Review

A Translational Approach to Lung Cancer Research: From EGFRs to Wnt and Cancer Stem Cells

Adam Yagui-Beltrán, MD, and David M. Jablons, MD

Lung cancer remains the main cause of all cancer deaths in the United States. The prognosis for non-small cell lung cancer, despite advances in current therapies, is disappointing. Fortunately, we are steadily gaining significant insights into the heterogeneous molecular pathogenesis of lung cancer, which seems to occur in a stepwise manner, mainly secondary to tobacco smoking. With the emerging power of gene expression signatures for individual lung tumors and with the advancing field of stem cell biology and the paradigm of cancer stem cells, we are most certainly paving the way to developing novel tools for the early detection, chemoprevention, and treatment of these vastly morbid pathologies with enormous global burden. We will explore some of these issues and highlight how we are starting to translate them into clinically relevant tools for lung cancer patients. (Ann Thorac Cardiovasc Surg 2009; 15: 213–220)

Key words: non-small cell lung cancer, angiogenesis, epidermal growth factor receptor, novel targeted therapy, stem cell

Introduction

Lung cancer annually causes more than 1 million deaths worldwide.[1)] Despite efforts aimed at improving survival, delayed diagnosis and high relapse result in dismal prognosis. Overall lung cancer 5-year survival rates have only marginally changed over the past few decades; the current 5-year survival rate is about 15% in the United States and lower in developing countries.[1)] Many of the genetic and epigenetic anomalies found in lung cancer are also present in normal and preneoplastic tissue, suggesting a multistep process of epithelial carcinogenesis contemporary to tobacco smoking.[2)] Good examples of the translation of pulmonary molecular events to the clinic include of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs). The growing field of stem cell research is becoming relevant in the understanding of lung carcinogenesis and to the development of novel targeted therapies.[3–7)] Identifying indi-viduals at high risk of lung cancer is crucial, and various biomarkers such as hypermethylation of certain lung cancer genes are being tested clinically.[8)]

The Molecular Basis of Lung Cancer

Lung carcinogenesis, like in the breast and colon, also follows a multistep oncogenic process.[9–11)] Atypical adenomatous hyperplasia (AAH), a premalignant lesion thought to be a precursor to bronchioalveolar carcinoma (BAC), is frequently adjacent to invasive adenocarcinoma.[12–15)] The elaborate collection of genetic abnormalities of disrupted pathways caused by environmental carcinogens, such as tobacco, result in the heterogeneous nature of lung cancers.

The Clinical Relevance of the Growth Factor Receptor Tyrosine Kinase

The tyrosine kinase activity of the EGFR was character-

From Department of Surgery, The University of California San Francisco, Helen Diller Family Comprehensive Cancer Center, San Francisco, USA

