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Clinical Cancer Research

Molecular Pathways: Targeting *NRG1* Fusions in Lung Cancer

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Abstract

The four members of the ERBB (HER) family of transmembrane receptor tyrosine kinases are frequently activated in cancer by several mechanisms, such as mutation, amplification, or autocrine ligand–receptor stimulation. We recently identified gene fusions involving the ERBB ligand gene, *NRG1*, which represent a novel mechanism for ERBB pathway deregulation. These fusions lead to expression and presentation of the EGF-like domain of NRG1 on the cell surface, which binds to ERBB3 in an autocrine and juxtacrine manner, thus inducing the formation of ERBB2–ERBB3 heterodimers, and subsequent activation of the PI3K–AKT and MAPK signaling pathways. These fusion genes were exclusively detected in lung adenocarcinomas of never smokers of the invasive mucinous subtype, which usually presents as a multifocal and unresectable disease, for which no effective treatment exists. Considering the large amount of drugs that target ERBB2 (HER2) and ERBB3 (HER3), and which are currently in different stages of clinical development, detecting and targeting NRG1 fusions in invasive mucinous lung adenocarcinomas may represent a therapeutic opportunity for this aggressive disease. *Clin Cancer Res; 21(9); 1989–94. ©2014 AACR.*

Background

ERBB1 (EGFR; ref. 1), ERBB2 (HER2; ref. 2), ERBB3 (3, 4), and ERBB4 (5) constitute the ERBB family of transmembrane receptor tyrosine kinases (RTK). These RTKs are composed of a large extracellular ligand-binding domain, a transmembrane domain, a small intracellular juxtamembrane domain, a kinase domain, and a C-terminal tail (6). Upon ligand binding, these receptors form hetero- or homodimers, which induce the phosphorylation of their intrinsic kinase domain, resulting in the activation of the downstream PI3K–AKT and MAPK pathways (7–9). There are 13 polypeptide extracellular ligands for these RTK, all of them carrying an EGF-like consensus sequence, consisting of six cysteine residues. These ligands can be subdivided in those that specifically bind EGFR and ERBB4 (such as EGF, TGF-a, amphiregulin, epiregulin, BTC, HB-EGF, and EPR), and the neuregulins (NRG), which bind to ERBB3 (NRG1 and NRG4) and to ERBB4 (NRG1–NRG4; ref. 10). ERBB receptors are expressed in many cells of the epithelial, mesenchymal, and neuronal lineages, in which they have diverse roles in development, proliferation, and differentiation (11). Aberrant tyrosine kinase activity of ERBB receptors owing to gene amplification and mutations (EGFR and ERBB2;refs. 12–14), or autocrine ligand–receptor stimulation (10) can result in growth stimulation and tumorigenesis in various tumor types, including cancers of the breast, lung, brain, head and neck, and colon (12).

Contrary to ERBB1 and ERBB4, ERBB2 and ERBB3 are nonautonomous receptors and therefore their activation relies on their heterodimerization with other ERBB receptors: ERBB2 lacks the capacity to interact with growth factor ligands (15) but is the preferred dimerization partner of the other three receptors (16). ERBB3 is generally considered to be kinase deficient (17), although it has a low level of kinase activity (1,000-fold less than EGFR; refs. 18, 19). Interestingly, the heterodimer ERBB2–ERBB3 is considered the most transforming and mitogenic one (20–22), as suggested by the following: first, ERBB2-containing heterodimers undergo slow endocytosis, recycling more frequently back to the cell surface (23–25). Similarly, upon ligand binding and heterodimerization, ERBB3 undergoes tyrosine phosphorylation and can recruit PI3K but not GRB2, enabling ERBB3 to evade ligand-induced degradation (23, 26); and second, ERBB2–ERBB3 leads to the activation of both the PI3K–AKT and the MAPK pathways, strongly enhancing cellular proliferation and evasion of apoptosis (20, 21).

Because the main ligands for ERBB3 are NRGs, these molecules also activate ERBB2–ERBB3 heterodimers. In fact, coexpression of ERBB2 and ERBB3 constitutes a high-affinity receptor for these ligands (27). NRGs are signaling proteins mainly expressed in the nervous system, the heart, and the breast (28). They comprise a large family of growth factors that induce dimerization and activation of ERBB receptors (29). NRGs are encoded by four individual genes (*NRG1–4*), of which *NRG1* is the best characterized. *NRG1* generates six types of proteins (I–VI), and at least 31 isoforms (29). All of these isoforms have an extracellular EGF-like domain that induces activation of ERBB RTKs. Contrary to most NRG1 isoforms that undergo proteolytic cleavage resulting in the release of the EGF-like domain, type III NRG1s have a membrane-tethered EGF-like domain that restricts the signaling to cell–cell interactions mainly, although a small amount of the EGF-like domain of these isoforms has been found secreted into the medium (30).

