Cysteine dioxygenase 1 (CDO1) is differentially expressed in non-small cell lung cancer.

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Non-small cell lung cancer (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 gene encoding cysteine dioxygenase 1, CDO1⁶, in tumors from patients with NSCLC. CDO1 may be of relevance to the initiation, progression or maintenance of non-small cell lung cancers.

Keywords: non-small cell lung cancer, NSCLC, CDO1, systems biology of NSCLC, targeted therapeutics in NSCLC.

Over 80% of patients diagnosed with non-small cell lung cancer (NSCLC) will expire within 5 years⁷. 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 in lung cancers by mining independently published microarray datasets^{3,4,5}. We observed significant differential expression of CDO1 in NSCLC tumors, suggesting CDO1 may be important to the biology of non-small cell lung cancers.

Methods

We utilized microarray datasets GSE74706³, GSE43458⁴, 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 (platform: GPL13497) with $n=18$ 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. GSE62113 was generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology (platform: GPL14951) with $n=9$ control lung tissue and $n=7$ NSCLC tumors. Tumors were of adenocarcinoma type.

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 CDO1 expression was significantly different when comparing normal lung tissue (control) to 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 perturbed in NSCLC tumors.

CDO1 is differentially expressed in NSCLC.

We observed significant differential expression of the gene encoding cysteine dioxygenase 1, CDO1 in human NSCLC tumors when compared to the lung³. When sorting each transcript measured by microarray based on significance of difference in expression between NSCLC tumors and the normal lung, CDO1 ranked 9 out of 34183 total transcripts. Differential expression of CDO1 was statistically significant (Table 1; $p=2.78E-15$).

We queried a second dataset⁴ to determine if we could validate CDO1 differential expression in independent microarray data. We again observed significant differential expression of the CDO1 gene 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, CDO1 ranked 159 out of 33252 total transcripts (Table 2). Differential expression of CDO1 in

1 this second dataset was also statistically significant (Table 2; $p=3.3E-22$).

2 We queried a third dataset⁵ to validate our findings and again observed significant
3 differential expression of CDO1 in human NSCLC tumors when compared to normal lung.
4 When sorting each gene by significance of difference in expression between NSCLC tumors and
5 the normal lung, CDO1 ranked 186 out of 29376 total transcripts (Table 3). Differential
6 expression of CDO1 in this third dataset was also statistically significant (Table 3; $p=2.16E-05$).
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11 CDO1 expression is significantly decreased in NSCLC tumors.

12 We obtained exact mRNA expression levels for the differentially expressed CDO1
13 transcript in each of three microarray datasets queried, in normal lung tissue and in primary
14 NSCLC tumors. We also performed a statistical test to determine whether the difference in
15 expression of CDO1 was statistically significant when comparing NSCLC tumors to the normal
16 lung. CDO1 was expressed at lower levels across each dataset in NSCLC tumors (Figures 1-3);
17 decreased tumor expression of CDO1 was statistically significant in each case (Figure 1:
18 $p<0.0001$; Figure 2: $p<0.0001$; Figure 3: $p=0.0002$). We calculated a mean fold change in
19 CDO1 expression of 0.8344 ± 0.0665 (Table 2) and 0.8452 ± 0.0617 (Table 3) when comparing
20 NSCLC tumor to normal lung.
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24 Thus, we identified CDO1 as a differentially expressed gene in human non-small cell
25 lung cancer and found that CDO1 was expressed at significantly lower levels in NSCLC tumors
26 as compared to the normal lung.
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Discussion

Cysteine dioxygenase functions in cysteine metabolism, specifically to catalyze the oxidation of L-cysteine to cysteine sulfinic acid during conversion of cysteine to sulfate^{7,8}.

Cdo1-deficient mice manifest markedly decreased levels of taurine and hypotaurine, supporting a role for Cdo1 in taurine biosynthesis as well⁹. CDO1 is a metalloenzyme, with each molecule binding one atom of iron (Fe^{2-}); mouse and human CDO1 share 91% sequence identity⁸.