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Address reprint requests to Adam Yagui-Beltrán, MD: Department of Surgery, The University of California San Francisco, Helen Diller Family Comprehensive Cancer Center, 1600 Divisadero St., Box 1724, San Francisco, California 94143, USA.
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ized more than two decades ago.[16] The EGFR (HER1) is part of a bigger family of transmembrane receptors, including HER2, HER3, and HER4. They activate the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3, 4, 5 kinase (PI3k)/protein kinase B (PKB) signaling pathways, among others;[17] a disruption of these results in abnormal epithelial development, angiogenesis, and malignancy in most organs.[18,19] Moreover, high EGFR protein levels have been demonstrated in human lung, ovarian, and upper and lower gastrointestinal cancers.[18–22] HER1 and HER2 are overexpressed in about 70% and 30% of non-small cell lung cancers (NSCLCs), respectively. Tobacco-related dysplastic lung tissue has increased EGFR expression compared to hyperplastic and metaplastic lesions, suggesting a stage-dependent involvement in lung cancer.[23–25] Gefitinib and erlotinib are adenosine triphosphate (ATP) competitive inhibitors of the EGFR tyrosine kinase domain. Phase I trials of gefitinib demonstrated benefits in NSCLC. Two large phase II trials, Iressa Dose Evaluation in Advanced Lung Cancers (IDEALs) 1 and 2 in patients previously treated with conventional chemotherapy[26] showed response rates in the range of 9%–19%, compared to only 7% for docetaxel. The efficacy of erlotinib in NSCLC was illustrated in the Br.21 trial[27] by a higher median survival compared to placebo (6.7 months vs. 4.7 months) as well as a higher 1-year survival (31.2% vs. 21.5%). Interestingly, two large randomized trials, The Iressa NSCLC Trial Assessing Combination Treatment (INTACT) 1 and 2, showed that when gefitinib was used together with a first-line platinum-based agent, no survival advantage over chemotherapy alone was shown.[28,29] This may be explained by the antagonism between the cytotoxic effect of chemotherapy and the cytostatic (causing G_1 arrest) effects of the EGFR-TKI. The results of trials of sequential chemotherapy and EGFR-TKI will help clarify this. Combining erlotinib with conventional cytotoxic drugs, as in the case of gefitinib, did not prove to be useful. However, when erlotinib was combined with traditional chemotherapy in nonsmokers, a survival benefit was observed.[29,30] Mutations in the intracellular EGFR (tyrosine kinase) (EGFRTK) domain are common in never-a-smoker females of East Asian origin responding well to gefitinib.[31–34] The frequency of EGFR mutations is thought to be 39% and 48% among Japanese and Taiwanese patients, respectively, and around 3% to 9% in non-Asian U.S. patients. A new TK domain mutation (substitution of methionine for threonine at position 790 [T790M]) was found in four out of the seven patients studied.[35,36] It is interesting that the T790M mutation is analogous to a secondary mutation in bcr-abl causing resistance to imatinib in chronic myeloid leukemia (CML) patients.[37,38] These examples demonstrate that TKIs play a beneficial role in certain NSCLC patient subgroups. However, they prompt more genetic and clinical studies to understand and exploit the heterogeneity of EGFRTK domain mutations to eventually design more-efficient therapeutic TKIs.

Monoclonal Antibodies against EGFR Tyrosine Kinase

Cetuximab has been shown to confer clinical benefits through disease control in 24.13% of the EGFR-expressing NSCLC patients treated.[39] Data from phase I and II trials indicate that cetuximab combined with a first-line platinum-based agent is well tolerated, with rash being the only side effect.[40–42] The Lung Cancer Cetuximab Study (LUCAS) trial suggests that in first-line treatment of advanced NSCLC, the combination of cetuximab with cisplatin/vinorelbine shows beneficial clinical response with reasonable safety profiles compared to chemotherapy alone.[43,44] Such results led in 2004 to a large phase III multicenter trial, the First-Line ErbituX in lung cancer (FLEX) study, evaluating the efficacy of cetuximab in combination with cisplatin/vinorelbine *vs*. cisplatin/vinorelbine alone in patients with advanced untreated NSCLC. Accrual with 1,124 patients was completed in July 2007, and the specific clinical outcomes are being awaited.

Angiogenesis in Lung Cancer

Folkman et al. observed that tumors cannot grow beyond 2 mm without supporting vascularization.[45] Endothelial cells produce vascular endothelial cell growth factor (VEGF), a mitogenic factor with a myriad of physiological functions,[46] both during lung development in early life and during homeostasis in adulthood. Not surprisingly, abnormalities in the VEGF pathway can result in acute and chronic lung disease. Various VEGF families have been characterized: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental factor. VEGF ligands upon binding activate three structurally similar type III receptor tyrosine kinases: VEGF-receptor (VEGFR)-1, VEGFR-2, and VEGFR-3. Alternative gene splicing leads to several isoforms; these in turn may combine with the various receptors in a biologically heterogeneous manner. This is being exploited to develop targeted anti-

angiogenic therapies with minimal side effects.

