35% reduction compared with NRG-1 (P < 0.01). The decrease in HER2 activation was not corrected by increas-
ing nNRG-1 concentration (Fig. 2B). Whereas recombinant NRG-1 induced HER2 phosphorylation in the MCF-7 cell line in a concentration-dependent manner (Fig. 2B), nNRG-1 was unable to induce HER2 phosphorylation at any concentration tested.

Increasing nNRG-1 exposure time did not overcome the loss of HER2 activation. nNRG-1 was able to minimally activate HER2 at long exposure times with HER2 phosphorylation peaking 28 min after exposure, 4.57 ± 0.81-fold over baseline (Fig. 2C, top). In contrast, addition of 10 nM NRG-1 to MCF-7 cells caused maximal HER activation at 7 min, 11.3 ± 2.84-fold over baseline (Fig. 2C, top; P < 0.01 compared with nNRG-1 activation).

NRG-1 nitration affects receptor binding. The inability of nNRG-1 to induce HER2 phosphorylation, and the importance of the nitrated tyrosine residues in binding HER3 in vitro, suggested that nitration had altered NGR-1’s ability to bind HER3 and activate the HER2/HER3 heterodimer. We performed competition experiments with NRG-1 and nNRG-1 to determine the extent to which nNRG-1 retained the ability to compete with NRG-1 for receptor binding and activation. MCF-7 cells were preincubated with nNRG-1 (0, 0.5, 5, or 50 nM) for 15 min, followed by the addition of NRG-1 (5 nM). Cells were lysed after 7 min, and equal quantities of total protein were Western blotted for phosphorylated HER2 (pHER2) and HER2. As shown in Fig. 3A, increasing concentrations of nNRG-1 did not affect NRG-1’s ability to activate HER2/HER3, as no effect was seen on the pHER2 signal. For comparison, MCF-7 cells were stimulated with decreasing concentrations of NRG-1 (5, 2.5, or 0.5 nM) to correlate with the effective NRG-1 concentration if nNRG-1 competed for HER3 binding (Fig. 3B). The pHER2 signal resulting from stimulation with 0.5 nM NRG-1 was 1.85-fold lower than that of 5 nM NRG-1 stimulation and approximates HER2 phosphorylation that should result if nNRG-1 (50 nM) competed with NRG-1 (5 nM) for receptor binding. These data further support that nNRG-1 did not compete with NRG-1 for receptor binding.

To confirm the functional impact of NRG-1 nitration, we studied nNRG-1’s effect on proliferation. MCF-7 cells were cultured with 1 nM NRG-1 or 1–50 nM nNRG-1. As expected, NRG-1 increased proliferation compared with unstimulated cells; cell mass was 2.0 ± 0.9-fold higher at 3 days and 2.44 ± 0.8-fold higher at 4 days compared with unstimulated cells (Fig. 4A; P < 0.001). In contrast, nNRG-1 failed to induce proliferation at any concentration tested. As expected, with its loss of receptor binding, nNRG-1 did not inhibit NRG-1-induced proliferation. Figure 4B shows that 1 nM NRG-1 increased proliferation with no effect of nNRG-1 (50 nM) on NRG-1-induced proliferation (Fig. 4B).

NRG-1 nitration occurs in vivo. To determine the extent of NRG-1 nitration in vivo, we constructed an epitope-tagged,
full-length NRG-1 cDNA (NRG-1-FLAG). The protein coded by this construct could be identified through its FLAG epitope, avoiding the problem caused by alteration of the NRG-1 epitope through nitration (see Fig. 1). The FLAG sequence has no tyrosine residues and can be identified by a FLAG antibody regardless of NRG-1’s nitration status. This cDNA was cloned into the pcDNA3 expression vector and used for in vivo nitration studies.

A549 cells were transfected with the pcDNA3.NRG-1-FLAG expression plasmid and stimulated with serum-free medium containing a combination of IL-1β, TNF-α, and IFN-γ to induce iNOS expression. In prior studies, this combination of cytokines caused nitration of endogenous proteins (2). After 72 h, the culture medium was collected, concentrated, NRG-1-FLAG-immunoprecipitated using FLAG or a NT antibody, and Western blotted for FLAG. As shown in Fig. 5, cytokine stimulation induced NRG-1 nitration with a 155% increase in NT content ($P < 0.05$).

A549 cells were also stimulated with cytokines in the presence of PEG-SOD (2,000 U/l), a potent antioxidant that dismutates superoxide to oxygen and hydrogen peroxide. PEG-SOD greatly decreased cytokine-induced nitration, with nitration almost remaining at baseline (Fig. 5).

**DISCUSSION**

The current study examined our hypothesis that nitration of growth factors may represent a posttranslational mechanism to further regulate their function. Using NRG-1 to test our hypothesis, we found that NRG-1’s EGF-like domain was nitrated following treatment with TNM. Analysis by mass spectrometry revealed that three tyrosine residues, 208, 224, and 230, were nitrated. Of particular interest are Tyr208 and Tyr224. Alanine-scanning mutagenesis studies of NRG-1, in which specific amino acids were mutated to alanine, have demonstrated that these two tyrosine residues are critically important for the binding of NRG-1 to receptor (17). Significantly, nitration altered NRG-1’s immunoreactivity such that an antibody specifically directed against its EGF-like domain no longer recognized the protein. Using the MCF-7 cell line, nitration also altered NRG-1 function such that its activation of HER2/HER3 was greatly decreased. In competition experiments conducted with NRG-1 and nNRG-1, nNRG-1 did not compete with NRG-1 for HER2/HER3 activation. Functionally, nitration eliminated NRG-1’s ability to induce proliferation of the MCF-7 breast cancer cell line. Last, nitration of NRG-1 occurred in vivo, as media collected from cells stimulated with a mixture of cytokines known to induce nitration through induction of iNOS displayed increased levels of nNRG-1.

