FXYD1 is differentially expressed in non-small cell lung adenocarcinomas.

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Non-small cell lung adenocarcinoma (NSCLC) is a leading cause of death in the United States and worldwide^{1,2}. We mined published microarray data^{3,4,5} to discover genes associated with NSCLC. We identified significant differential expression of the FXYD domain-containing ion transport regulator 1 FXYD1⁶ in tumors from patients with NSCLC. FXYD1 may be of relevance to the initiation, progression or maintenance of non-small cell lung cancers.

Keywords: non-small cell lung adenocarcinoma, non-small cell lung cancer, NSCLC, FXYD1, systems biology of NSCLC, targeted therapeutics in NSCLC.

Over 80% of patients diagnosed with non-small cell lung cancer (NSCLC) will expire within 5 years². Improved understanding of the fundamental transcriptional biology of NSCLC tumors is important for discovery of novel therapeutic targets. We performed a systems-level, unbiased transcriptome analysis of human NSCLC tumors to identify genes whose expression was most different as compared to the lung by mining independent published microarray datasets^{3,4,5}. We observed significant differential expression of the FXYD domain-containing ion transport regulator 1 (FXYD1) in NSCLC tumors, suggesting FXYD1 may be important to the biology of non-small cell lung adenocarcinomas.

Methods

We utilized microarray datasets GSE74706³, GSE33532⁴, and GSE62113⁵ for this differential gene expression analysis of NSCLC tumors in conjunction with GEO2R. GSE74706 was generated using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology (platform: GPL13497) with $n=18$ control lung tissue and $n=10$ NSCLC tumors. GSE33532 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology (platform: GPL570) with $n=20$ control lung tissue and $n=10$ NSCLC tumors. GSE62113 was generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology (platform: GPL14951) with $n=9$ control lung tissue and $n=7$ NSCLC tumors.

The Benjamini and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of 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 FXYD1 expression was significantly different when comparing normal lung tissue (control) to NSCLC tumors using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We interrogated three independently published microarray datasets^{3,4,5} to determine in a blind fashion and at the transcriptome-level genes whose expression was most significantly perturbed in NSCLC tumors.

FXYD1 is differentially expressed in NSCLC.

We observed significant differential expression of the gene encoding the FXYD domain-containing ion transport regulator, FXYD1, in human NSCLC tumors when compared to the lung³. When sorting each gene by significance of difference in expression between NSCLC tumor and the normal lung, FXYD1 ranked 93 out of 34183 total transcripts. Differential expression of FXYD1 was statistically significant (Table 1; $p=1.12E-12$).

We queried a second dataset⁴ to determine if we could validate FXYD1 differential expression in independent microarray data. We again observed significant differential expression of the FXYD1 in human NSCLC tumors when compared to the lung. When sorting each gene by significance of difference in expression between NSCLC tumor and the normal lung, FXYD1 ranked 81 out of 25906 total transcripts. Differential expression of FXYD1 in this second

dataset was also statistically significant (Table 2; $p=3E-15$).

We queried a third dataset⁵ to validate our findings and again observed significant differential expression of FXYD1 in human NSCLC tumors when compared to normal lung. When sorting each gene by significance of difference in expression between NSCLC tumor and the normal lung, FXYD1 ranked 214 out of 29376 total transcripts. Differential expression of FXYD1 in this third dataset was also statistically significant (Table 3; $p=2.92E-05$).

FXYD1 expression is significantly decreased in NSCLC tumors.

We obtained exact mRNA expression levels for the differentially expressed FXYD1 transcript in each of three microarray datasets queried, in normal lung tissue and in primary NSCLC tumors. We also performed a statistical test to determine whether the difference in expression of FXYD1 was statistically significant when comparing NSCLC tumors to the normal lung. FXYD1 was expressed at lower levels across each dataset (Figures 1-3); decreased expression of FXYD1 was statistically significant in each case (Figure 1: $p<0.0001$; Figure 2: $p<0.0001$; Figure 3: $p<0.0001$). We calculated a mean fold change in FXYD1 expression of 0.6947 ± 0.0701 (Table 2) and 0.8174 ± 0.0284 (Table 3) when comparing NSCLC tumor to normal lung.

Thus, we identified FXYD1 as a differentially expressed gene in human non-small cell lung adenocarcinoma and found that FXYD1 was expressed at significantly lower levels in NSCLC tumors as compared to the normal lung.

