SDPR is differentially expressed in non-small cell lung adenocarcinomas.

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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 serum deprivation protein response gene SDPR⁵ in primary tumors from patients with NSCLC. SDPR 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, SDPR, 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 serum deprivation protein response gene SDPR in primary tumors from patients with NSCLC, suggesting SDPR 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 auto-detected, and annotation platform was NCBI-generated. A statistical test was performed to

1 evaluate whether SDPR expression was significantly different between normal lung tissue and
2 NSCLC tumors using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for
3 all statistical analyses (Version 8.4.0)(455).
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5 **Results**

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

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13 We observed significant differential expression of the gene encoding serum deprivation
14 protein response, SDPR, in human NSCLC tumors when compared to the lung³. When sorting
15 each gene by significance of difference in expression between NSCLC tumor and the normal
16 lung, SDPR ranked 92 out of 34183 total transcripts. Differential expression of SDPR was
17 statistically significant (Table 1; $p=1.09E-12$).
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20 We queried a second dataset⁴ to determine if we could validate SDPR differential
21 expression in independent microarray data. We again found significant differential expression of
22 SDPR in human NSCLC tumors when compared to the normal lung. When sorting each gene by
23 significance of difference in expression between NSCLC tumor and the normal lung, SDPR
24 ranked 18 out of 33252 total transcripts. Differential expression of SDPR in this second
25 dataset was also statistically significant (Table 2; $p=1.56E-29$).
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SDPR expression is significantly decreased in NSCLC tumors.

We obtained exact mRNA expression levels for the differentially expressed SDPR 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 SDPR was statistically significant when comparing NSCLC tumors to the normal lung. SDPR was expressed at lower levels in NSCLC tumors across each dataset (Figures 1-2); decreased tumor expression of SDPR was statistically significant in each case (Figure 1: $p < 0.0001$; Figure 2: $p < 0.0001$). We calculated a mean fold change of 0.7686 ± 0.0779 (Table 2) in SDPR expression when comparing NSCLC tumor to normal lung in one dataset.

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

Discussion

Serum deprivation protein response (SDPR) was identified based on high messenger RNA expression in serum-starved NIH3T3 cells⁵. Tissue expression of SDPR is widespread but relatively highest in the heart and lungs⁵. SDPR is identical to PS-p68, a phosphatidylserine-binding protein isolated from platelets⁵. A major described function for SDPR is in the formation of invaginated regions of the plasma membrane known as caveolae⁶. Deficiency of SDPR results in loss of caveolae demonstrating its requirement for this process. SDPR can directly interact with the polymerase I and transcript release factor, PTRF; interactions between PTRF

1 and SDPR are important for recruitment of PTRF to caveolae⁶. Over-expression of SDPR results
2 in curvature of the plasma membrane as well as plasma membrane tubulation⁶. Thus, SDPR is
3 important for membrane curvature of the plasma membrane⁶.
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5 Roles for SDPR have been documented in human cancer. A study utilizing the MCF10
6 cell line and variants in a model of breast cancer progression *in vivo* to discover genes important
7 for suppression of metastasis identified SDPR as a metastasis suppressor gene⁷. SDPR
8 expression in MIVpQ breast cancer cells resulted in significant inhibition of lung metastasis
9 colonization⁷. Mechanisms by which SDPR could exert its anti-metastatic effects included
10 impairment of migration, as SDPR expression in LM2 cells reduced migration across an
11 endothelial cell layer *in vitro* by 92.8%, and enhanced cell death by apoptosis⁷. The influence of
12 SDPR on apoptosis appeared to be mediated through regulation of expression of Bcl-2 family
13 proteins, with increased expression of pro-apoptotic proteins Bad, Bim and Bid and decreased
14 expression of anti-apoptotic protein Bcl-x_L⁷. Decreased expression of SDPR in a variety of
15 cancer cell lines could be attributed to hypermethylation of the SDPR gene at CpG sites adjacent
16 to a CpG island⁷. 5-azacytidine treatment resulted in enhanced expression of SDPR in 5 out of 6
17 breast cancer cell lines, supporting an epigenetic mechanism of SDPR silencing in cancer cells⁷.
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19 In tumors from patients with papillary thyroid cancer, SDPR expression was significantly
20 downregulated and decreased SDPR expression was correlated with increased tumor size, more
21 extensive lymph node metastasis and advanced tumor staging⁸. Expression of SDPR (also
22 known as Cavin-2) could discriminate between liposarcomas based on degree of differentiation;
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SDPR expression was relatively highest in well-differentiated, relatively lower in less aggressive liposarcomas and nearly undetectable in aggressive myxoid and dedifferentiated liposarcomas⁹. Differential expression of non-coding anti-sense transcripts from the SDPR locus (SDPR-AS) have been reported in renal cell carcinoma (RCC)¹⁰ and in non-small cell lung carcinoma¹¹. SDPR-AS expression resembled a gradient based on tumor stage, with highest expression in normal tissues and lowest expression in Stage IV RCC. In lung adenocarcinoma, one study found relatively decreased expression of SDPR in tumors from 150 patients when compared to adjacent benign lung tissue, and decreased SDPR expression in tumors was significantly correlated with tumor size, lymph node metastasis and TNM tumor stage¹². Thus, SDPR is important for membrane curvature at the plasma membrane and formation of caveolae; SDPR can function in a tumor and metastasis suppressive manner by impairing cell migration and proliferation, and promoting cell death by apoptosis, and SDPR expression is down-regulated in a number of human cancers, with decreased SDPR expression correlated with worse clinical outcomes including tumor stage.