Monoclonal Antibody against Angiogenesis

VEGF is overexpressed in many cancers, including lung cancer.[47,48] Its expression in macrophages in the lung cancer microenvironment correlates to poor prognosis. Thus modulating angiogenesis in cancer is indeed an attractive therapeutic strategy. Bevacizumab, a recombinant humanized monoclonal VEGF antibody, has shown a synergistic survival advantage in colorectal cancer.[49] A lung cancer phase II trial studying the synergy of bevacizumab and paclitaxel/carboplatin showed a significant improvement in treatment response:[50] an overall response rate (ORR) of 31.5% *vs.* 18.8% in the bevacizumab arm compared to chemotherapy alone. Furthermore, a longer disease-free interval (7.4 months *vs.* 4.2) and overall survival time (17.7 months *vs.* 14.9) was seen in the bevacizumab group. The Eastern Cooperative Oncology Group (ECOG) phase III trial of paclitaxel/carboplatin with or without bevacizumab in untreated stage IIIB or metastatic NSCLC[50] showed a 27% *vs.* 10% response rate for the bevacizumab/chemotherapy group. Progression-free survival for the former group was 6.4 *vs.* 4.5 months, and the median survival was 12.5 *vs.* 10.2 months for the chemotherapy-alone arm. This is ECOG's gold standard treatment for advanced NSCLC. Other studies are under way to assess the role of bevacizumab combined with EGFR TKIs.[51]

Miscellaneous Antiangiogenesis Agents

The number of molecules under current clinical investigation include the platelet-derived growth factor (PDGF), the platelet-derived endothelial cell growth factor (PD-ECGF), various integrins, angiopoietins, and fibroblast growth factors (FGF)-1 and FGF-2.[52,53] Below is a brief description of the ones most relevant to lung cancer.

VEGF-Trap (aflibercept)

Aflibercept is a soluble antiangiogenic decoy receptor composed of segments of extracellular domains VEGFR-1, VEGFR-2, and placental growth factor (PLGF) fused to the constant region (Fc) of human immunoglobulin (Ig) G1. Regeneron and Sanofi-Aventis have commenced an oncology development program to evaluate aflibercept with chemotherapy. Efficacy analysis showed evidence of tumor reduction and prolonged stable disease after aflibercept treatment as a single agent.[54] Under way is a phase III study of aflibercept with second-line docetaxel for metastatic NSCLC (Sanofi-Aventis: NCT00532155).

SU11248 (sunitinib)

FDA-approved sunitinib targets various TK domains, including those of VEFGR, PDGF receptor (PDGFR), and c-Kit.[55] SU11248 in NSCLC is being assessed.[56]

ZD6474

This is a low molecular weight inhibitor of the TK domain of EGFR and VEGFR-2. Its benefit in lung cancer was shown in a phase I and phase II study in metastatic tumors when used in conjunction with docetaxel.[57] Further trials will elucidate the role of ZD6474 either as a single agent or in combination with other chemotherapeutic agents.

BAY 43-9006 (sorafenib)

This agent is a potent TKI of VEGFR-2, VEGFR-3, B-Raf, and PDGFR-β. Several phase III clinical studies are assessing this agent in NSCLC; under way is a phase III randomized, double-blinded, placebo-controlled study sponsored by Bayer (NCT00449033) estimated to be concluded in 2010. It aims to evaluate the efficacy of cisplatin, gemcitabine, and sorafenib to cisplatin, gemcitabine, cisplatin, and placebo as first-line therapy for advanced NSCLC (specifically stage IIIb with pleural effusion and stage IV disease).

AG-013736

AG-013736 is an oral agent against a variety of TKs, such as VEGFR-1, VEGFR-2, VEGFR-3, c-Kit, and PDGFR-β. A phase I trial in metastatic NSCLC showed benefits in disease stabilization, and phase II trials are under way.[58]

The Therapeutic Potential of Exploiting Epigenetic Changes in Lung Cancer

About 80 genes are hypermethylated, often simultaneously within an individual lung tumor.[59] These epigenetic changes can be translated into practical tools for early diagnosis. For example, the detection of hypermethylated DNA of the $p16^{INK4a}$ gene in the sputum of patients with higher risks of developing lung cancer is useful. Approaches include identifying hypermethylated genes in lung tumors using a genome-wide strategy to create novel diagnostic and therapeutic tools.[60]

