The gene encoding tensin 1, TNS1, is differentially expressed in non-small cell lung cancer and associates with patient survival.

Shahan Mamoor¹

¹shahanmamoor@gmail.com

East Islip, NY USA

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 tensin 1, encoded by TNS1, was among the genes whose expression was most quantitatively different in tumors from patients with NSCLC as compared to the lung. TNS1 expression was significantly decreased in NSCLC tumors as compared to the lung, and lower expression of TNS1 in patient tumors was significantly associated with worse overall survival. TNS1 may be important for initiation or progression of non-small cell lung cancer in humans.

Keywords: TNS1, 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 TNS1 in
10 adenocarcinoma tumors from patients with NSCLC, suggesting TNS1 may be important for
11 NSCLC tumor initiation or progression.

12 **Methods**

13 We utilized microarray datasets GSE33532², GSE43458³ and GSE74706⁴ for this
14 differential gene expression analysis of NSCLC tumors in conjunction with GEO2R. GSE33532
15 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology; for this
16 analysis, we used $n=20$ control lung tissue and $n=10$ NSCLC tumors, and the analysis was
17 performed using platform GPL570. GSE43458 was generated using Affymetrix Human Gene
18 1.0 ST Array technology; for this analysis, we used with $n=30$ control lung tissue and $n=80$
19 NSCLC tumors, and the analysis was performed using platform GPL6244. GSE74706 was
20 generated using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology; for

1 this analysis, we used $n=18$ control lung tissue and $n=10$ NSCLC tumors, and the analysis was
2 performed using platform GPL13497. All tumors utilized for differential gene expression
3 analysis here were of the adenocarcinoma type.

4
5 The Benjamini and Hochberg method of p -value adjustment was used for ranking of
6 differential expression but raw p -values were used to assess statistical significance of global
7 differential expression. Log-transformation of data was auto-detected, and the NCBI
8 generated category of platform annotation was used. A statistical test was performed to evaluate
9 whether TNS1 expression was significantly between normal lung tissue and NSCLC tumors
10 using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical
11 analyses of differential gene expression in NSCLC tumors (Version 8.4.0)(455). For Kaplan-
12 Meier survival analysis, we used the Kaplan-Meier plotter online tool⁶ for correlation of TNS1
13 mRNA expression levels with overall survival in $n=1925$ non-small cell lung cancer patients.
14
15
16
17
18

19 **Results**

20 We harnessed the power of multiple, independently published microarray datasets^{2,3,4} to
21 discover in an unbiased fashion and at the transcriptome-level the most striking gene expression
22 features of NSCLC tumors.
23
24

25 TNS1 is differentially expressed in non-small cell lung cancers.

26 We found significant differential expression of TNS1 in NSCLC tumors when compared
27 to the lung² (Table 1). When sorting each of the transcripts measured based on significance of
28

1 difference in expression of TNS1 between NSCLC tumors and the normal lung, TNS1 ranked 11
2 out of 25906 total transcripts (Table 1). Differential expression of TNS1 in NSCLC tumors was
3 statistically significant (Table 1; $p=2.67E-18$).
4

5 We queried a second microarray dataset³ to determine if we could validate differential
6 expression of TNS1 in non-small cell lung cancers (Table 2). We again found significant
7 differential expression of TNS1 in NSCLC tumors of the adenocarcinoma type when compared
8 to the normal lung (Table 2). When sorting each of the transcripts measured based on
9 significance of difference in expression of TNS1 between NSCLC tumors and the normal lung,
10 TNS1 ranked 234 out of 33252 total transcripts (Table 2). Differential expression of TNS1 in
11 NSCLC tumors was statistically significant (Table 2; $p=3.44E-20$).
12
13
14

15 Analysis of a third microarray dataset⁴ revealed significant differential expression of
16 TNS1 in NSCLC tumors of the adenocarcinoma type. When sorting each of the transcripts
17 measured based on significance of difference in expression of TNS1 between NSCLC tumors
18 and the normal lung, in this dataset, TNS1 ranked 60 out of 34183 total transcripts (Table 3).
19 Differential expression of TNS1 in NSCLC tumors was statistically significant (Table 3;
20 $p=3.28E-13$).
21
22

23 TNS1 is expressed at significantly lower levels in NSCLC tumors as compared to the lung.
24

25 We obtained exact mRNA levels for TNS1 from NSCLC tumors and from the lung to
26 directly compare TNS1 expression between tumor and control lung tissue and assess for
27 statistical significance. TNS1 was expressed at significantly lower levels in NSCLC tumors as
28

1 compared to the normal lung in each dataset queried (Figure 1: $p < 0.0001$, Figure 2: $p < 0.0001$,
2 Figure 3: $p < 0.0001$). We calculated a mean fold change of 0.8378 ± 0.0235 (Table 1) and
3
4 0.8788 ± 0.0576 (Table 2) in TNS1 expression when comparing NSCLC tumors to the lung.

