1 **CD36 is differentially expressed in non-small cell lung adenocarcinomas.**

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6 Non-small cell lung adenocarcinoma (NSCLC) is the leading cause of cancer death in the United
7 States^{1,2}. We mined published microarray data^{3,4} to discover genes associated with NSCLC
8 tumors. We identified significant differential expression of CD36 in primary tumors from
9 patients with NSCLC. CD36 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, CD36,
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 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 found significant differential expression of the scavenger receptor CD36 in primary tumors from patients with NSCLC, suggesting CD36 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 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. GSE62113 was generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology with $n=9$ control lung tissues and $n=7$ NSCLC tumors; analysis performed using platform GPL14951.

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 auto-detected, and annotation platform was NCBI-generated. A statistical test was performed to evaluate whether CD36 expression was significantly different 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 (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 different in NSCLC tumors.

CD36 is differentially expressed in NSCLC.

We observed significant differential expression of the gene encoding the scavenger receptor CD36 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, CD36 ranked 140 out of 34183 total transcripts. Differential expression of CD36 was statistically significant (Table 1; $p=5.24E-12$).

We queried a second dataset⁴ to determine if we could validate CD36 differential expression in independent microarray data. We again found significant differential expression of CD36 in human NSCLC tumors when compared to the normal lung. When sorting each gene by significance of difference in expression between NSCLC tumor and the normal lung, CD36

ranked 70 out of 25906 total transcripts. Differential expression of CD36 in this second dataset was also statistically significant (Table 2; $p=1.51\text{E-}15$).

We queried a third dataset⁵ to validate our findings and again observed significant differential expression of CD36 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, CD36 ranked 75 out of 29376 total transcripts. Differential expression of CD36 in this third dataset was also statistically significant (Table 3; $p=1.75\text{E-}06$).

CD36 expression is significantly decreased in NSCLC tumors.

We obtained exact mRNA expression levels for the differentially expressed CD36 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 CD36 was statistically significant when comparing NSCLC tumors to the normal lung. CD36 was expressed at lower levels in NSCLC tumors across each dataset (Figures 1-3); decreased tumor expression of CD36 was statistically significant in each case (Figure 1: $p<0.0001$; Figure 2: $p<0.0001$; Figure 3: $p=0.0004$). We calculated a mean fold change in CD36 expression of 0.5829 ± 0.0864 (Table 2) and 0.8261 ± 0.0689 (Table 3) when comparing NSCLC tumor to normal lung.

Thus, we identified CD36 as a differentially expressed gene in human non-small cell lung adenocarcinoma and found that CD36 was expressed at significantly lower levels in NSCLC tumors as compared to the normal lung.

Discussion

CD36 is a cell surface receptor of the scavenger receptor (SR) family, also known as SR-BII, glycoprotein IV and the fatty acid translocase⁶. CD36 is expressed on cells of the immune system including macrophage, dendritic cells and platelets, as well as on adipocytes and endothelial cells⁶. CD36 can function similar to a channel, enabling translocation of long chain fatty acids across the plasma membrane but can also act as a receptor in innate immune signal transduction pathways. Classically defined ligands for CD36 include the oxidized form of low density lipoprotein (oxLDL)⁷, thrombospondin-1⁸, erythrocytes infected with the malaria parasite *Plasmodium falciparum*⁹, as well as collagen¹⁰. CD36 has been described as part of an innate immune receptor system that includes the Toll-like receptor (TLR) heterodimer TLR2/TLR6, for the pathogen-associated molecular pattern (PAMP) lipoteichoic acid but not for the PAMPs and TLR2 ligands PAM₂CSK₄ and PAM₃CSK₄, demonstrating a specific role for CD36 in recognition of some but not all TLR2 ligands¹¹. In conjunction with TLR4 and TLR6, CD36 has also been described as a receptor for amyloid- β particles, resulting in inflammatory signaling in microglia including activation of NF- κ B and production of nitric oxide¹².

CD36 also has functions in fatty acid metabolism, specifically in fatty acid oxidation. Soleus (calf) muscle fibers from mice over-expressing CD36 under the control of the muscle creatine kinase promoter possess increased capacity for fatty acid oxidation in response to muscle contraction, and these CD36-transgenic mice are leaner than wild-type mice, with decreased adipose tissue content and lower blood levels of triglycerides and fatty acids¹³.

1 Adipocytes from CD36-null mice manifest decreased transport of fatty acids. Fasting cholesterol
2 levels in the plasma, specifically of high density fraction, are significantly increased in CD36-
3 null mice; while plasma triacylglycerol levels are also increased, this increase is of the very low
4 density fraction¹⁴.

6 CD36 receptor has multiple described roles in human cancer. In glioblastoma¹⁵, ovarian
7 cancer¹⁶ and gastric cancer¹⁷, CD36 expression is up-regulated. A recent study demonstrated that
8 expression of CD36 is specifically up-regulated in regulatory T-cells that reside within tumors
9 (intratumoral T_{reg}), supporting adaptation of T_{reg} to a tumor microenvironment rich in lactic acid
10 through effects on the mitochondria via the peroxisome proliferator-activated receptor β
11 signaling pathway¹⁸. In lung cancer, one study has documented decreased expression of CD36 in
12 human lung tumors with increased methylation of CD36¹⁹. Thus, CD36 can function in transport
13 or recognition of a multitude of ligands, supporting activation of inflammatory signaling; CD36
14 can influence metabolic processes including fatty acid oxidation in muscle tissue, and in cancer,
15 CD36 expression is perturbed across tissue type with at least one report demonstrating influence
16 of CD36 on metabolic status of the tumor microenvironment.

22 In summary, we probed multiple independent published microarray datasets to identify in
23 an unbiased manner genes whose expression is most different in non-small cell lung
24 adenocarcinomas and discovered differential and significantly decreased expression of CD36 in
25 primary tumors from patients with NSCLC. CD36 may be relevant to the biology of NSCLC.

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Rank	ID	p-value	t	B	Gene
140	A_23_P111583	5.24E-12	-1.12E+01	17.471904	CD36

Table 1: CD36 is differentially expressed in non-small cell lung adenocarcinomas.

The rank of differential expression, probe ID, p-value of global differential expression, t, a moderated 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
70	228766_at	1.51E-15	-14.709715	25.4758936	0.5829 ± 0.0864	CD36	CD36 molecule

Table 2: CD36 is differentially expressed in non-small cell lung adenocarcinomas.

The rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression when comparing tumor to lung, the fold change (FC) in CD36 expression when comparing tumor to lung, and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene
75	ILMN_1796094	1.75E-06	-7.4263506	5.376528	0.8261 ± 0.0689	CD36

Table 3: CD36 is differentially expressed in non-small cell lung adenocarcinomas.

The rank of differential expression, probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression when comparing tumor to lung, the fold change (FC) in CD36 expression when comparing tumor to lung, and gene name are listed in this chart.

Figure 1: CD36 expression is significantly decreased in NSCLC tumors.

Expression of CD36 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 CD36 expression between the groups, a *p*-value, is listed above.

Figure 2: CD36 expression is significantly decreased in NSCLC tumors.

Expression of CD36 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 CD36 expression between the groups, a *p*-value, is listed above.

Figure 3: CD36 expression is significantly decreased in NSCLC tumors.

Expression of CD36 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 CD36 expression between the groups, a *p*-value, is listed above.