Table 1. Immunotherapy in lung cancer

Trial	Year	Antigen type	Notes	Clinical outcome	Reference
Phases I/II ALVAC (n = 3 lung, 15 gastrointestinal)	2000	CEA	Nonreplicating canarypoxvirus expressing both human CEA and the B7.1 costimulatory molecule.	SD in 3/18	Liu et al.[70]
Phase I (n = 19 NSCLC)	2004	Allogenic cell lines	NSCLC modified to express B7.1	SD in 5 and PR in 1.	Giangreco et al.[71]
Phase IIB BLP25 (n = 171 NSCLC)	2005	MUC1	MUC1 peptide with liposome adjuvant.	Evidence of survival benefit.	Butts et al.[72]
Phase II trial (n = 86 NSCLC)	2006	Autologous tumor cells	GVAX: autologous tumor cells with GM-CSF secreting bystander cells.	Marginal prolonged remission was seen. No significant tumor response.	Nemunaitis et al.[61]
Phase II trial (n = 182 stage Ib/II NSCLC)	2007	MAGE-A3	Multicenter, double-blinded placebo-controlled study. Efficacy of MAGE-A3 immunotherapeutic as adjuvant therapy in stage IB/ II NSCLC.	Some equivocal clinical benefits; further trials pending.	Vansteenkiste et al.[73]
Phases I/II trial (n = 83 NSCLC)	2007	EGF	Pooled data from three nonrandomized studies. Neisseria meningitides P64k protein conjugated to a chemical adjuvant and EGF.	Survival benefit of 3.5 months for vaccinated patients compared to nonrandomized non-vaccinated.	González et al.[74]

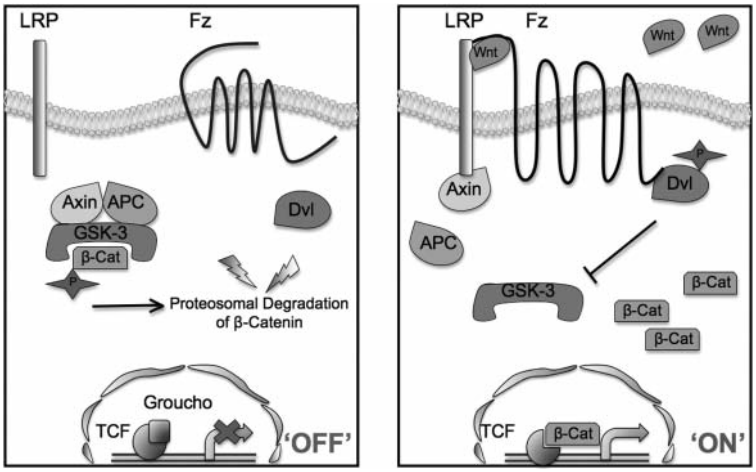
CEA, carcinoembryonic antigen; SD, stable disease, NSCLC, non-small cell lung cancer; PR, partial response; BLP, β-lipoprotein; MUC1, mucin 1; GM-CSF, granulocyte macrophage colony-stimulating factor; EGF, epidermal growth factor.

The Therapeutic Potential of Exploiting Immunomodulation in Lung Cancer

Two current immunotherapy strategies focus on active vaccination and adoptive T cell transfer. A recent phase I and phase II multicenter clinical trial using granulocyte macrophage colony-stimulating factors in advanced NSCLC demonstrated promising results that provide not only proof of principle, but also the basis for further studies.[61,62] Adoptive T cell transfer plays a role in the treatment of malignant melanoma; it is a process that entails the isolation and subsequent *in vitro* expansion of tumor infiltrating T cells followed by their reinfusion into the patient.[63,64] The challenge relies on the difficulty of isolating enough tumor-reactive T cells from lung cancer patients. A summary of clinical trials evaluating the role of immunotherapy in lung cancer is shown in Table 1.

