1 **STX11 is differentially expressed in non-small cell lung adenocarcinomas.**

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6 Non-small cell lung adenocarcinoma (NSCLC) is a leading cause of death in the United States
7 and worldwide^{1,2}. We mined published microarray data^{3,4} to discover genes associated with
8 NSCLC. We identified significant differential expression of syntaxin 11 (STX11) in tumors from
9 patients with NSCLC. STX11 may be of relevance to the initiation, progression or maintenance
10 of non-small cell lung cancers.

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24 Keywords: non-small cell lung adenocarcinoma, non-small cell lung cancer, NSCLC, STX11,
25 systems biology of NSCLC, targeted therapeutics in NSCLC.

Over 80% of patients diagnosed with non-small cell lung cancer (NSCLC) will expire within 5 years⁵. 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}. We observed significant differential expression of STX11 in NSCLC tumors, suggesting STX11 may be important to the biology of non-small cell lung adenocarcinomas.

Methods

We utilized microarray datasets GSE33532³ and GSE43458⁴ for this differential gene expression analysis of NSCLC tumors in conjunction with GEO2R. 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. GSE43458 was generated using Affymetrix Human Gene 1.0 ST Array technology (platform: GPL6244) with $n=30$ control lung tissue and $n=80$ 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 STX11 expression was significantly different when comparing normal lung tissue from control subjects to tumor tissue from NSCLC patients, 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 harnessed the power of multiple, independently published microarray datasets^{3,4} to determine in a blind fashion and at the transcriptome-level genes whose expression was most significantly perturbed in NSCLC tumors.

STX11 is differentially expressed in NSCLC.

We observed significant differential expression of STX11 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, STX11 ranked 55 out of 25906 total transcripts. Differential expression of STX11 was statistically significant (Table 1; $p=5.73\text{E-}16$).

Analysis of a second microarray dataset⁴ again revealed significant differential of STX11 in primary tumors from patients diagnosed with NSCLC. When sorting each gene by significance of difference in expression between NSCLC tumor and the normal lung, STX11 ranked 179 out of 33252 total transcripts. Differential expression of STX11 in this second dataset was also statistically significant (Table 2; $p=1.2\text{E-}21$).

STX11 expression is significantly decreased in NSCLC tumors.

We obtained exact mRNA expression levels for the differentially expressed STX11 transcript in both 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 STX11 was statistically significant when comparing NSCLC tumors to the normal lung. STX11

1 was expressed at lower levels across each dataset (Figures 1-2); decreased expression of STX11
2 was statistically significant in each case (Figure 1: $p<0.0001$; Figure 2: $p<0.0001$). We
3 calculated a mean fold change in STX11 expression of 0.6351 ± 0.0793 (Table 1) and $0.8140 \pm$
4 0.0846 (Table 2) respectively, when comparing NSCLC tumor to normal lung.
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7 Thus, we identified STX11 as a differentially expressed gene in human non-small cell
8 lung adenocarcinoma and found that STX11 was expressed at significantly lower levels in
9 NSCLC tumors as compared to the normal lung.
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12 Discussion

13 Syntaxin-11 is a member of the syntaxin family of proteins utilized in cells for vesicular
14 fusion as part of a broader class of molecules known as soluble NSF attachment (SNAP) receptor
15 (SNARE) proteins. STX11, as a target-SNARE (t-SNARE) functions in vesicular tethering and
16 fusion in conjunction with other SNARE proteins known as vesicle-SNARES (v-SNARES)^{6,7}.
17 STX11 is most homologous to STX1 and STX2 and spans 287 amino acid in length⁶. Unlike
18 other syntax family members, STX11 does not possess a hydrophobic transmembrane domain at
19 its carboxy-terminus. Instead, STX11 contains a short, cysteine-rich tail at its carboxy-
20 terminus⁶. Tissue expression of STX11 can be detected most abundantly in the heart and
21 placenta, with expression found in most other tissues except for the brain⁶. STX11 is found co-
22 localized along with the mannose-6-phosphate receptor at the trans-Golgi network and late
23 endosomes⁷. STX11 can interact with the t-SNARE SNAP-23 as well as the t-SNARE VAMP⁷.
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28 STX11 expression can also be observed in immune cells, and in macrophages, STX11 can

1 interact with the SNARE protein Vti1b in modulating of fusion between late endosomes and
2 lysosomes through regulation of assembly of complexes containing Vti1b⁸. Macrophages
3 depleted of STX11 possess enlarged LAMP1-positive late endocytic structures⁸. Natural killer
4 (NK) cells and cytotoxic T-lymphocytes from mice deficient in STX11 are defective in
5 granulation and secrete higher than normal levels of interferon gamma, and neutrophils from
6 STX11-deficient mice are impaired in exocytosis⁹. STX11 is important for platelet secretion¹⁰.
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8 Mutation of STX11 results in type 4 familial hemophagocytic lymphohistiocytosis (FHL), an
9 autosomal recessive disorder featuring unregulated T-cell and macrophage activation and
10 cytokine secretion¹¹. Consistent with STX11 mutation resulting in FHL, STX11-deficient mice
11 develop hemophagocytic lymphohistiocytosis (HLH) following infection with lymphocytic
12 choriomeningitis virus (LCMV), with defects in granulation and cytolytic activity in CD8+ T-
13 lymphocytes which expressed high levels of inhibitory receptors PD-1 and LAG-3¹². In the
14 context of cancer, STX11 has been described as a tumor suppressor in primary thyroid T-cell
15 lymphomas (PTTL)¹³. 67% of PTTL possess loss of 6q24.2, where STX11 is located; expression
16 of STX11 corrected proliferation defects in T-cell lines possessing STX11 alteration like
17 Su9T01¹³. We could not identify information describing a function for STX11 in NSCLC.
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23 Differential expression of STX11 in NSCLC should be validated in larger and separate
24 cohorts of patients with NSCLC. If validated, mouse models of non-small cell lung cancers can
25 be utilized to assess the effect of conditional expression of STX11 in NSCLC tumor post-
26 development and on tumor cell proliferation and cancer progression *in vivo*.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
55	235670_at	5.73E-16	-15.237354	26.4246999	0.6351 ± 0.0793	STX11	syntaxin 11

Table 1: STX11 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
179	8122457	1.2E-21	-11.938206	38.704493	0.8140 ± 0.0846	STX11	syntaxin 11

Table 2: STX11 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 STX11 expression when comparing tumor to lung, and gene name are listed in this chart.

Figure 1: STX11 expression is significantly decreased in NSCLC tumors.

Expression of STX11 is graphically represented in control, normal lung tissue (left) and in primary tumors from patients with non-small cell adenocarcinoma (right); mean mRNA expression level is marked in each group and the result of a statistical test evaluating significance of difference in STX11 expression between the groups, a *p*-value, is listed above.

Figure 2: STX11 expression is significantly decreased in NSCLC tumors.

Expression of STX11 is graphically represented in control, normal lung tissue (left) and in primary tumors from patients with non-small cell adenocarcinoma (right); mean mRNA expression level is marked in each group and the result of a statistical test evaluating significance of difference in STX11 expression between the groups, a *p*-value, is listed above.