Leucine-rich repeat neuronal 3, LRRN3, 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 the leucine-rich repeat neuronal 3, LRRN3, was among those whose expression was most different in human NSCLC tumors as compared to the lung. LRRN3 expression levels were significantly decreased in NSCLC tumors as compared to the lung, and lower expression of LRRN3 in patient tumors was significantly associated with worse overall survival. LRRN3 may be important for initiation or progression of non-small cell lung cancer in humans.

Keywords: LRRN3, leucine-rich repeat neuronal 3, 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 LRRN3 in
10 adenocarcinoma tumors from patients with NSCLC, suggesting LRRN3 may be important for
11 NSCLC tumor initiation or progression.

12 **Methods**

13 We utilized microarray datasets, GSE43458², GSE33532³ 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 LRRN3 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 tool⁶ for correlation of LRRN3 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.

LRRN3 is differentially expressed in non-small cell lung cancers.

We found significant differential expression of the gene encoding leucine-rich repeat neuronal 3, LRRN3, in NSCLC tumors when compared to the lung² (Table 1). When sorting

each of the transcripts measured by microarray based on significance of difference in LRRN3 expression between NSCLC tumors and the normal lung, in this dataset, LRRN3 ranked 68 out of 25906 total transcripts, equating to 99.7% differential expression (Table 1). Differential expression of LRRN3 in NSCLC tumors was statistically significant (Table 1; $p=2.62E-25$).

We queried a second microarray dataset³ to determine if we could validate differential expression of LRRN3 in non-small cell lung cancers (Table 2). We found significant differential expression of LRRN3 in NSCLC tumors of the adenocarcinoma type when compared to the normal lung (Table 2). When sorting each of the transcripts measured based on significance of difference in expression of LRRN3 between NSCLC tumors and the normal lung, LRRN3 ranked 39 of 33252 total transcripts (Table 2), equating to 99.9% differential expression. Differential expression of LRRN3 in NSCLC tumors was statistically significant (Table 2; $p=1.65E-16$).

Analysis of a third microarray dataset⁴ again revealed significant differential expression of LRRN3 in NSCLC tumors of the adenocarcinoma type. When sorting each of the transcripts measured based on significance of difference in expression of LRRN3 between NSCLC tumors and the normal lung, LRRN3 ranked 154 out of 34183 total transcripts (Table 3), equating to 99.5% differential expression. Differential expression of LRRN3 in NSCLC tumors was statistically significant (Table 3; $p=7.36E-12$).

LRRN3 is expressed at significantly lower levels in NSCLC tumors as compared to the lung.

We obtained exact mRNA levels for LRRN3 from NSCLC tumors and from the lung to

1 directly compare LRRN3 expression between tumor and control lung tissue and assess for
2 statistical significance. LRRN3 was expressed at significantly lower levels in NSCLC tumors as
3 compared to the normal lung in each dataset queried, respectively (Figure 1: $p<0.0001$, Figure 2:
4 $p<0.0001$, and Figure 3: $p<0.0001$). We calculated a mean fold change of 0.7665 ± 0.0790 and
5 0.6284 ± 0.0491 in LRRN3 expression when comparing NSCLC tumors to the lung (Table 1 and
6 Table 2, respectively).

7 LRRN3 expression in NSCLC tumors correlates with overall survival.

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10 We performed Kaplan-Meier survival analysis using LRRN3 mRNA expression in
11 NSCLC tumors coupled with paired overall survival data from each patient, in 1925 NSCLC
12 patients in total, to determine whether LRRN3 tumor expression was correlated with survival
13 outcomes in NSCLC. We found that patients whose tumors expressed lower levels of LRRN3
14 possessed significantly shorter overall survival than patients with high tumor expression of
15 LRRN3 (Figure 4). Median overall survival (OS) of patients in the low expression cohort was
16 62 months, while median OS in patients in the high LRRN3 expression cohort was 95.5 months
17 (Table 4); this difference in median OS based on LRRN3 tumor expression in NSCLC was
18 statistically significant (Figure 4; logrank p -value: $1e-05$, hazard ratio: 0.71 (0.61 - 0.83); false
19 discovery rate=0.01).

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21 Thus, blind comparative transcriptome analysis of non-small cell lung cancers revealed
22 differential expression of transcripts encoded by the LRRN3 gene as among the most significant
23 transcriptional features of NSCLC tumors, and LRRN3 expression was significantly correlated
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1 with patient outcomes, as patients with lower tumor expression of LRRN3 possessed
2 significantly worse overall survival.
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4 **Discussion**