The Therapeutic Potential of Targeting Wnt in Lung Cancer

Wnt signaling (Fig. 1) is crucial during the development and homeostasis of the pulmonary tree.[65–68] Wnt exerts a regulatory function in the stem cell compartment of many organs.[69,70] The clonogenic nature of human lung cancer was described almost 30 years ago. Clinical lung adenocarcinomas and small cell lung cancers (SCLC) were found to have a rare cellular population (< 1.5%) with the capacity to form agar colonies. When intracranially inoculated into athymic mice, they grew into originating malignant lesions. There is a link between the high expression of ABC transporters and drug resistance. Side population (SP) cells isolated by the efflux of Hoechst 33342 by ABC transporters with stem-cell characteristics have been demonstrated in both lung cell lines and clinical NSCLC. These data together with the heterogeneity and regionality of lung cancer demonstrated in



Yagui-Beltrán et al. 'The Wingless Gene: From Embryogenesis to Stem Cell Self-Renewal' Book: Stem Cells and Cancer.
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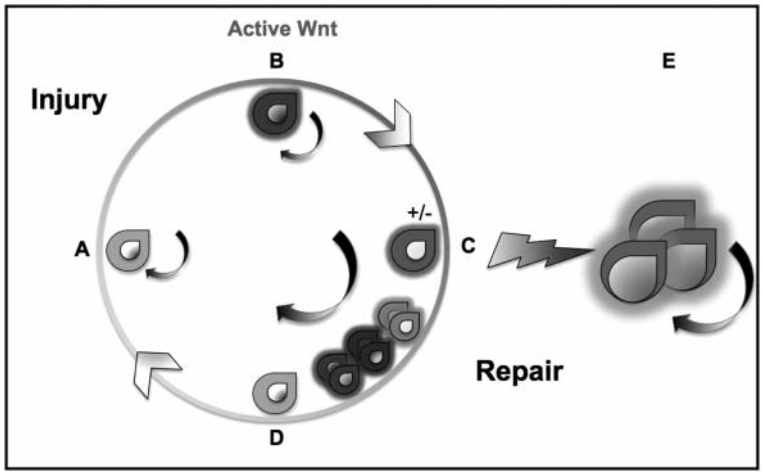
Fig. 1. The canonical Wnt transduction pathway.
Proteosomal degradation of β-catenin via its phosphorylation occurs in the absence of Wnt ligands. Downstream, Wnt target genes are maintained repressed ('OFF'). Degradation of active β-catenin is reduced upon the binding of Wnt's. Accumulation and translocation of β-catenin into the nucleus lead to binding to T cell factors and activation of target genes ('ON').
APC, adenomatous polyposis coli; Dvl, disheveled; GSK, glycogen synthase kinase; TCF, T cell factor.



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Beachy PA, Karhadkar SS, Berman DM. Tissue repair and stem cell renewal in carcinogenesis. Nature 2004; 432: 324–31.

Fig. 2. Tissue homeostasis and carcinogenesis through stem cell cycling.
A: Quiescent stem cell (SC) with inactive Wnt.
B: Upon tissue trauma, Wnt transduction leads to activation of homeostatic SCs.
C: These cells produce more pluripotent SCs as well as progenitor cells with limited proliferative power.
D,A: That produces specialized differentiated cells (shown in orange, purple, and blue) to regenerate the tissue. Upon repair, SCs cycle into a quiescent state.
E: Accumulation of oncogenic events may 'lock' activated SCs in a permanent Wnt-driven state, leading to cancer stem cells.

murine models[71] suggest the involvement of stem cells in the human lung (Fig. 2). The dissection of the precise molecular mechanisms by which Wnt regulates the initiation, malignant transformation, and metastatic spread of lung progenitor, or stem-cell like cells becomes mandatory because of its enormous therapeutic potential.

Future Perspectives

In this article was have highlighted some of the most exciting translational therapeutic developments using various strategies with the aim to target lung cancer more precisely. We expect that in the next decade our understanding of the specific mechanisms governing lung carcinogenesis within individual pulmonary microenvironments will grow further. Thus we will be able to successfully translate these mechanisms into novel clinically and relevant tools for the diagnosis, screening, and management of lung cancer patients. We greatly hope that through the identification and targeting of unique and phenotypically defined lung cancer stem-cell populations that possess the potential to result in tumor recurrence, we will be able to design new targeted therapies that will circumvent many of the side effects of current cytotoxics with implicit improvement in the quality of life for our lung cancer patients and increased disease-free survival rates.

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