S1PR1 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 sphingosine-1-phosphate receptor S1PR1 in tumors from patients with NSCLC. S1PR1 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, S1PR1, systems biology of NSCLC, targeted therapeutics in NSCLC.

Over 80% of patients diagnosed with non-small cell lung cancer (NSCLC) will expire within 5 years⁶. 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 S1PR1 in NSCLC tumors, suggesting S1PR1 may be important to the biology of non-small cell lung adenocarcinomas.

Methods

We utilized microarray datasets GSE74706³, GSE33532⁴, and GSE43458⁵ 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. 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 S1PR1 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^{3,4,5} to determine in a blind fashion and at the transcriptome-level genes whose expression was most significantly perturbed in tumors from patients with non-small cell lung cancer.

S1PR1 is differentially expressed in NSCLC.

We observed significant differential expression of the gene encoding the sphingosine-1-phosphate receptor, S1PR1 in human NSCLC tumors when compared to the lung². When sorting each gene by significance of difference in expression between NSCLC tumor and the normal lung, S1PR1 ranked 80 out of 34183 total transcripts. Differential expression of S1PR1 was statistically significant (Table 1; $p=7.03E-13$).

We queried a second dataset³ to determine if we could validate S1PR1 differential expression in independent microarray data. We again observed significant differential expression of S1PR1 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, S1PR1 ranked 171 out of 25906 total transcripts. Differential expression of S1PR1 in this second dataset was also statistically significant (Table 2; $p=9.54E-14$).

1 Analysis of a third microarray dataset⁴ again revealed significant differential of S1PR1 in
2 primary tumors from patients diagnosed with NSCLC. When sorting each gene by significance
3 of difference in expression between NSCLC tumor and the normal lung, S1PR1 ranked 38 out of
4 33252 total transcripts. Differential expression of S1PR1 in this third dataset was also
5 statistically significant (Table 3; $p=2.2E-27$).
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8 S1PR1 expression is significantly decreased in NSCLC tumors.
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10 We obtained exact mRNA expression levels for the differentially expressed S1PR1
11 transcript in each of three microarray datasets queried, in normal lung tissue and in primary
12 NSCLC tumors. We also performed a statistical test to determine whether the difference in
13 expression of S1PR1 was statistically significant when comparing NSCLC tumors to the normal
14 lung. S1PR1 was expressed at lower levels across each dataset (Figures 1-3); decreased
15 expression of S1PR1 was statistically significant in each case (Figure 1: $p<0.0001$; Figure 2:
16 $p<0.0001$; Figure 3: $p<0.0001$). We calculated a mean fold change in S1PR1 expression of
17 0.7203 ± 0.0672 (Figure 2) and 0.8353 ± 0.0702 (Figure 3) respectively, when comparing tumors
18 from patients with NSCLC to normal lung.
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22 Thus, we identified S1PR1 as a differentially expressed gene in human non-small cell
23 lung adenocarcinoma and found that S1PR1 was expressed at significantly lower levels in
24 NSCLC tumors as compared to the normal lung.
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Discussion

S1PR1 is one of five heterotrimeric G-protein-coupled receptors for the sphingosine-1-phosphate lipid mediator^{7,8}. S1PR1, also known as endothelial differentiation gene (EDG-1) can function as a chemotactic receptor for the platelet-derived growth factor (PDGF), facilitating chemotaxis towards PDGF⁹. S1PR1 can influence lymphocyte migration *in vivo* as administration of an S1PR agonist, FTY720 results in lymphopenia associated with accumulation of lymphocytes in the lymph nodes¹⁰. Marginal zone B-cells re-localize to lymphoid follicles upon administration of FTY720; genetic studies in mice revealed that S1PR1 but not S1PR3 is required for localization of B-cells to the splenic marginal zone¹¹. FTY720 treatment fails to induce re-localization of B-cells to lymphoid follicles in Cxcl13-deficient mice, demonstrating the importance of Cxcl13 in competing with S1PR signaling for recruitment of marginal zone B-cells to either the marginal or the lymphoid follicles¹¹. Entry of peripheral T-lymphocytes into the tissues is also controlled by S1PR1, as S1PR1 agonism blocks tissue T-cell entry into the afferent lymphatic system¹².

Aside from controlling trafficking and migration of lymphocytes, S1PR1 is described to be important for angiogenesis and for endothelial cell interactions. S1PR1 is important for interactions between mural cells and endothelial cells (EC) and for N-cadherin trafficking as well as adhesion between EC and mural cells involving the actin cytoskeleton¹³. S1PR1 has been described as a negative regulator of angiogenesis; loss of S1PR1 results in angiogenic hypersprouting and loss of regular VE-cadherin localization at endothelial cell junctions; S1PR1 also regulates VEGFR2 signaling induced by VE-cadherin¹⁴. Thus, S1PR1 is important for

vascular stabilization. Endothelial cell knockdown of S1PR1 resulted in increased endothelial

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1 permeability induced by activation of the protease-activated receptor PAR-1¹⁵.

2 S1PR1 is expressed in endothelial cells during angiogenesis of the embryo and in
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4 vascular cells of adult mice¹⁶. In tumors, S1PR1 is expressed in intratumoral capillaries and
5 large tumor vessels during tumor-associated angiogenesis¹⁶. Depletion of S1PR1 using small
6 interfering RNA (siRNA)-liposome complexes resulted in significant decreases in number of
7 tumor blood vessels and in tumor volume in Lewis lung carcinoma cells in nude mice, as well as
8 coverage of tumor vessels by mural cells¹⁶. Thus, S1PR1 appears to be important for tumor
9 angiogenesis. A transporter of S1PR1, Spn2, has been demonstrated to be important in the A549
10 non-small cell lung cancer cell line for cell viability and migration¹⁷.

13 In summary, we probed multiple independent published microarray datasets to search for
14 differentially expressed genes in non-small cell lung cancer and found significantly decreased
15 expression of the sphingosine-1-phosphate receptor S1PR1 in tumors from patients with NSCLC.
16 S1PR1 may be relevant to the biology of NSCLC.
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Rank	ID	p-value	t	B	Gene
80	A_23_P404481	7.03E-13	-1.21E+01	19.4380442	S1PR1

Table 1: S1PR1 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
171	204642_at	9.54E-14	-12.595444	21.376778	0.7203 ± 0.0672	S1PR1	sphingosine-1-phosphate receptor 1

Table 2: S1PR1 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 S1PR1 expression when comparing tumor to lung, and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
38	7903393	2.2E-27	-14.495344	51.730125	0.8353 ± 0.0702	S1PR1	sphingosine-1-phosphate receptor 1

Table 3: S1PR1 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 S1PR1 expression when comparing tumor to lung, and gene name are listed in this chart.

Figure 1: S1PR1 expression is significantly decreased in NSCLC tumors.

Expression of S1PR1 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 S1PR1 expression between the groups, a *p*-value, is listed above.

Figure 2: S1PR1 expression is significantly decreased in NSCLC tumors.

Expression of S1PR1 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 S1PR1 expression between the groups, a *p*-value, is listed above.

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