The CDO1 promoter is differentially methylated in tumor tissues from patients with cancers of the lung, breast, stomach, bladder and esophagus; in cancer cell lines from these tissues, expression of CDO1 was decreased, suggesting that CDO1 expression can be silenced by promoter methylation in human cancer¹⁰. Ectopic expression of CDO1 in the HCT116 and DLD1 cancer cell lines resulted in decreased cell proliferation, as well as decreased colony forming potential *in vitro*¹⁰. In nude mice grafted with CDO1-over-expressing DLD1 cells, tumor volume was significantly decreased indicating that CDO1 can exert tumor suppressive influence in an experimental model *in vivo*¹⁰. Conversely, silencing CDO1 using small interfering RNA (siRNA) in HEK293 cells resulted in increased cell proliferation, together demonstrating that CDO1 could control cell growth *in vitro*¹⁰.

Investigators studying the metabolic functions of the transcription factor NRF2 found that NRF2 supported increased intracellular cysteine levels through CDO1; in NSCLC tumors expressing a mutant version of the NRF2 regulator Keap1, the CDO1 promoter was preferentially hyper-methylated, resulting in CDO1 silencing and limiting of cysteine

1 metabolism to sulfite¹¹. In the Keap1 mutant NSCLC cell line A549, CDO1 expression impaired
2 NSCLC cancer cell proliferation in the absence of NRF2 but not in the presence of NRF2,
3 suggesting that in Keap1 mutant NSCLC, NRF2 and CDO1 could cooperate to support cancer
4 cell proliferation¹¹.
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6 At least four studies have shown that in sputum¹², plasma^{12,13}, and tumor tissue^{11,14} from
7 patients with lung cancer, hyper-methylation of the CDO1 promoter can be observed. In clear
8 cell renal cell cancer, CDO1 promoter methylation was significantly correlated with worse
9 patient survival¹⁵, and in lymph node- and estrogen receptor-positive breast cancer, CDO1
10 promoter methylation could predict distant metastasis in patients receiving anthracycline-based
11 adjuvant chemotherapy¹⁶. Thus, CDO1 has enzymatic activity in cysteine metabolism, its
12 promoter is hyper-methylated in multiple human cancers including in some non-small cell lung
13 cancers, and appears to exert tumor suppressive influence through control of cell proliferation.
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15 We probed multiple independent published microarray datasets to search for
16 differentially expressed genes in patients with non-small cell lung cancers and discovered
17 significantly decreased and differential expression of CDO1 in tumors from patients with
18 NSCLC. CDO1 may be relevant to the biology of NSCLC.
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Rank	ID	p-value	t	B	Gene
9	A_23_P30294	2.78E-15	-1.51E+01	24.790308	CDO1

Table 1: CDO1 is differentially expressed in non-small cell lung cancers.

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
159	8113641	3.3E-22	-12.183355	39.977325	0.8344 ± 0.0665	CDO1	cysteine dioxygenase type 1

Table 2: CDO1 is differentially expressed in non-small cell lung cancers.

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 CDO1 expression when comparing tumor to lung, and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene
186	ILMN_2234187	2.16E-05	-5.9903756	2.977137	0.8452 ± 0.0617	CDO1

Table 3: CDO1 is differentially expressed in non-small cell lung cancers.

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 CDO1 expression when comparing tumor to lung, and gene name are listed in this chart.

Figure 1: CDO1 expression is significantly decreased in NSCLC tumors.

Expression of CDO1 is graphically represented in control, normal lung tissue (left) and in primary tumors from patients with non-small cell lung cancers of the adenocarcinoma type (right); mean mRNA expression level is marked in each group and the result of a statistical test evaluating significance of difference in CDO1 expression between the groups, a *p*-value, is listed above.

Figure 2: CDO1 expression is significantly decreased in NSCLC tumors.

Expression of CDO1 is graphically represented in control, normal lung tissue (left) and in primary tumors from patients with non-small cell lung cancers of the adenocarcinoma type (right); mean mRNA expression level is marked in each group and the result of a statistical test evaluating significance of difference in CDO1 expression between the groups, a *p*-value, is listed above.

Figure 3: CDO1 expression is significantly decreased in NSCLC tumors.

Expression of CDO1 is graphically represented in control, normal lung tissue (left) and in primary tumors from patients with non-small cell lung cancers of the adenocarcinoma type (right); mean mRNA expression level is marked in each group and the result of a statistical test evaluating significance of difference in CDO1 expression between the groups, a *p*-value, is listed above.