The junctional adhesion molecule 2, JAM2, 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 the junctional adhesion molecule 2, JAM2, also known as vascular endothelial (VE)-JAM, was among those whose expression was most different in human NSCLC tumors as compared to the lung. JAM2 expression levels were significantly decreased in NSCLC tumors as compared to the lung, and lower expression of JAM2 in patient tumors was significantly associated with worse overall survival. JAM2 may be important for initiation or progression of non-small cell lung cancer in humans.

Keywords: JAM2, junctional adhesion molecule 2 vascular endothelial JAM, VE-JAM, 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 JAM2 in
10 adenocarcinoma tumors from patients with NSCLC, suggesting JAM2 may be important for
11 NSCLC tumor initiation or progression.

12 **Methods**

13 We utilized microarray datasets, GSE43458², GSE33532³ and GSE74706⁴ for this
14 differential gene expression analysis of NSCLC tumors in conjunction with GEO2R. GSE43458
15 was generated using Affymetrix Human Gene 1.0 ST Array technology; for this analysis, we
16 used with $n=30$ control lung tissue and $n=80$ NSCLC tumors, and the analysis was performed
17 using platform GPL6244. GSE33532 was generated using Affymetrix Human Genome U133
18 Plus 2.0 Array technology; for this analysis, we used $n=20$ control lung tissue and $n=10$ NSCLC
19 tumors, and the analysis was performed using platform GPL570. GSE74706 was generated
20 using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology; for this

analysis, we used $n=18$ control lung tissue and $n=10$ NSCLC tumors, and the analysis was performed using platform GPL13497. 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 JAM2 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 JAM2 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.

JAM2 is differentially expressed in non-small cell lung cancers.

We found significant differential expression of the gene encoding the junctional adhesion molecule 2, JAM2, in NSCLC tumors when compared to the lung² (Table 1). When

1 sorting each of the transcripts measured by microarray based on significance of difference in
2 JAM2 expression between NSCLC tumors and the normal lung, in this dataset, JAM2 ranked
3 241 out of 25906 total transcripts, equating to 99.1% differential expression (Table 1).
4
5 Differential expression of JAM2 in NSCLC tumors was statistically significant (Table 1;
6 $p=4.8E-20$).
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8 We queried a second microarray dataset³ to determine if we could validate differential
9 expression of JAM2 in non-small cell lung cancers (Table 2). We found significant differential
10 expression of JAM2 in NSCLC tumors of the adenocarcinoma type when compared to the
11 normal lung (Table 2). When sorting each of the transcripts measured based on significance of
12 difference in expression of JAM2 between NSCLC tumors and the normal lung, JAM2 ranked
13 77 of 33252 total transcripts (Table 2), equating to 99.8% differential expression. Differential
14 expression of JAM2 in NSCLC tumors was statistically significant (Table 2; $p=2.72E-15$).
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17 Analysis of a third microarray dataset⁴ again revealed significant differential expression
18 of JAM2 in NSCLC tumors of the adenocarcinoma type. When sorting each of the transcripts
19 measured based on significance of difference in expression of JAM2 between NSCLC tumors
20 and the normal lung, JAM2 ranked 75 out of 34183 total transcripts (Table 3), equating to 99.8%
21 differential expression. Differential expression of JAM2 in NSCLC tumors was statistically
22 significant (Table 3; $p=5.34E-13$).
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25 JAM2 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 JAM2 from NSCLC tumors and from the lung to

1 directly compare JAM2 expression between tumor and control lung tissue and assess for
2 statistical significance. JAM2 was expressed at significantly lower levels in NSCLC tumors as
3 compared to the normal lung in each dataset queried, respectively (Figure 1: $p < 0.0001$; Figure 2:
4 $p < 0.0001$; and Figure 3: $p < 0.0001$). We calculated a mean fold change of 0.8194 ± 0.0817 and
5 0.7565 ± 0.0534 in JAM2 expression when comparing NSCLC tumors to the lung (Table 1 and
6 Table 2, respectively).