Nitration or similar posttranslational modifications have been shown to regulate the function of a number of proteins. MnSOD (6), GAPDH, and heat shock protein 90 (35) were nitrated on tyrosine residues under conditions in which iNOS was upregulated and nitric oxide production increased.
nitrated, normal protein function was altered and in some cases eliminated. A recent report by Vadseth et al. (32) indicated clots formed from nitrated fibrinogen could be more easily deformed by mechanical stress than those from nonnitrated fibrinogen. Although more easily deformed, increased fibrinogen nitration could lead to a prothrombotic state by accelerating fibrin clot formation. In airway epithelial cells, nitration inactivated MnSOD and led to an increase in apoptosis (6). Heme oxygenase-1, a protein that catalyzes the degradation of heme to produce iron, carbon monoxide, and biliverdin, has recently been shown to be inhibited by both TNM and peroxynitrite nitration in vitro (18). Osna et al. (23) have demonstrated that peroxynitrite and SIN-1, a nitric oxide donor, dose dependently modulated the activity of the proteasome, an enzyme complex that degrades misfolded and oxidized proteins. Myofibrillar contractile function, both cardiac and diaphragmatic, is severely decreased (21, 31) following incubation with peroxynitrite or SIN-1. Last, nitric oxide synthase-mediated formation of peroxynitrite during hypoxia in guinea pig brain decreases Na\(^+\)-K\(^+\)-ATPase activity by 36–65% (27). It is clear, therefore, that nitration can have a dramatic impact on protein function. In the majority of the cases cited above, nitration was caused by peroxynitrite, the reaction product of nitric oxide and superoxide. Of note, the active domain of NRG-1 contains seven amino acids (6 cysteine and 1 histidine residues) known to be sensitive to peroxynitrite (8, 16). In this study, cytokine-induced nitration of NRG-1 was decreased by the addition of PEG-SOD, an inhibitor of superoxide. These results implicate peroxynitrite as the species responsible for NRG-1 nitration in this model.

Following nitration, alterations in protein function, predominantly loss of function, have been observed in a number of disease states. In cardiac and renal ischemia, for example, tyrosine residues in MnSOD are nitrated, resulting in its inactivation (7). With the loss of MnSOD protection against oxidant damage, further injury can occur through the action of reactive nitrogen species. During sepsis, lipopolysaccharide (LPS) reduced liver glutamine synthetase (GS) activity greater than 50%. This reduction in activity correlated with nitration of 1 histidine residues (known to be sensitive to peroxynitrite (8, 16)). In this study, cytokine-induced nitration of NRG-1 was decreased by the addition of PEG-SOD, an inhibitor of superoxide. These results implicate peroxynitrite as the species responsible for NRG-1 nitration in this model.

To our knowledge there are no reports of the specific effects of nitration on growth factors. These proteins are clearly regulated by posttranslational modification, however. For example, modification of EGFR by stimulatory G protein subunits (Go) leads to ligand-dependent degradation of the receptor (36). High level nitric oxide production, as occurs with iNOS, is attributed with anti-tumor and antiproliferative effects (1, 3, 25, 28), although the precise mechanisms are not well defined. Posttranslational modification and inactivation of growth factors by nitration may be one mechanism of the nitric oxide effects.

In vivo, multiple cellular pathways exist by which growth factors and other proteins may become nitrated. In our studies, we employed inflammatory cytokines (IL-1β, TNF-α, IFN-γ) to induce iNOS and the subsequent generation of nitrogen species such as peroxynitrite (2, 14). Reactive nitrogen species generated via this pathway have been demonstrated in human airway epithelial cells (9, 13) and can nitrate amino acids, particularly tyrosine residues (2, 20). Our results demonstrate that NRG-1 can become nitrated by this or a similar pathway in vivo. In pulmonary epithelium, NRG-1 and iNOS are both expressed on the epithelium’s apical surface (24, 34). Nitric oxide and peroxynitrite generated by iNOS, therefore, can potentially interact with NRG-1 and abolish NRG-1 function.

The relative importance of this posttranslational process in regulating NRG-1 function is not clear. In addition, receptor nitration may also contribute to the overall effect. NRG-1 binds to domains I and II of the HER3 extracellular domain (29), and a tyrosine residue (Tyr50) in this region is involved in binding (29). Nitration of this tyrosine could further impact the binding characteristics of NRG-1 and influence receptor activation. In previous reports, we (19, 22, 26) have shown that modulation of the NRG-1/HER2/HER3 axis plays an important role in epithelial cell proliferation and recovery from lung injury. Nitration of growth factors may represent a posttranslational mechanism by which signaling is further regulated to affect outcome of pulmonary disease states, such as tumor proliferation, or chronic inflammatory states, such as asthma.

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