Discussion

FXYD1, a polypeptide 72 amino acids in length (also known as phospholemman [PLM]) is among the smallest proteins known to form a membrane ion channel with expression observed in skeletal and heart muscle⁶. Injection of *Xenopus* oocytes with FXYD1 results in development of a chloride-selective current, featuring a sigmoidal delay and pulsed amplitude increase⁷. A central function of FXYD1 is in the regulation of the Na,K-ATPase (NKA); FXYD1 directly interacts with NKA α - β isoenzymes. This interaction can occur in the Golgi apparatus or plasma membrane, and results in reductions of both external K⁺ affinity and internal Na⁺ affinity for NKA. FXYD1 interaction with NKA isozymes can be found in skeletal and cardiac muscle as well; together, FXYD1 was deemed to function in regulation of muscle contraction⁸. FXYD1 can be post-translationally modified through phosphorylation by protein kinases A and C (PKA and PKC); phosphorylation of FXYD1 by PKA results in increased apparent affinity of α 1/ β 1 and α 2/ β 1 NKA isoenzymes for Na⁺⁹. PKC phosphorylation appears to regulate FXYD1 activity differently than PKA, increasing turnover number of α 2/ β 1 isoenzymes resulting in increased maximal Na,K-pump current, and only affecting α 2/ β 1 isozymes and not α 1/ β 1 isoenzymes⁹. Ischemia, or reduced blood flow results in activation of NKA; association between PKA and FXYD1 increases during ischemia¹⁰. FXYD1 is an important regulator of Aquaporin-2 (AQP-2) transport; the daytime urine of FXYD1-deficient mice is more dilute than in wild-type mice as a result of more rapid loss of AQP2 from the apical membrane¹¹, with localization and

1 distribution through the cytoplasm instead¹¹. Thus, FXYD1 is important for contractility and
2 excitability of muscle, can be regulated through PKA/PKC phosphorylation and its loss, in mice,
3 affects urinary output due to redistribution of Aquaporin-2.
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5 We could identify one report of FXYD1 function in the lung. In lung alveolar epithelial
6 cells, FXYD1 can function as a negative regulator of NKA and contribute to persistence of
7 edema; FXYD1 expression is significantly increased in the lungs of patients with acute
8 respiratory distress syndrome (ARDS) and the transforming growth factor β could induce
9 expression of FXYD1 in A549 human lung alveolar epithelial cells¹². We could not identify
10 literature describing a function for FXYD1 in non-small cell lung cancer.
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13 We probed multiple independent published microarray datasets to search for
14 differentially expressed genes in patients with non-small cell lung adenocarcinoma and
15 discovered significantly decreased expression of FXYD1 in tumors from patients with NSCLC.
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17 FXYD1, among the smallest membrane ion channels known to man, may be relevant to the
18 biology of NSCLC.
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Rank	ID	p-value	t	B	Gene
93	A_33_P3228435	1.12E-12	-1.19E+01	18.9855569	FXYD1

Table 1: FXYD1 is differentially expressed in non-small cell lung adenocarcinomas.

The rank of differential expression, probe ID, p-value of global differential expression, t, a t-statistic, B, the log-odds of differential expression when comparing tumor to lung, and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
81	205384_at	3E-15	-14.342234	24.7983365	0.6947 ± 0.0701	FXYP1	FXYP domain containing ion transport regulator 1

Table 2: FXYP1 is differentially expressed in non-small cell lung adenocarcinomas.

The rank of differential expression, probe ID, p-value of global differential expression, t, a t-statistic, B, the log-odds of differential expression when comparing tumor to lung, the fold change in FXYP1 expression when comparing tumor to lung, and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Symbol
214	ILMN_2345292	2.92E-05	-5.8287062	2.687424	0.8174 ± 0.0284	FXYD1

Table 3: FXYD1 is differentially expressed in non-small cell lung adenocarcinomas.

The rank of differential expression, probe ID, p-value of global differential expression, t, a t-statistic, B, the log-odds of differential expression when comparing tumor to lung, the fold change in FXYD1 expression when comparing tumor to lung, and gene name are listed in this chart.

Figure 1: FXYD1 expression is significantly decreased in NSCLC tumors.

Expression of FXYD1 is graphically represented in control, normal lung tissue (left) and in primary tumors from patients with non-small cell lung adenocarcinoma (right); mean mRNA expression level is marked in each group and the result of a statistical test evaluating significance of difference in FXYD1 expression between the groups, a *p*-value, is listed above.

Figure 2: FXYD1 expression is significantly decreased in NSCLC tumors.

Expression of FXYD1 is graphically represented in control, normal lung tissue (left) and in primary tumors from patients with non-small cell lung adenocarcinoma (right); mean mRNA expression level is marked in each group and the result of a statistical test evaluating significance of difference in FXYD1 expression between the groups, a *p*-value, is listed above.

Figure 3: FXYP1 expression is significantly decreased in NSCLC tumors.

Expression of FXYP1 is graphically represented in control, normal lung tissue (left) and in primary tumors from patients with non-small cell lung adenocarcinoma (right); mean mRNA expression level is marked in each group and the result of a statistical test evaluating significance of difference in FXYP1 expression between the groups, a *p*-value, is listed above.