FHL1 is differentially expressed in non-small cell lung cancer and associates with patient survival.

Shahan Mamoor¹

¹shahanmamoor@gmail.com

East Islip, NY USA

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 FHL1 was among the genes whose expression was most quantitatively different in tumors from patients with NSCLC as compared to the lung. FHL1 expression was significantly decreased in NSCLC tumors as compared to the lung, and lower expression of FHL1 in patient tumors was significantly associated with worse overall survival. FHL1 may be important for initiation or progression of non-small cell lung cancer in humans.

Keywords: FHL1, 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 FHL1 in
10 adenocarcinoma tumors from patients with NSCLC, suggesting FHL1 may be important for
11 NSCLC tumor initiation or progression.

12 **Methods**

13 We utilized microarray datasets GSE33532³, GSE43458⁴ and GSE74706⁵ for this
14 differential gene expression analysis of NSCLC tumors in conjunction with GEO2R. GSE33532
15 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology; for this
16 analysis, we used $n=20$ control lung tissue and $n=10$ NSCLC tumors, and the analysis was
17 performed using platform GPL570. GSE43458 was generated using Affymetrix Human Gene
18 1.0 ST Array technology; for this analysis, we used with $n=30$ control lung tissue and $n=80$
19 NSCLC tumors, and the analysis was performed using platform GPL6244. GSE74706 was
20 generated using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology; for

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

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5 The Benjamini and Hochberg method of p -value adjustment was used for ranking of
6 differential expression but raw p -values were used to assess statistical significance of global
7 differential expression. Log-transformation of data was auto-detected, and the NCBI
8 generated category of platform annotation was used. A statistical test was performed to evaluate
9 whether FHL1 expression was significantly between normal lung tissue and NSCLC tumors
10 using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical
11 analyses of differential gene expression in NSCLC tumors (Version 8.4.0)(455). For Kaplan-
12 Meier survival analysis, we used the Kaplan-Meier plotter online tool⁶ for correlation of FHL1
13 mRNA expression levels with overall survival in $n=1925$ non-small cell lung cancer patients.
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19 **Results**

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

23 FHL1 is differentially expressed in non-small cell lung cancers.

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26 We found significant differential expression FHL1 in NSCLC tumors when compared to
27 the lung² (Table 1). When sorting each of the transcripts measured based on significance of
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1 difference in expression of FHL1 between NSCLC tumors and the normal lung, FHL1 ranked 10
2 out of 25906 total transcripts (Table 1). Differential expression of FHL1 in NSCLC tumors was
3 statistically significant (Table 1; $p=2.48E-18$).
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5 We queried a second microarray dataset⁴ to determine if we could validate differential
6 expression of FHL1 in non-small cell lung cancers (Table 2). We again found significant
7 differential expression of FHL1 in NSCLC tumors of the adenocarcinoma type when compared
8 to the normal lung (Table 2). When sorting each of the transcripts measured based on
9 significance of difference in expression of FHL1 between NSCLC tumors and the normal lung,
10 FHL1 ranked 64 out of 33252 total transcripts (Table 2). Differential expression of FHL1 in
11 NSCLC tumors was statistically significant (Table 2; $p=1.49E-25$).
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15 Analysis of a third microarray dataset³ revealed significant differential expression of
16 FHL1 in NSCLC tumors of the adenocarcinoma type. When sorting each of the transcripts
17 measured based on significance of difference in expression of FHL1 between NSCLC tumors
18 and the normal lung, in this dataset, FHL1 ranked 73 out of 34183 total transcripts (Table 3).
19 Differential expression of FHL1 in NSCLC tumors was statistically significant (Table 3;
20 $p=5.06E-13$).
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23 FHL1 is expressed at significantly lower levels in NSCLC tumors as compared to the lung.
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25 We obtained exact mRNA levels for FHL1 from NSCLC tumors and from the lung to
26 directly compare FHL1 expression between tumor and control lung tissue and assess for
27 statistical significance. FHL1 was expressed at significantly lower levels in NSCLC tumors as
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1 compared to the normal lung in each dataset queried (Figure 1: $p < 0.0001$, Figure 2: $p < 0.0001$,
2 Figure 3: $p < 0.0001$). We calculated a mean fold change of 0.5842 ± 0.0841 (Table 1) and
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4 0.7478 ± 0.1011 (Table 2) in FHL1 expression when comparing NSCLC tumors to the lung.

