Pathway Targets to Explore in the Treatment of Non-small Cell Lung Cancer

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Introduction: With a 5-year survival rate of only 16%, improvements in therapeutic strategies for the treatment of non-small cell lung cancer (NSCLC) are still warranted. Published prognostic gene expression classification signatures in NSCLC overlap poorly across studies. We hypothesized that different NSCLC microarray datasets will share common pathways leading to the identification of new targets for therapeutic development.

Methods: Per gene mean expression fold change ratios were calculated from 7 publicly-available microarray datasets consisting of a total of 725 profiled samples. This was followed by mapping of differentially expression genes into functionally annotated biologic pathways using Ingenuity pathway analysis software. Common pathways containing genes whose expression levels differed between phenotypic classes defined by NSCLC cases and/or histology, pathologic stage, and gender were determined.

Results: Several significant common pathways overlapping these datasets were identified in silico. One of which, Leukocyte Extravasation Signaling pathway, includes targeted agents such as imatinib, dasatinib, and temozolomide currently under exploration in NSCLC trials. The remaining pathways have targets with few drugs developed or under development in cancer therapeutics. Comparison of pathways derived from Eastern versus Western population datasets revealed several differing targets and potential target drugs.

Conclusion: This in silico pathway exploration in NSCLC warrants further investigation and could open the door to potential new therapeutics that might improve NSCLC patient outcomes. This strategy can be applied to other cancer types.

Key Words: Gene expression profiling, Non-small cell lung cancer, Pathway analysis, In silico.

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Lung cancer is the most common cause of cancer mortality in the world, with an estimated 1.18 million new cases annually.[1] Non-small cell lung cancer (NSCLC) represents approximately 88% of lung cancer cases,[2] of which approximately 75% represent advanced-stage disease. Recent treatment advances for advanced NSCLC include the addition of bevacizumab to chemotherapy in nonsquamous histology[3,4] and the Food and Drug Administration (FDA) approval of three agents for second-line therapy.[5–7] However, the 5-year survival rate for all NSCLC is a disappointing 16%,[8] necessitating further improvement in therapeutic strategies. Several prognostic gene expression signatures in NSCLC have been reported, but reproducibility across studies has been poor.[9] While not fully elucidated, possible explanations include differences in microarray platforms, processing of material, patient cohorts, and/or methods of analysis.[10] If these different microarray datasets share perturbations in common pathways, however, identification of these commonalities may lead to the identification of new and possibly unrealized targets for NSCLC treatment.

METHODS

Microarray datasets from Duke University (Duke)[11] were downloaded as text files prenormalized by the MAS5 algorithm.[12,13] Microarray data sets from the Samsung Medical Center (Korea)[14] were downloaded as text files prenormalized using the GC Robust Multi-array Average algorithm.[15]

Microarray datasets from the Dana-Farber Cancer Institute,[16] Taipei Veterans General Hospital (Taipei),[17] University of Duesseldorf (Germany),[18] University of Michigan (Michigan),[19] and Expression Project for Oncology (ExpO)[20] were downloaded as raw Affymetrix.cel files and normalized using the Robust Multichip Average method[21] as implemented in the Affymetrix Expression Console v1.1.

For all datasets, small cell and carcinoid lung cancer samples were excluded and per gene mean expression fold change ratios were separately calculated from the remaining samples of each dataset. When the appropriate clinical annotations were available, fold changes were calculated for lung cancer versus normal lung, adenocarcinoma (AC) versus squamous cell carcinoma (SCC), early-stage (stage I–II) versus late-stage (stage III–IV), and male versus female.

Fold changes greater than 2 or less than 0.5 were analyzed in a functional annotation and pathway database using Ingenuity pathway analysis (IPA).[22] Common pathways significantly perturbed when comparing NSCLC expression profiles of differing histologic class, pathologic stage, and gender were determined. Canonical pathways were interrogated, and *p*-values from all performed hypothesis tests were pooled and adjusted by the method of Benjamini and Hochberg (as implemented in the R/bioconductor multi-

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test package[23]) to estimate the False Discovery Rate (FDR).[24] Those pathways with an estimated FDR less than 0.05 were recorded. When two or more institutions shared significant canonical IPA pathway results by category (lung cancer versus normal tissue; AC versus SCC; early-stage versus late-stage; or male versus female), the pathway was annotated as a "common pathway." Common pathways were ranked according to the number of datasets in which the pathway was significantly perturbed and were interrogated for the presence of known drug targets and known oncogenes.

