The StAR-related lipid transfer protein STARD8 is differentially expressed in non-small cell lung adenocarcinomas.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Non-small cell lung adenocarcinoma (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 StAR-related lipid transfer protein STARD8 in primary tumors from patients with NSCLC. STARD8 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, STARD8, 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 independently published microarray datasets^{3,4}. We found significant differential expression of the steroidogenic acute regulatory protein (StAR)-related lipid transfer protein STARD8 in primary tumors from patients with NSCLC, suggesting STARD8 may be important to the biology of non-small cell lung adenocarcinomas.

Methods

We utilized microarray datasets GSE74706³ and GSE43458⁴ 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. GSE43458 was generated using Affymetrix Human Gene 1.0 ST Array technology with $n=30$ control lung tissues and $n=80$ NSCLC tumors; analysis performed using platform GPL6244.

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 STARD8 expression was significantly different between normal lung tissue and
3 NSCLC tumors using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for
4 all statistical analyses (Version 8.4.0)(455).

7 **Results**

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

13 STARD8 is differentially expressed in NSCLC.

15 We observed significant differential expression of STARD8 in human NSCLC tumors
16 when compared to the lung³. When sorting each gene by significance of difference in expression
17 between NSCLC tumor and the normal lung, STARD8 ranked 170 out of 34183 total transcripts.
18 Differential expression of STARD8 was statistically significant (Table 1; $p=1.04E-11$).

20 We queried a second dataset⁴ to determine if we could validate STARD8 differential
21 expression in independent microarray data. We again found significant differential expression of
22 STARD8 in human NSCLC tumors when compared to the normal lung. When sorting each gene
23 by significance of difference in expression between NSCLC tumor and the normal lung,
24 STARD8 ranked 143 out of 33252 total transcripts. Differential expression of STARD8 in this
25 second dataset was also statistically significant (Table 2; $p=1.1E-22$).
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STARD8 expression is significantly decreased in NSCLC tumors.

We obtained exact mRNA expression levels for the differentially expressed STARD8 transcript in both 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 STARD8 was statistically significant when comparing NSCLC tumors to the normal lung. STARD8 was expressed at lower levels in NSCLC tumors across each dataset (Figures 1-2); decreased tumor expression of STARD8 was statistically significant in each case (Figure 1: $p < 0.0001$; Figure 2: $p < 0.0001$). We calculated a mean fold change of 0.8592 ± 0.0553 (Table 2) in STARD8 expression when comparing NSCLC tumor to normal lung in one dataset.

Thus, we identified STARD8 as a differentially expressed gene in human non-small cell lung adenocarcinoma and found that STARD8 was expressed at significantly lower levels in NSCLC tumors as compared to the normal lung.

Discussion

STARD8 belongs to a family of StAR-related lipid transfer (START) domain-containing proteins that bear relation to the steroidogenic acute regulatory protein, STAR, which functions in the transport of cholesterol molecules to the mitochondria in cells producing steroid hormone⁵.

STARD8, also known as deleted in liver cancer (DLC-3), belongs to the RhoGAP subfamily of START domain-containing proteins and contains an amino-terminal sterile alpha motif (SAM), a carboxy-terminal START domain as well as a RhoGAP domain⁵. Two transcripts are produced by the STARD8/DLC-3 gene, including the DLC-3 α and DLC-3 β

1 isoforms which differ at the 5' end, with the DLC-3 β isoform lacking the sterile alpha motif⁵.
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3 Messenger RNA for STARD8 is decreased in cancers of the lung, uterus, kidneys, ovaries and
4 breast⁵. Expression of STARD8 in the MCF-7 human breast cancer cell line results in reduction
5 of cell proliferation, colony formation in soft agar, as well as anchorage-independent growth *in*
6 *vitro*⁶. In the triple negative breast cancer (TNBC) cell line MDA-MB-231, STARD8 expression
7 is significantly decreased and 68/100 TNBC tumor samples surveyed expressed relatively low
8 levels of STARD8 at the protein level⁷. As in MCF-7 cells, expression of STARD8 in MDA-
9 MB-231 impaired cell proliferation and colony formation *in vitro*⁷. STARD8 expression resulted
10 in significant up-regulation of glycogen synthase kinase 3 β as well as down-regulation of β -
11 catenin, suggesting STARD8 could influence the Wnt signaling pathway⁷. Decreased expression
12 of STARD8 has also been found in gastric cancer cell lines and tissues, with decreased STARD8
13 expression correlating with lymph node metastasis and TNM tumor staging⁸. Thus, STARD8
14 appears to exert tumor-suppressive influence in some human cancers.
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20 In summary, we probed independent published microarray datasets^{3,4} to identify in an
21 unbiased manner genes whose expression is most different in non-small cell lung
22 adenocarcinomas and found that a transcript produced by the STARD8 gene was among those
23 whose expression changed most significantly between NSCLC tumors and normal lung tissue;
24 STARD8 expression was significantly decreased in primary tumors from patients with NSCLC.
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26 STARD8 may be relevant to the biology of NSCLC.
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Rank	ID	p-value	t	B	Gene
170	A_23_P387630	1.04E-11	-1.08E+01	16.7981761	STARD8

Table 1: STARD8 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
143	8168028	1.1E-22	-12.393526	41.065287	0.8592 ± 0.0553	STARD8	StAR related lipid transfer domain containing 8

Table 2: STARD8 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 STARD8 expression when comparing tumor to lung, and gene name are listed in this chart.

Figure 1: STARD8 expression is significantly decreased in NSCLC tumors.

Expression of STARD8 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 STARD8 expression between the groups, a *p*-value, is listed above.

Figure 2: STARD8 expression is significantly decreased in NSCLC tumors.

Expression of STARD8 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 STARD8 expression between the groups, a *p*-value, is listed above.