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6 Leucine rich repeat neuronal 3, LRRN3, was originally described in rats to function in the
7 internalization of the epidermal growth factor in clathrin-coated vesicles⁷. Like other neuronal
8 LRR (LRRN) genes, LRRN3 contains 11 or 12 leucine-rich repeat (LRR) domains, an
9 immunoglobulin-like domain and a fibronectin type III-like domain⁷. The carboxy-terminus of
10 LRRN3 also contains two endocytosis motifs⁷. The 30 amino acids most carboxy-terminal in
11 LRRN3 function to enhance MAP kinase signaling⁷. The carboxy-terminus, which contains the
12 endocytosis motifs, binds to clathrin-coated adaptor protein complexes⁷. LRRN3-deficient mice
13 are reported to possess severe developmental delays and behavioral abnormalities, as well as
14 structural changes⁸. In a study of transcriptome changes associated with decline of gray matter
15 thickness in the whole brain during aging, LRRN3 was found to be among the most differentially
16 expressed genes⁹. Descriptions of LRRN3 functions are otherwise limited.
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21 We could not identify any reports describing a role for LRRN3 in non-small cell lung
22 cancer. In serous ovarian cancer, LRRN3 was identified as among the top genes whose
23 expression was associated with poor patient prognosis using Cancer Genome Atlas data from
24 stage 3 and stage 4 tumors¹⁰.
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26 We found, by mining multiple independently published microarray datasets, that the
27 leucine-rich repeat neuronal 3, LRRN3, was among the genes most differentially expressed in the
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1 primary tumors of patients with non-small cell lung cancer; LRRN3 was expressed at
2 significantly lower levels in NSCLC tumors as compared to the lung, and decreased expression
3 of LRRN3 was significantly associated with worse overall survival in NSCLC patients. LRRN3
4 may be of relevance to the biology of tumor initiation or progression in patients with NSCLC of
5 the adenocarcinoma type, the most common type of the leading cause of cancer death in the
6 United States and worldwide.
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References

1. Siegel, R.L., Miller, K.D. and Jemal, A., 2019. Cancer statistics, 2019. *CA: a cancer journal for clinicians*, 69(1), pp.7-34.
2. Meister, M., Belousov, A., Xu, E.C., Schnabel, P., Warth, A. and Hoofmann, H., 2014. Intra-tumor heterogeneity of gene expression profiles in early stage non-small cell lung cancer. *J Bioinf Res Stud*, 1, p.1.
3. Marwitz, S., Depner, S., Dvornikov, D., Merkle, R., Szczygiał, M., Müller-Decker, K., Lucarelli, P., Wäsch, M., Mairbörl, H., Rabe, K.F. and Kugler, C., 2016. Downregulation of the TGFβ pseudoreceptor BAMBI in non-small cell lung cancer enhances TGFβ signaling and invasion. *Cancer research*, 76(13), pp.3785-3801.
4. Kabbout, M., Garcia, M.M., Fujimoto, J., Liu, D.D., Woods, D., Chow, C.W., Mendoza, G., Momin, A.A., James, B.P., Solis, L. and Behrens, C., 2013. Ets2 mediated tumor suppressive function and met oncogene inhibition in human non-small cell lung cancer. *Clinical cancer research*, 19(13), pp.3383-3395.
5. Lung Cancer - Non-Small Cell: Statistics. <https://www.cancer.net/cancer-types/lung-cancer-non-small-cell/statistics>.
6. Gyorffy, B., Surowiak, P., Budczies, J. and Lanczky, A., 2013. Online survival analysis software to assess the prognostic value of biomarkers using transcriptomic data in non-small-cell lung cancer. *PloS one*, 8(12), pp.e82241-e82241.
7. Fukamachi, K., Matsuoka, Y., Ohno, H., Hamaguchi, T. and Tsuda, H., 2002. Neuronal leucine-rich repeat protein-3 amplifies MAPK activation by epidermal growth factor through a carboxyl-terminal region containing endocytosis motifs. *Journal of Biological Chemistry*, 277(46), pp.43549-43552.
8. Nakamura, M., Hatayama, M., Matsunaga, H., Nakagawa, S. and Aruga, J., 2018. Role of Lrrn3, a brain-enriched transmembrane protein in neural development. In *Proceedings for Annual Meeting of The Japanese Pharmacological Society WCP2018 (The 18th World Congress of Basic and Clinical Pharmacology)* (pp. PO2-1). Japanese Pharmacological Society.
9. Kochunov, P., Charlesworth, J., Winkler, A., Hong, L.E., Nichols, T.E., Curran, J.E., Sprooten, E., Jahanshad, N., Thompson, P.M., Johnson, M.P. and Kent Jr, J.W., 2013. Transcriptomics of cortical gray matter thickness decline during normal aging. *Neuroimage*, 82, pp.273-283.

1 10. Willis, S., Villalobos, V.M., Gevaert, O., Abramovitz, M., Williams, C., Sikic, B.I. and
2 Leyland-Jones, B., 2016. Single gene prognostic biomarkers in ovarian cancer: A meta-
3 analysis. *PloS one*, 11(2), p.e0149183.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
68	8135488	2.62E-25	-13.556575	47.020284	0.7665 ± 0.0790	LRRN3	leucine rich repeat neuronal 3

Table 1: LRRN3 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 LRRN3 expression in NSCLC tumors as compared to the lung, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
39	209841_s_at	1.65E-16	-15.937748	27.6421054	0.6284 ± 0.0491	LRRN3	leucine rich repeat neuronal 3

Table 2: LRRN3 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 LRRN3 expression in NSCLC tumors as compared to the lung, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene
154	A_23_P31376	7.36E-12	-1.1E+01	17.1370657	LRRN3

Table 3: LRRN3 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, and gene are listed in this chart.

Figure 1: LRRN3 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of LRRN3 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 LRRN3 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 2: LRRN3 transcript is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of LRRN3 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 LRRN3 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 3: LRRN3 transcript is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of LRRN3 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 LRRN3 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 4: LRRN3 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 LRRN3 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 LRRN3 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)
62	95.5

Table 4: LRRN3 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 LRRN3 in tumors is listed in this chart.