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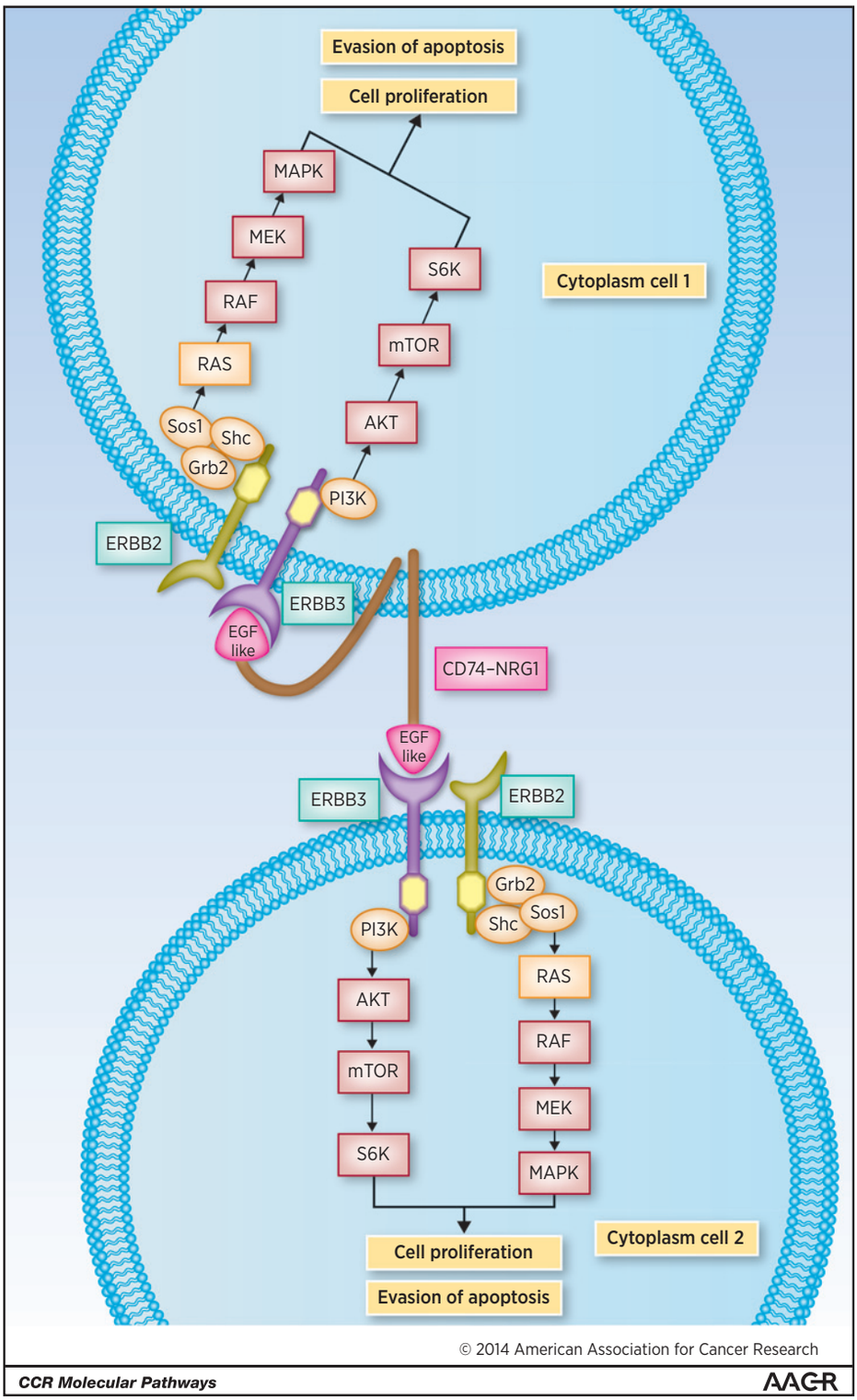

Figure 1.
Schematic representation of the molecular pathways activated by CD74–NRG1. CD74–NRG1 provides the ligand for ERBB3 receptors inducing its heterodimerization with ERBB2, and subsequent activation of the PI3K–AKT and MAPK pathways, in an autocrine (cytoplasm cell 1) and juxtacrine (cytoplasm cell 2) manner, resulting in an abnormal cell proliferation. The mechanism of action is expected to be the same for SCL3A2–NRG1. In the CD74–NRG1 fusion protein, the part coming from CD74 is colored in brown and the part coming from NRG1, in pink. Kinase domains of ERBB2 and ERBB3 receptors are colored in yellow. Adaptor and enzymes are represented in orange and members of the signaling cascade, in red.