7 JAM2 expression in NSCLC tumors correlates with overall survival.

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10 We performed Kaplan-Meier survival analysis using JAM2 mRNA expression in NSCLC
11 tumors coupled with paired overall survival data from each patient, in 1925 NSCLC patients in
12 total, to determine whether JAM2 tumor expression was correlated with survival outcomes in
13 NSCLC. We found that patients whose tumors expressed lower levels of JAM2 possessed
14 significantly shorter overall survival than patients with high tumor expression of JAM2 (Figure
15 3). Median overall survival (OS) of patients in the low expression cohort was 57 months, while
16 median OS in patients in the high JAM2 expression cohort was 95 months (Table 4); this
17 difference in median OS based on JAM2 tumor expression in NSCLC was statistically
18 significant (Figure 3; logrank p -value: 1.2×10^{-8} , hazard ratio: 0.62 (0.52 - 0.73); false discovery
19 rate=0.01).

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21 Thus, blind comparative transcriptome analysis of non-small cell lung cancers revealed
22 differential expression of JAM2 as among the most significant transcriptional features of NSCLC
23 tumors, and JAM2 expression was significantly correlated with patient outcomes, as patients
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1 with lower tumor expression of JAM2 possessed significantly worse overall survival.

3 **Discussion**

4 Vascular endothelial junctional adhesion molecule, VE-JAM, also known as junctional
5 adhesion molecule 2, or JAM2, belongs to the immunoglobulin superfamily, and was identified
6 based on up-regulation during co-culture of tumor cells with endothelial cells. JAM2 is
7 expressed in endothelial cells, in vessels of the lymphatic system and in Peyer's patches and high
8 endothelial venules, and in the heart, and can be found localized to cell-cell contacts at tight
9 junctions^{7,8}. Aside from its role in sealing regions of cell-cell contacts, JAM2 can function to
10 support migration of lymphocytes across endothelial cell barriers⁹, and the integrin $\alpha 4\beta 1$ is an
11 interaction partner of JAM2¹⁰. JAM2 contains extracellular immunoglobulin-like folds, a
12 transmembrane domain, as well as a PDZ domain¹¹. Another interaction partner of JAM2 is
13 JAM3, with the ectodomain of JAM3 binding the JAM2 Fc¹¹, and JAM3 cooperates with JAM2
14 in T-cell adhesion, as well as interaction with other cells of the immune system including natural
15 killer (NK) cells and dendritic cells¹². Thus, JAM2 functions both in sealing of endothelial cell
16 boundaries to support barrier integrity and in transendothelial migration of immune cells.

17 In the context of the lung, induction of JAM2 expression has been described in
18 streptozotocin-induced diabetic mice, and JAM2 expression could also be induced in alveolar
19 organoids generated from mesenchymal stem cells *in vitro*¹³. JAM3 has been associated with
20 lymphatic microvessel density, lymph node metastasis and decreased overall and metastasis-free
21 survival in patients with non-small cell lung cancer¹⁴. Depletion experiments demonstrated that

JAM3 could support expression of the vascular endothelial growth factor C (VEGF-C) in NSCLC cell lines¹⁴.

We found, by mining multiple independently published microarray datasets, that the endothelial cell adhesion molecule, JAM2, was among the genes most differentially expressed in the primary tumors of patients with non-small cell lung cancer; JAM2 was expressed at significantly lower levels in NSCLC tumors as compared to the lung, and decreased expression of JAM2 was significantly associated with worse overall survival in NSCLC patients. JAM2 may be of relevance to the biology of tumor initiation or progression in patients with NSCLC of the adenocarcinoma type, 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	FC	Gene	Gene name
241	8068024	4.8E-20	-11.240662	35.064353	0.8194 ± 0.0817	JAM2	junctional adhesion molecule 2

Table 1: JAM2 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 JAM2 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
77	219213_at	2.72E-15	-14.392843	24.8924837	0.7565 ± 0.0534	JAM2	junctional adhesion molecule 2

Table 2: JAM2 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 JAM2 expression in NSCLC tumors as compared to the lung, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene
75	A_32_P310335	5.34E-13	-1.23E+01	19.7059647	JAM2

Table 3: JAM2 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, and gene are listed in this chart.

Figure 1: JAM2 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of JAM2 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 JAM2 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 2: JAM2 transcript is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of JAM2 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 JAM2 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 3: JAM2 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of JAM2 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 JAM2 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 4: JAM2 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 JAM2 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 JAM2 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)
57	95

Table 4: JAM2 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 JAM2 in tumors is listed in this chart.