Fam107A 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 family with sequence similarity 107A, Fam107A was among those whose expression was most different in human NSCLC tumors as compared to the lung. Fam107A expression levels were significantly decreased in NSCLC tumors as compared to the lung, and lower expression of Fam107A in patient tumors was significantly associated with worse overall survival. Fam107A may be important for initiation or progression of non-small cell lung cancer in humans.

Keywords: Fam107A, 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 the gene
10 encoding Fam107A in adenocarcinoma tumors from patients with NSCLC, suggesting Fam107A
11 may be important for NSCLC tumor initiation or progression.
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16 **Methods**

17 We utilized microarray datasets GSE74706², GSE43458³, GSE33532⁴ and for this
18 differential gene expression analysis of NSCLC tumors in conjunction with GEO2R. GSE74706
19 was generated using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology;
20 for this analysis, we used $n=18$ control lung tissue and $n=10$ NSCLC tumors, and the analysis
21 was performed using platform GPL13497. GSE43458 was generated using Affymetrix Human
22 Gene 1.0 ST Array technology; for this analysis, we used with $n=30$ control lung tissue and $n=80$
23 NSCLC tumors, and the analysis was performed using platform GPL6244. GSE33532 was
24 generated using Affymetrix Human Genome U133 Plus 2.0 Array technology; for this analysis,
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we used $n=20$ control lung tissue and $n=10$ NSCLC tumors, and the analysis was performed using platform GPL570. 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 Fam107A 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 Fam107A 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.

Fam107A is differentially expressed in non-small cell lung cancers.

We found significant differential expression of the gene encoding family with sequence

1 similarity 107A, Fam107A, in NSCLC tumors when compared to the lung² (Table 1). When
2 sorting each of the transcripts measured based on significance of difference in expression of
3 Fam107A between NSCLC tumors and the normal lung, in this dataset, Fam107A ranked 27 out
4 of 34183 total transcripts (Table 1). Differential expression of Fam107A in NSCLC tumors was
5 statistically significant (Table 1; $p=5.6E-14$).
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8 We queried a second microarray dataset³ to determine if we could validate differential
9 expression of Fam107A in non-small cell lung cancers (Table 2). We found significant
10 differential expression of Fam107A in NSCLC tumors of the adenocarcinoma type when
11 compared to the normal lung (Table 2). When sorting each of the transcripts measured based on
12 significance of difference in expression of Fam107A between NSCLC tumors and the normal
13 lung, Fam107A ranked 72 of 33252 total transcripts (Table 2). Differential expression of
14 Fam107A in NSCLC tumors was statistically significant (Table 2; $p=3.54E-25$).
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17 Analysis of a third microarray dataset⁴ again revealed significant differential expression
18 of Fam107A in NSCLC tumors of the adenocarcinoma type. When sorting each of the
19 transcripts measured based on significance of difference in expression of Fam107A between
20 NSCLC tumors and the normal lung, Fam107A ranked 26 out of 25906 total transcripts (Table
21 3). Differential expression of Fam107A in NSCLC tumors was statistically significant (Table 3;
22 $p=4.44E-17$).
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26 Fam107A is expressed at significantly lower levels in NSCLC tumors as compared to the lung.
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28 We obtained exact mRNA levels for Fam107A from NSCLC tumors and from the lung to

1 directly compare Fam107A expression between tumor and control lung tissue and assess for
2 statistical significance. Fam107A 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: $p < 0.0001$, Figure 3: $p < 0.0001$). We calculated a mean fold change of 0.8413 ± 0.0528 and
5 0.5990 ± 0.0867 in Fam107A expression when comparing NSCLC tumors to the lung (Table 2
6 and Table 3, respectively).

7 Fam107A expression in NSCLC tumors correlates with overall survival.

8 We performed Kaplan-Meier survival analysis using Fam107A 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 Fam107A tumor expression was correlated with survival
11 outcomes in NSCLC. We found that patients whose tumors expressed lower levels of Fam107A
12 possessed significantly shorter overall survival than patients with high tumor expression of
13 Fam107A (Figure 4). Median overall survival (OS) of patients in the low expression cohort was
14 56.5 months, while median OS in patients in the high Fam107A expression cohort was 103
15 months (Table 4); this difference in median OS based on Fam107A tumor expression in NSCLC
16 was statistically significant (Figure 4; logrank p -value: $2.9e-10$; hazard ratio: 0.63 (0.55 - 0.73);
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 Fam107A gene as among the most
20 significant transcriptional features of NSCLC tumors, and Fam107A expression was significantly
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1 correlated with patient outcomes, as patients with lower tumor expression of Fam107A possessed
2 significantly worse overall survival.