RESULTS

Clinical characteristics of profiled samples ($n = 725$) are summarized in Table 1. Age, gender, ethnicity, stage, histology, and pack-years of cigarette smoking were included when available. Dana-Farber Cancer Institute, Michigan, and Taiwan datasets comprise primarily ACs and include more women than men. Duke, Korea, and ExpO datasets lacked profiling of normal lung tissue.

Several common pathways were identified in silico as significantly affected among these datasets using IPA. Table 2 lists the pathways with an estimated FDR less than 5% among the datasets, while Table 3 lists the shared pathways among the datasets. No significant pathways were identified in any of the clinical annotation comparisons in the Korea dataset. In the lung cancer versus normal tissue comparison, there were two common pathways shared by at least two datasets—Leukocyte Extravasation Signaling (Figure 1) and Coagulation System (Figure 2). Targets in these two pathways and their respective potential targeting drugs are included in Table 4. Imatinib,[25] dasatinib,[26] and temozolomide[27] are potential drugs for the Leukocyte Extravasation Signaling pathway, targeting ABL/c-Kit (imatinib) and Src (dasatinib and temozolomide). All three of these agents are or have been studied in NSCLC clinical trials[28] and are FDA approved agents in other tumor types. Possible drug targets in the Coagulation System pathway include Factor Xa, Factor IIa, and SERPINC1, which could potentially be treated with available antiheparinase agents.

Acute Phase Response Signaling (Figure 3 supplementary section) and Complement System pathways are affected in four datasets comparing AC versus SCC. The Acute Phase Response Signaling pathway includes mTOR, c-RAF, and TNFα targets, for which drugs such as everolimus, sorafenib, and thalidomide have been FDA approved for cancer treatment and are currently being explored in NSCLC clinical trials[28] (thalidomide studies are now closed). In the Complement System pathway, eculizumab is listed as a potential agent, which targets C5, C5a, and C5b, and is FDA approved for the treatment of paroxysmal nocturnal hemoglobinuria.

Acute Phase Response Signaling and LPS/IL-1 mediated inhibition of Retinoid X Receptor (RXR) function are significant in two datasets comparing males versus females. The LPS/IL-1 Mediated Inhibition of RXR Function pathway includes the target, RXRα, for which drugs such as bexarotene has been studied in NSCLC clinical trials.[29,30]

TABLE 1. Clinical Characteristics of Analyzed NSCLC Specimens

Clinical Characteristic	Michigan Dataset $n = 96$ Including 10 Normal Lung	DFCI Dataset[a] $n = 228$ Including 17 Normal Lung	Duke Dataset $n = 111$	Germany Dataset $n = 25$ Including 5 Normal Lung	ExpO Dataset $n = 69$	Korean Dataset $n = 138$	Taiwan Dataset $n = 58$ Including 30 Normal Lung
Age (range)	N/A	33–88	N/A	45–80	40–90[b]	37–82	N/A
Gender							
Female/male/(N/A)	51/35 (0)	102/73 (34)	47/64 (0)	4/16 (0)	20/49 (0)	34/104 (0)	22/5 (1)
Ethnicity	N/A	N/A	N/A	N/A			
Caucasian					64	—	—
Asian					2	138	28
Hispanic					1	—	—
Native American					1	—	—
Stage				N/A		N/A	N/A
Stage I	67	107	67		30		
Stage II	—	33	18		8		
Stage III	19	15	21		7		
Stage IV	—	19	5		3		
Histology							
AC	86	190	58	10	15	62	27
SCC	—	21	53	10	34	76	
Other NSCLC	—	—	—	—	20	—	1
Pack years (range)[b]	0–160	0–140	N/A	N/A	11–65[c]	N/A	N/A
Array platform	Affymetrix Hu6800	Affymetrix U95Av2	Affymetrix HG U133 Plus 2.0	Affymetrix Human HG-focus target	Affymetrix HG U133 Plus 2.0	Affymetrix HG U133 Plus 2.0	Affymetrix U133A

[a] Includes 51 replicates of profiled samples.
[b] Age range listed by 10 yr span, specific ages not available.
[c] Pack years range listed by 5 yr span, specific pack years smoked not available.
N/A, not available; AC, adenocarcinoma; SCC, squamous cell carcinoma; Other NSCLC, other non-small cell lung cancer subtypes, DFCI, Dana-Farber Cancer Institute.

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