ERBB3 is not frequently affected by mutation or amplification in cancer (14); in contrast, overproduction of ligands is another mechanism by which cancers aberrantly activate ERBB receptors. Several examples point toward the existence of an autocrine loop between ERBB3 and NRG1: in ovarian tumors that overexpressed NRG1 and ERBB3, inactivation of ERBB3 suppressed cancer

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Table 1. Overview of ERBB3 inhibitors

Name (antibody)	Target	Biologic effect	Clinical status
MM-121 (SAR256212)	ERBB3	In combination with cetuximab, it blocks reactivation of ERBB3 in mice EGFR T790M-L858R, resulting in a sustained and durable response (31, 55, 56)	–Completed phase I safety and dose-finding trial –Several phase I and phase II studies ongoing –Refs 62–66
MM-111	ERBB2 and ERBB3	Antitumor activity in preclinical models that are dependent on ERBB2 overexpression (57)	–Completed phase I safety and dose-finding trial –Several phase II studies ongoing –Refs 52, 53
MM-141	IGF-IR and ERBB3	Prevents PI3K/AKT/mTOR network adaptation by blocking the redundant survival pathways and degrading the RTK complexes (58)	–Ongoing phase I study to establish the recommended dose and schedule –Ref. 67
MEHD7945A	EGFR and ERBB3	It has superior antitumor activity against multiple tumor models compared with monospecific antibodies (59)	–Completed phase I safety and dose-finding trial, with evidence of anti-tumor activity –Several phase I and phase II studies ongoing –Ref. 68
LJM716	ERBB3	It inhibits ligand-induced and ligand-independent ERBB3 dimerization (60)	–Completed phase I safety and dose-finding trial, with evidence of antitumor activity –Several phase I and phase II studies ongoing –Refs 69, 70
RG7116 (RO5479599)	ERBB3	It inhibits xenograft tumor growth and downregulates cell surface ERBB3. Its efficacy is further enhanced in combination with anti-ERBB2 (pertuzumab) antibodies (61)	–Completed phase I safety and dose-finding trial, with evidence of antitumor activity –Several phase I studies ongoing –Ref. 71
U3-1287 (AMG 888, patritumab)	ERBB3	It blocks the upregulation of total and phosphorylated ERBB3 that follows treatment with lapatinib and trastuzumab, and enhances the antitumor action of the combination against trastuzumab-sensitive and -resistant cells (48)	–Completed phase I safety and dose-finding trial –Several phase I, phase II, and phase III studies ongoing –Ref. 72

growth in laboratory models (31). Similarly, an NRG1-mediated autocrine loop inducing ERBB3 activation was also discovered in head and neck cancer cells lacking *ERBB2* amplification, and these cells were particularly sensitive to the EGFR/ERBB2 kinase inhibitor, lapatinib (32). Finally, analyses of prostate cancer cells and tumors suggest the existence of a paracrine loop involving NRG1 and the ERBB2–ERBB3 heterodimer (33).

We recently discovered recurrent *CD74–NRG1* gene fusions, which result in the expression of the NRG1 III-β3 isoform in the otherwise NRG1-negative invasive mucinous subtype of lung adenocarcinomas (34). CD74–NRG1 activates ERBB3 as well as PI3K–AKT signaling (34), thus promoting anchorage-independent growth of NIH-3T3 cells (ref. 35; Fig. 1). Furthermore, NRG1 fusion–mediated signaling could be suppressed by clinically approved kinase inhibitors (35). *NRG1* fusions are preferentially found in women who had never smoked, and whose tumors are negative for other known mutant oncogenic gene mutations such as those in *KRAS* [frequently mutated in invasive mucinous adenocarcinoma (IMA)], or *EGFR* (34, 35). In another study, *CD74–NRG1*-positive tumors displayed uncommon radiologic features in comparison with what it is usually seen in IMA (36).