3 4 **Discussion**

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6 Fam107A, also known as TU3A, was identified in an investigation of an area of genomic
7 loss in renal cell carcinoma on chromosome 3⁷. A gene product encoding 144 amino acids was
8 isolated, translated from a transcript of 3 kilobases with ubiquitous expression. Fam107A
9 contains a domain of unknown function at the amino-terminus termed DUF1151, a centrally
10 located nuclear localization signal, and two motifs within the DUF1151: a histidine-arginine-
11 glutamic acid (HRE) sequence, and a proline-glutamic acid (PE) motif⁸. A study of the brain,
12 specifically of the radial glia found that FAM107A was expressed in the ventricular zone at
13 gestational weeks 13.5, and that its expression was up-regulated in outer radial glia cells⁹.

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17 Fam107A is described as being perturbed at the genomic level or at the level of
18 expression in human cancers. Of five renal cell carcinoma cell lines examined in the study in
19 which Fam107A was identified, two had lost expression of Fam107A/TU3A⁷. Whole exome
20 sequencing of a family with five members who were diagnosed with nodular sclerosis classical
21 Hodgkin's lymphoma revealed a missense mutation in Fam107A resulting in a mutation of
22 arginine at position 76 to glutamine, and patient-derived lymphoblastoid cell lines revealed lack
23 of Fam107A expression¹⁰. Fam107A (also known as DRR1) was also identified as a
24 differentially expressed gene in a study of low-grade gliomas that recurred as secondary
25 glioblastomas¹¹. Fam107A expression was significantly lower in secondary glioblastomas and in
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1 primary glioblastomas when compared to diffuse astrocytomas¹¹. Hyper-methylation of the
2 Fam107A locus was found in 22 out of 53 renal cell carcinoma cancers and Fam107A hyper-
3 methylation correlated with worse disease-specific survival¹². We found one study describing a
4 role for Fam107A in non-small cell lung cancer, with decreased expression of Fam107A in
5 NSCLC tumors as compared to normal adjacent lung tissue, and with promoter methylation of
6 Fam107A detectable in a minority of cases surveyed¹³. Thus, Fam107A contains a domain of
7 unknown function, DUF1151, its expression is lost or decreased in a number of cancers and
8 hyper-methylation of the Fam107A locus has also been documented in cancer.

12 We found, by mining multiple independently published microarray datasets, that
13 Fam107A was among the genes most differentially expressed in the primary tumors of patients
14 with non-small cell lung cancer; Fam107A was expressed at significantly lower levels in NSCLC
15 tumors as compared to the lung, and decreased expression of Fam107A was significantly
16 associated with worse overall survival in NSCLC patients. Fam107A may be of relevance to the
17 biology of tumor initiation or progression in patients with NSCLC of the adenocarcinoma type,
18 the most common type of the leading cause of cancer death in the United States and worldwide.
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Rank	ID	p-value	t	B	Gene
27	A_23_P84860	5.6E-14	-1.34E+01	21.8980757	FAM107A

Table 1: Fam107A 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 Fam107A 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
72	8088415	3.54E-25	-13.498127	46.723991	0.8413 ± 0.0528	FAM107A	family with sequence similarity 107 member A

Table 2: Fam107A 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.

Rank	ID	p-value	t	B	FC	Gene	Gene name
26	209074_s_at	4.44E-17	-16.706572	28.9259813	0.5990 ± 0.0867	FAM107A	family with sequence similarity 107 member A

Table 3: Fam107A 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 Fam107A expression in NSCLC tumors as compared to the lung, gene and gene name are listed in this chart.

Figure 1: Fam107A is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of Fam107A 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 Fam107A expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 2: Fam107A transcript is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of Fam107A 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 Fam107A expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 3: Fam107A transcript is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of Fam107A 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 Fam107A expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 4: Fam107A 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 Fam107A 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 Fam107A 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)
56.5	103

Table 4: Fam107A 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 Fam107A in tumors is listed in this chart.