LRRC36 is differentially expressed in non-small cell lung cancer and associates with patient survival.

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer death in the United States¹. We mined published microarray data^{2,3,4} to identify differentially expressed genes in NSCLC. We found that the gene encoding LRRC36 was among those whose expression was most different in human NSCLC tumors as compared to the lung. LRRC36 expression levels were significantly decreased in NSCLC tumors as compared to the lung, and lower expression of LRRC36 in patient tumors was significantly associated with worse overall survival. LRRC36 may be important for initiation or progression of non-small cell lung cancer in humans.

Keywords: LRRC36, NSCLC, non-small cell lung cancer, systems biology of NSCLC, targeted therapeutics in NSCLC.

1 In 2016, lung cancer resulted in the death of 158,000 Americans; 81% of all patients
2 diagnosed with lung cancer will expire within 5 years⁵. Non-small cell lung cancer (NSCLC) is
3 the most common type of lung cancer, diagnosed in 84% of patients with lung cancer, and 76%
4 of all patients with NSCLC will expire within 5 years⁵. The rational development of targeted
5 therapeutics to treat patients with NSCLC can be supported by an enhanced understanding of
6 fundamental transcriptional features of NSCLC tumors. To discover genes associated with
7 NSCLC tumors in an unbiased fashion and at the systems-level, we mined independently
8 published microarray data^{2,3,4} to compare global gene expression profiles of NSCLC tumors to
9 that of the normal lung. We found recurrent and significant differential expression of LRRC36 in
10 adenocarcinoma tumors from patients with NSCLC, suggesting LRRC36 may be important for
11 NSCLC tumor initiation or progression.

12 **Methods**

13 We utilized microarray datasets, GSE43458², GSE33532⁴ and GSE74706⁴ for this
14 differential gene expression analysis of NSCLC tumors in conjunction with GEO2R. GSE43458
15 was generated using Affymetrix Human Gene 1.0 ST Array technology; for this analysis, we
16 used with $n=30$ control lung tissue and $n=80$ NSCLC tumors, and the analysis was performed
17 using platform GPL6244. GSE33532 was generated using Affymetrix Human Genome U133
18 Plus 2.0 Array technology; for this analysis, we used $n=20$ control lung tissue and $n=10$ NSCLC
19 tumors, and the analysis was performed using platform GPL570. GSE74706 was generated
20 using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology; for this

analysis, we used $n=18$ control lung tissue and $n=10$ NSCLC tumors, and the analysis was performed using platform GPL13497. All tumors utilized for differential gene expression analysis here were of the adenocarcinoma type.

The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether LRRC36 expression was significantly between normal lung tissue and NSCLC tumors using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in NSCLC tumors (Version 8.4.0)(455). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁵ for correlation of LRRC36 mRNA expression levels with overall survival in $n=1925$ non-small cell lung cancer patients.

Results

We harnessed the power of multiple, independently published microarray datasets^{2,3,4} to discover in an unbiased fashion and at the transcriptome-level the most striking gene expression features of NSCLC tumors.

LRRC36 is differentially expressed in non-small cell lung cancers.

We found significant differential expression of LRRC36 in NSCLC tumors when compared to the lung² (Table 1). When sorting each of the transcripts measured by microarray

1 based on significance of difference in LRRC36 expression between NSCLC tumors and the
2 normal lung, in this dataset, LRRC36 ranked 248 out of 25906 total transcripts, equating to
3 99.0% differential expression (Table 1). Differential expression of LRRC36 in NSCLC tumors
4 was statistically significant (Table 1; $p=6.29\text{E-}20$).

5
6 We queried a second microarray dataset³ to determine if we could validate differential
7 expression of LRRC36 in non-small cell lung cancers (Table 2). We found significant
8 differential expression of LRRC36 in NSCLC tumors of the adenocarcinoma type when
9 compared to the normal lung (Table 2). When sorting each of the transcripts measured based on
10 significance of difference in expression of LRRC36 between NSCLC tumors and the normal
11 lung, LRRC36 ranked 45 of 33252 total transcripts (Table 2), equating to 99.9% differential
12 expression. Differential expression of LRRC36 in NSCLC tumors was statistically significant
13 (Table 2; $p=2.68\text{E-}16$).

14
15 Analysis of a third microarray dataset⁴ again revealed significant differential expression
16 of LRRC36 in NSCLC tumors of the adenocarcinoma type. When sorting each of the transcripts
17 measured based on significance of difference in expression of LRRC36 between NSCLC tumors
18 and the normal lung, LRRC36 ranked 793 out of 34183 total transcripts, equating to 97.7%
19 differential expression (Table 3). Differential expression of LRRC36 in NSCLC tumors was
20 statistically significant (Table 3; $p=1.29\text{E-}08$).

21
22 LRRC36 is expressed at significantly lower levels in NSCLC tumors as compared to the lung.

23
24 We obtained exact mRNA levels for LRRC36 from NSCLC tumors and from the lung to
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1 directly compare LRRC36 expression between tumor and control lung tissue and assess for
2 statistical significance. LRRC36 was expressed at significantly lower levels in NSCLC tumors
3 as compared to the normal lung in each dataset queried, respectively (Figure 1: $p < 0.0001$, Figure
4 2: $p < 0.0001$, and Figure 3: $p < 0.0001$). We calculated a mean fold change of 0.8024 ± 0.0874
5 and 0.6090 ± 0.0253 in LRRC36 expression when comparing NSCLC tumors to the lung (Table
6 1 and Table 2, respectively).