6 FHL1 expression in NSCLC tumors correlates with overall survival.

7 We performed Kaplan-Meier survival analysis using FHL1 mRNA expression in NSCLC
8 tumors coupled with paired overall survival data from each patient, in 1925 NSCLC patients in
9 total, to determine whether FHL1 tumor expression was correlated with survival outcomes in
10 NSCLC. We found that patients whose tumors expressed lower levels of FHL1 possessed
11 significantly shorter overall survival than patients with high tumor expression of FHL1 (Figure
12 4). Median overall survival (OS) of patients in the low expression cohort was 53 months, while
13 median OS in patients in the high FHL1 expression cohort was 108.27 months (Table 4); this
14 difference in median OS based on FHL1 tumor expression in NSCLC was statistically significant
15 (Figure 4; logrank p -value: $1.6e-12$; hazard ratio: 0.58 (0.5 -0.68)).
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20 Thus, blind comparative transcriptome analysis of non-small cell lung cancers revealed
21 differential expression of FHL1 as among the most significant transcriptional features of NSCLC
22 tumors; we found that FHL1 expression was significantly correlated with patient outcomes, as
23 patients with lower tumor expression of FHL1 possessed significantly worse overall survival.
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Discussion

FHL1, the four and a half LIM domains protein 1, was named based on its possession of a LIM domain, a zinc finger motif contained in LIN11, ISL1 and MEC3^{7,8}. FHL1-deficient mice are born in normal mendelian ratios but the size of the heart is significantly decreased⁹. In cardiomyocytes, FHL1 senses biomechanical stress through a region of the titin protein known as the N2B stretch sensor domain, existing in a complex in sarcomeres⁹. Mutations in FHL1 are the cause of Emery-Dreifuss muscular dystrophy¹⁰. Moreover, a proteomic analysis demonstrated the existence of mutant FHL1 protein in human reducing body myopathy¹¹. Consistent with a role for mutation of FHL1 in a form of muscular dystrophy and in human myopathy, FHL1 can regulate skeletal muscle mass¹².

FHL1 is implicated in a number of human cancers. FHL1 has been reported to function in an oncogenic manner following phosphorylation by Src at tyrosine residue 149 and 272, associating with kindlin-2 at focal adhesions and resulting in nuclear translocation of the BCLAF1 transcription factor¹³. In nude mice injected with the H1299 NSCLC cell line expressing phosphomimetic mutation Y149-Y272D in FHL1, both tumor volume and growth rate were significantly increased as compared to nude mice injected with H1299 cells expressing wild-type FHL1¹³. However, a separate study described tumor suppressive functions of FHL1; FHL1 could interact with Smad2, Smad3 and Smad4 in a pathway together with casein kinase 1 δ (CK1 δ) in a TGF- β -like pathway, and together CK1 δ and FHL1 could impair proliferation of HepG2 human hepatoma cancer cells¹⁴. In a study of 110 gastric cancer patients, both mRNA

1 and protein level of FHL1 was found to be significantly decreased in tumors¹⁵. Consistent with
2 our analysis, lower expression of FHL1 was associated with shorter survival. Importantly,
3 decreased expression of FHL1 was associated with increased distant metastasis and serosal layer
4 invasion¹⁶. The level of FHL1 mRNA was significantly decreased in human hepatocellular
5 carcinoma tumors as compared to normal liver tissue, and H3K27me3 was significantly enriched
6 at the FHL1 promoter in HCC cell lines; ectopic expression of FHL1 in the Hep3B cell line
7 resulted in decreased cell growth and migration¹⁷. We identified one study describing a role for
8 FHL1 in human lung cancer, with 90% of tumors from 80 patients with lung cancer displaying
9 decreased expression of FHL1 at the protein level by immunohistochemistry and
10 immunoblotting¹⁸.

15 We found, by mining multiple independently published microarray datasets, that FHL1
16 was among the genes most differentially expressed in the primary tumors of patients with non-
17 small cell lung cancer; FHL1 was expressed at significantly lower levels in NSCLC tumors as
18 compared to the lung, and decreased expression of FHL1 was significantly associated with worse
19 overall survival in NSCLC patients. FHL1 may be of relevance to the biology of tumor initiation
20 or progression in patients with NSCLC of the adenocarcinoma type, the most common type of
21 the leading cause of cancer death in the United States and worldwide.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
10	210299_s_at	2.48E-18	-18.496769	31.7196362	0.5842 ± 0.0841	FHL1	four and a half LIM domains 1

Table 1: FHL1 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 FHL1 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
64	8170119	1.49E-25	-13.666854	47.578392	0.7478 ± 0.1011	FHL1	four and a half LIM domains 1

Table 2: FHL1 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 FHL1 expression in NSCLC tumors as compared to the lung, and gene are listed in this chart.

Rank	ID	p-value	t	B	Gene
73	A_33_P3849275	5.06E-13	-1.23E+01	19.760007	FHL1

Table 3: FHL1 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 gene name are listed in this chart.

Figure 1: FHL1 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of FHL1 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 FHL1 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 2: FHL1 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of FHL1 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 FHL1 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 3: FHL1 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of FHL1 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 FHL1 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 4: FHL1 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 FHL1 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 FHL1 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)
53	108.27

Table 4: FHL1 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 FHL1 in tumors is listed in this chart.