The platelet endothelial cell adhesion molecule PECAM1 is differentially expressed in non-small cell lung cancer.

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer death in the United States^{1,2}. We mined published microarray data^{3,4} to discover genes associated with NSCLC tumors. We identified significant differential expression of the platelet endothelial cell adhesion molecule PECAM1⁵ in primary tumors from patients with NSCLC. PECAM1 may be of relevance to the initiation, progression or maintenance of non-small cell lung cancers.

Keywords: non-small cell lung cancer, NSCLC, PECAM1, 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}. We found significant differential expression of the platelet endothelial cell adhesion molecule PECAM1 in primary tumors from patients with NSCLC, suggesting PECAM1 may be important to the biology of non-small cell lung cancers.

Methods

We utilized microarray datasets GSE74706³ and GSE33532⁴ 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 with $n=18$ control lung tissues and $n=10$ NSCLC tumors; analysis performed using platform GPL13497. GSE33532 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=20$ control lung tissues and $n=10$ NSCLC tumors; analysis performed using platform GPL570. NSCLC tumors 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 whether differential expression was statistically significant relative to the measured transcriptome. Log-transformation of data was

1 auto-detected, and annotation platform was NCBI-generated. A statistical test was performed to
2 evaluate whether PECAM1 expression was significantly different between normal lung tissue
3 and NSCLC tumors using a two-tailed, unpaired t-test with Welch's correction. We used PRISM
4 for all statistical analyses (Version 8.4.0)(455).

7 **Results**

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

13 PECAM1 is differentially expressed in NSCLC.

15 We observed significant differential expression of the gene encoding the platelet
16 endothelial cell adhesion molecule PECAM1 in human NSCLC tumors when compared to the
17 lung³. When sorting each gene by significance of difference in expression between NSCLC
18 tumor and the normal lung, PECAM1 ranked 95 out of 34183 total transcripts. Differential
19 expression of PECAM1 was statistically significant (Table 1; $p=1.23E-12$).

21
22 We queried a second dataset⁴ to determine if we could validate PECAM1 differential
23 expression in independent microarray data. We again found significant differential expression of
24 PECAM1 in human NSCLC tumors when compared to the normal lung. When sorting each
25 gene by significance of difference in expression between NSCLC tumor and the normal lung,
26 PECAM1 ranked 73 out of 25906 total transcripts. Differential expression of PECAM1 in
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1 this second dataset was also statistically significant (Table 2; $p=1.8E-15$).

2
3 PECAM1 expression is significantly decreased in NSCLC tumors.

4 We obtained exact mRNA expression levels for the differentially expressed PECAM1
5 transcript in both microarray datasets queried, in normal lung tissue and in primary NSCLC
6 tumors. We also performed a statistical test to determine whether the difference in expression of
7 PECAM1 was statistically significant when comparing NSCLC tumors to the normal lung.
8
9 PECAM1 was expressed at lower levels in NSCLC tumors across each dataset (Figures 1-3);
10 decreased tumor expression of PECAM1 was statistically significant in each case (Figure 1:
11 $p<0.0001$ and Figure 2: $p<0.0001$). We calculated a mean fold change of 0.8395 ± 0.0426 in
12
13 PECAM1 expression when comparing NSCLC tumor to normal lung (Table 2).
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15 Thus, we identified PECAM1 as a differentially expressed gene in human non-small cell
16 lung adenocarcinoma using published microarray data and found that PECAM1 was expressed at
17 significantly lower levels in NSCLC tumors as compared to the normal lung.
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20 **Discussion**

21 Platelet endothelial cell adhesion molecule, PECAM1, also known as CD31, was
22 characterized by generation of antibodies against human endothelial cells grown on dehydrated
23 collagen gels, and these antibodies were assessed for their ability to bind the intercellular
24 junctional zones⁷. An antibody known as hec7 was generated which stained the borders of
25 endothelium and of confluent endothelial cell monolayers, recognizing a 135 kilodalton
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1 plasmalemmal protein on western blot, with expression on nearly all endothelial vessels
2 examined including arteries, vessels, and capillaries⁷. PECAM1 expression can also be found in
3 cells of the blood, including platelets, monocytes, granulocytes and some lymphocytes⁸. An
4 antibody against PECAM1 but not LFA-3 could induce flux of radio-labeled albumin across an
5 endothelial cell monolayer suggesting PECAM1 functioned to maintain the integrity of vascular
6 barrier⁵. Homophilic PECAM1 binding at cell junctions is important for maintenance of barrier
7 integrity as a mutant PECAM1 (K89A), unable to form homophilic interactions, was unable to
8 fully maintain barrier integrity in response to thrombin exposure⁹. Together, the data support a
9 role for PECAM1 in maintenance of vascular barrier integrity.

13 A mouse PECAM1 antibody could block tumor growth and vascularity of three types of
14 cancer cell lines grown in mice including the A549 non-small cell lung cancer cell line¹⁰. This
15 suggested that PECAM1 could function in tumor angiogenesis¹⁰. Nude BALB/c mice
16 xenografted with a lung adenocarcinoma cell line transfected with chemically modified small
17 interfering RNA (siRNA) against PECAM1 displayed decreased tumor growth and decreased
18 tumor volume on day 10 after tail vein injection. Expression of the vascular endothelial growth
19 factor (VEGF) was also decreased in siPECAM1 tumor xenografts. Together, the data suggested
20 that silencing PECAM1 *in vivo* could effectively impair tumor growth and expression of a
21 marker associated with vasculature¹¹. A study of tumors from 55 patients with NSCLC,
22 identifying tumor microvessels by staining for PECAM-1 and another marker (factor VIII) found
23 that tumors from patients with stage IIIA/IIIB disease had significantly higher microvessel
24 counts than tumors from patients with Stage I-II disease¹². Moreover, the density of

1 microvessels was significantly higher in the tumors of patients with nodal metastasis as
2 compared to those without nodal metastasis¹². Thus, PECAM1 can mark microvessels in tumors
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4 from patients with NSCLC and in experimental models, PECAM1 inhibition by antibody
5 blockade or siRNA depletion can result in some level of therapeutic efficacy¹².
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7 By mining published microarray data^{3,4}, we found that the gene encoding the platelet
8 endothelial cell adhesion molecule PECAM1 was among the genes most differentially expressed
9 in tumors from patients with non-small cell lung cancer. PECAM1 may be of relevance to the
10 biology of NSCLC.
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7 2157-2162.

Rank	ID	p-value	t	B	Gene
95	A_23_P252471	1.23E-12	-1.19E+01	18.8922703	PECAM1

Table 1: PECAM1 is differentially expressed in non-small cell lung cancer.

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
73	208982_at	1.8E-15	-14.613896	25.3005706	0.8395 ± 0.0426	PECAM1	platelet and endothelial cell adhesion molecule 1

Table 2: PECAM1 is differentially expressed in non-small cell lung cancer.

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 PECAM1 expression when comparing tumor to lung, and gene name are listed in this chart.

Figure 1: PECAM1 expression is significantly decreased in NSCLC tumors.

Expression of PECAM1 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 PECAM1 expression between the groups, a *p*-value, is listed above.

Figure 2: PECAM1 expression is significantly decreased in NSCLC tumors.

Expression of PECAM1 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 PECAM1 expression between the groups, a *p*-value, is listed above.