7 LRRC36 expression in NSCLC tumors correlates with overall survival.

8 We performed Kaplan-Meier survival analysis using LRRC36 mRNA expression in
9 NSCLC tumors coupled with paired overall survival data from each patient, in 1925 NSCLC
10 patients in total, to determine whether LRRC36 tumor expression was correlated with survival
11 outcomes in NSCLC. We found that patients whose tumors expressed lower levels of LRRC36
12 possessed significantly shorter overall survival than patients with high tumor expression of
13 LRRC36 (Figure 4). Median overall survival (OS) of patients in the low expression cohort was
14 57.33 months, while median OS in patients in the high LRRC36 expression cohort was 105.4
15 months (Table 4); this difference in median OS based on LRRC36 tumor expression in NSCLC
16 was statistically significant (Figure 4; logrank p -value: $1.6e-9$; hazard ratio: 0.61 (0.52 - 0.72);
17 false discovery rate=0.01).

18 Thus, blind comparative transcriptome analysis of non-small cell lung cancers revealed
19 differential expression of transcripts encoded by the LRRC36 gene as among the most significant
20 transcriptional features of NSCLC tumors, and LRRC36 expression was significantly correlated
21

1 with patient outcomes, as patients with lower tumor expression of LRRC36 possessed
2 significantly worse overall survival.
3

4 **Discussion**

5
6 We could find little information describing functions for LRRC36, in normal biology or
7 in cancer. LRRC36 is located at 16q22.1 and has been described as uncharacterized, but is
8 phylogenetically most related to the centriolar satellite protein Cep72⁷. LRRC36 contains a
9 leucine-rich repeat (LRR) at the amino terminus and a coiled-coil domain at the carboxy-
10 terminus⁷. In mammalian cells *in vitro*, an LRRC36-green fluorescent protein (GFP) fusion
11 localizes to the centrosome. In a study of 186 primary tumors of the colon and 54 liver
12 metastases, LRRC36 was found to be significantly up-regulated in both primary tumors and liver
13 metastases⁸.
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17 We found, by mining multiple independently published microarray datasets, that a
18 relatively uncharacterized molecule, LRRC36, was among the genes most differentially
19 expressed in the primary tumors of patients with non-small cell lung cancer; LRRC36 was
20 expressed at significantly lower levels in NSCLC tumors as compared to the lung, and decreased
21 expression of LRRC36 was significantly associated with worse overall survival in NSCLC
22 patients. LRRC36 may be of relevance to the biology of tumor initiation or progression in
23 patients with NSCLC of the adenocarcinoma type, the most common type of the leading cause of
24 cancer death in the United States and worldwide.
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Rank	ID	p-value	t	B	FC	Gene	Gene name	% DE
248	7996546	6.29E-20	-11.189446	34.79623	0.8024 ± 0.0874	LRRC36	leucine rich repeat containing 36	99.0

Table 1: LRRC36 is differentially expressed in NSCLC tumors.

The rank of differential expression relative all transcripts measured, probe ID, *p*-value of global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, fold change of LRRC36 expression in NSCLC tumors as compared to the lung, gene, gene name and percent differential expression (% DE) are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name	% DE
45	220003_at	2.68E-16	-15.662658	27.1695328	0.6090 ± 0.0253	LRRC36	leucine rich repeat containing 36	99.9

Table 2: LRRC36 is differentially expressed in NSCLC tumors.

The rank of differential expression relative all transcripts measured, probe ID, *p*-value of global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, fold change of LRRC36 expression in NSCLC tumors as compared to the lung, gene, gene name and percent differential expression (% DE) are listed in this chart.

Rank	ID	p-value	t	B	Gene	% DE
793	A_23_P118042	1.29E-08	-7.82	9.7525852	LRRC36	97.7

Table 3: LRRC36 is differentially expressed in NSCLC tumors.

The rank of differential expression relative all transcripts measured, probe ID, *p*-value of global differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, gene and percent differential expression (% DE) are listed in this chart.

Figure 1: LRRC36 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of LRRC36 is graphically represented in the lung (left) and in NSCLC tumors of the adenocarcinoma type (right) with mean mRNA expression values marked and the result of a statistical test evaluating significance of difference in LRRC36 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 2: LRRC36 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of LRRC36 is graphically represented in the lung (left) and in NSCLC tumors of the adenocarcinoma type (right) with mean mRNA expression values marked and the result of a statistical test evaluating significance of difference in LRRC36 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 3: LRRC36 expression in NSCLC tumors significantly correlates with overall survival.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1925$ total patients stratified into two groups, based on low or high expression of LRRC36 in patient tumors. The log rank p -value denoting statistical significance of difference in overall survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on LRRC36 expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low expression cohort (months)	High expression cohort (months)
57.33	105.4

Table 4: LRRC36 expression in NSCLC tumors significantly correlates with patient survival.

The median overall survival of $n=1925$ NSCLC patients based on stratification into low or high expression of LRRC36 in tumors is listed in this chart.