Clinical–Translational Advances

Invasive mucinous adenocarcinoma accounts for 2% to 10% of all lung adenocarcinomas and is associated with an unfavorable clinical course because it usually presents as multifocal unresectable disease, for which no effective treatment exists (37–40). *KRAS* mutations were, until recently, the only recurrent oncogenic mutations identified in IMA, with a frequency ranging from 50% in Asians to 80% in Caucasians. *NRG1* fusions are the first potentially treatable oncogenic driver alteration associated with a specific lung adenocarcinoma subtype and in contrary to *KRAS* mutations, which are associated with tobacco use, *NRG1* fusions occur in *KRAS*-negative IMA of never smokers. Taking all together, the discovery of recurrent, oncogenic, and possibly targetable *NRG1* fusions may represent a therapeutic opportunity for these tumors (34, 35).

Because NRG1 fusions act through activation of ERBB2–ERBB3 heterodimers (34), blocking the activity of these receptors may be the best strategy to treat *NRG1*-rearranged tumors. Similar to EGFR and ERBB4, the extracellular domain of ERBB3 probably exists in two different conformations: a tethered autoinhibited form and an untethered ligand-bound form (41). In contrast, the crystal structure of ERBB2 reveals a constitutively activated conformation similar to that of ligand-bound EGFR. Although more mechanistic details exist for ERBB2 than for ERBB3 (14), and therefore more drugs are available for the former, blocking only this receptor might not be enough to completely shut down the signaling elicited by ERBB2–ERBB3. In fact, the effect of single-agent lapatinib or afatinib (irreversible small-molecule inhibitor of the ERBB2 and EGFR tyrosine kinases; ref. 42) has already been tested for *NRG1* fusions *in vitro*, and downstream signaling was not completely shut down, although an inhibitory effect was observed (35). Reactivation of ERBB3 has been observed as a resistance mechanism to EGFR and ERBB2 inhibitors (31, 43–49), and such mechanism might help *NRG1*-rearranged tumor cells escape exclusive targeting of ERBB2. A more successful approach could be to

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block ERBB2–ERBB3 heterodimerization. In this context, ligand-induced ERBB2–ERBB3–PI3K complexes can be disrupted by pertuzumab (monoclonal antibody that recognizes an epitope in the heterodimerization domain II of ERBB2; ref. 50), whereas ligand-independent complexes might be dissociated by trastuzumab (51). However, it is important to note that the observed clinical efficacy of all therapeutic inhibitors against ERBB2 has been limited to breast cancer with overexpression of this gene (14).

Several ERBB3 inhibitors showing promising preclinical data are currently in different phases of clinical trials. A selection of those, for which substantial preclinical and clinical data exist, is presented in Table 1. Several of these are being tested in combination with ERBB2-targeting drugs in breast and gastric ERBB2-positive tumors (refs. 52, 53; Clinical trials: NTC01602406, NTC02167854, NTC01512199). However, although the outcome of these clinical trials will help gauge the benefit that dual ERBB2 and ERBB3 inhibition might have in cancer patients, a clinical trial in a selected cohort of *NRG1* fusion–positive cases is necessary to determine the benefit for this specific group of patients. Finally, given the activation of the PI3K–AKT signaling pathway, its inhibition may represent another therapeutic strategy. Inhibitors targeting PI3K itself or AKT have been in clinical development for several years now. Unfortunately, identification of the right patient population is not as clear as anticipated from preclinical experiments (54). Thus, inhibition of the upstream ERBB family members, ERBB2 and ERBB3, might be more promising at this point.

Disclosure of Potential Conflicts of Interest

L. Fernandez-Cuesta and R.K. Thomas are listed as coinventors of a patent pending on NRG1 fusions in lung cancer, which is owned by the University of Cologne. R.K. Thomas is a founder and shareholder of Blackfield AG/New Oncology; reports receiving commercial research grants from AstraZeneca, EOS, and Merck; and is a consultant/advisory board member for AstraZeneca, Bayer, Blackfield AG/New Oncology, Boehringer Ingelheim, Clovis Oncology, Daiichi-Sankyo, Eli Lilly, Janssen, Merck, Puma, Roche, and Sanofi. No other potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: L. Fernandez-Cuesta, R.K. Thomas
Development of methodology: L. Fernandez-Cuesta, R.K. Thomas
Writing, review, and/or revision of the manuscript: L. Fernandez-Cuesta, R.K. Thomas
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L. Fernandez-Cuesta, R.K. Thomas
Study supervision: L. Fernandez-Cuesta, R.K. Thomas

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