In summary, we probed independent published microarray datasets^{3,4} to identify in an unbiased manner genes whose expression is most different in non-small cell lung adenocarcinomas and found that a transcript produced by the SDPR gene was among those whose expression changed most significantly between NSCLC tumors and normal lung tissue; SDPR expression was significantly decreased in primary tumors from patients with NSCLC. SDPR may be relevant to the biology of NSCLC.

References

1. Felip, E., Stahel, R.A. and Pavlidis, N.E.S.M.O., 2005. ESMO Minimum Clinical Recommendations for diagnosis, treatment and follow-up of non-small-cell lung cancer (NSCLC). *Annals of Oncology*, 16, pp.i28-i29.
2. Siegel, R.L., Miller, K.D. and Jemal, A., 2016. Cancer statistics, 2016. *CA: a cancer journal for clinicians*, 66(1), pp.7-30.
3. Marwitz, S., Depner, S., Dvornikov, D., Merkle, R., Szczygiel, M., Müller-Decker, K., Lucarelli, P., Wäsch, M., Mairbäurl, H., Rabe, K.F. and Kugler, C., 2016. Downregulation of the TGF β pseudoreceptor BAMBI in non-small cell lung cancer enhances TGF β signaling and invasion. *Cancer research*, 76(13), pp.3785-3801.
4. Kabbout, M., Garcia, M.M., Fujimoto, J., Liu, D.D., Woods, D., Chow, C.W., Mendoza, G., Momin, A.A., James, B.P., Solis, L. and Behrens, C., 2013. Ets2 mediated tumor suppressive function and met oncogene inhibition in human non-small cell lung cancer. *Clinical cancer research*, 19(13), pp.3383-3395.
5. Gustincich, S., Vatta, P., Goruppi, S., Wolf, M., Saccone, S., Della Valle, G., Baggiolini, M. and Schneider, C., 1999. The humanserum deprivation responsegene (sdpr) maps to 2q32-q33 and codes for a phosphatidylserine-binding protein. *Genomics*, 57(1), pp.120-129.
6. Hansen, C.G., Bright, N.A., Howard, G. and Nichols, B.J., 2009. SDPR induces membrane curvature and functions in the formation of caveolae. *Nature cell biology*, 11(7), pp.807-814.
7. Ozturk, S., Papageorgis, P., Wong, C.K., Lambert, A.W., Abdolmaleky, H.M., Thiagalingam, A., Cohen, H.T. and Thiagalingam, S., 2016. SDPR functions as a metastasis suppressor in breast cancer by promoting apoptosis. *Proceedings of the National Academy of Sciences*, 113(3), pp.638-643.
8. Wang, Q.X., Chen, E.D., Cai, Y.F., Zhou, Y.L., Dong, S.Y., Zhang, X.H., Wang, O.C. and Li, Q., 2019. Serum deprivation response functions as a tumor suppressor gene in papillary thyroid cancer. *Clinical genetics*, 96(5), pp.418-428.
9. Codenotti, S., Vezzoli, M., Poliani, P.L., Cominelli, M., Monti, E. and Fanzani, A., 2017. Cavin-2 is a specific marker for detection of well-differentiated liposarcoma. *Biochemical and biophysical research communications*, 493(1), pp.660-665.

- 1 10.Ni, W., Song, E., Gong, M., Li, Y., Yao, J. and An, R., 2017. Downregulation of lncRNA
2 SDPR-AS is associated with poor prognosis in renal cell carcinoma. *OncoTargets and*
3 *therapy*, 10, p.3039.
- 4 11.Zhu, J., Wang, L. and Liao, R., 2019. Long non-coding RNA SDPR-AS affects the
5 development of non-small cell lung cancer by regulating SDPR through p38 MAPK/ERK
6 signals. *Artificial cells, nanomedicine, and biotechnology*, 47(1), pp.3172-3179.
- 7 12.Wang, X., Arcani, D.M.C., Zhao, J., Xu, M., Zhou, X. and Yang, Y., 2020. Prognostic and
8 diagnostic significance of Cavin 2 in lung adenocarcinoma. *Archives of Medical Science:*
9 *AMS*, 16(5), p.1189.

Rank	ID	p-value	t	B	Gene
92	A_23_P72668	1.09E-12	-1.19E+01	19.0075967	SDPR

Table 1: SDPR 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
18	8057797	1.56E-29	-15.4889	56.605576	0.7686 ± 0.0779	SDPR	serum deprivation response

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

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

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

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

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