6 TNS1 expression in NSCLC tumors correlates with overall survival.

7 We performed Kaplan-Meier survival analysis using TNS1 mRNA expression in NSCLC
8 tumors coupled with paired overall survival data from each patient, in 1925 NSCLC patients in
9 total, to determine whether TNS1 tumor expression was correlated with survival outcomes in
10 NSCLC. We found that patients whose tumors expressed lower levels of TNS1 possessed
11 significantly shorter overall survival than patients with high tumor expression of TNS1 (Figure
12 4). Median overall survival (OS) of patients in the low expression cohort was 45.6 months,
13 while median OS in patients in the high TNS1 expression cohort was 89 months (Table 4); this
14 difference in median OS based on TNS1 tumor expression in NSCLC was statistically significant
15 (Figure 4; logrank p -value: $7.4e-16$; hazard ratio: 0.59 (0.52 - 0.67); false discovery rate: 0.01).
16
17
18
19

20 Thus, blind comparative transcriptome analysis of non-small cell lung cancers revealed
21 differential expression of TNS1 as among the most significant transcriptional features of NSCLC
22 tumors, and TNS1 expression was significantly correlated with patient outcomes, as patients with
23 lower tumor expression of TNS1 possessed significantly worse overall survival.
24
25
26
27
28

Discussion

Tensin 1, or tensin, was cloned as a cytoskeletal component of focal adhesions and actin-binding protein of containing a Src homology 2 (SH2) domain⁷. Recombinant tensin can interact with F-actin, and impair the rate at which F-actin polymerizes in actin filaments⁸ through binding at the filament barbed-end⁹. In the mouse, tensin is expressed in the kidney, the heart, lungs, liver, skeletal muscles and in lesser quantities in the brain, testis and spleen¹⁰. Tensin-null mice were born in normal Mendelian ratio and displayed no gross phenotypes aside from defects associated with the kidneys¹⁰. Kidneys from tensin-deficient mice were paler than in wild-type, at times larger, and possessed a variety of defects including dilated cysts in the medullar and cortex arising from the proximal tubules, enlargement of the Bowman's spaces in glomeruli, and focal infiltrates of an inflammatory nature in interstitial spaces¹⁰. Though tensin-deficient mice displayed kidney defects, only 3 of 44 mice found to possess illnesses associated with kidney failure¹⁰. In the kidneys, focal adhesions are intact in the tubules but lacking in cystic tubular cells¹⁰. While tensin-deficient mice do not manifest fertility-associated defects, mating between tensin-null males and tensin-null females fail to produce normal quantities of live pups¹⁰. Tensin-1 is heavily phosphorylated at least 50 serine and threonine sites and at least 62 tyrosine sites; phosphorylation at serine and threonine¹¹. Tensin can associate with focal adhesions through binding of its PTB motif to the tail of integrin $\beta 1$ at NPxY motifs¹². AMP-activated protein kinase (AMPK) is a negative regulator of tensin expression¹³. Association with actin occurs through domains at the amino-terminus and at a second domain spanning residues 889 to

1 1143; the SH2 domain and PTB domain are carboxy-terminal, from residues 1471-1744¹⁴. At
2 the leading-edge of migrating cells, tensin co-localizes with the p130Cas protein at focal
3 adhesions¹⁴. Ectopic expression of the region of tensin containing the SH2 and PTB domains,
4 which lacks actin-binding domains, reduces the maximum velocity, in microns/hour, of cell
5 migration, but only in cells expressing Cas, and reduces inward flux of Cas¹⁴. Together the data
6 suggested tensin could function to link the actin cytoskeleton to focal adhesions through Cas. A
7 second study supported a role for tensin in cell migration, as expression of tensin in HEK293
8 cells resulted in increased cells per field in an *in vitro* Boyden chamber cell migration assay
9 while the number of cells traversed per field was decreased in fibroblasts deficient in tensin¹⁵.
10 Another interaction partner of TNS1 is the Rho-GTPase activating protein (RhoGAP) deleted in
11 liver cancer, or DLC1; expression of a DLC1 mutant (Y442F) that is deficient in binding tensin
12 results in impaired of DLC1 capacity to exert tumor suppressive influence in *in vitro* transwell
13 migration and anchorage-independent growth assays¹⁶.
14

15 Tensin expression is implicated in biology of lungs as well as in human cancers.
16 A genome-wide association study of 20,288 Europeans found significant association between the
17 rs2571445 single nucleotide polymorphism at 2q35 in TNS1 with measures of pulmonary
18 function including forced expiratory volume per second¹⁷. In myofibroblasts of the lung, TNS1
19 expression is TGF- β inducible, and depletion of TNS1 impairs myofibroblast differentiation
20 induced by TGF- β . TNS1 expression was also important for generation of fibrillar adhesions and
21 extracellular matrix components including fibronectin and collagen I¹⁸. In human colorectal
22
23
24
25
26
27
28

1 cancer (CRC), higher expression of TNS1 has been demonstrated in CRC tumor tissue as
2 compared to normal colon, as well as in the RKO and SW620 CRC cell lines as compared to
3 normal colon cells¹⁹. Depletion of TNS1 in SW620 cells resulted in decreased cell proliferation
4 and an *in vitro* cell invasion assay¹⁹. Higher expression of TNS1 in CRC tumors was correlated
5 with worse overall survival¹⁹. TNS1 was among the genes whose expression changed most
6 significantly when comparing 2D and 3D culture conditions of the non-small cell lung cancer
7 (NSCLC) cell lines A549 and Colo699²⁰. In a study identifying genes fused to the anaplastic
8 lymphoma kinase (ALK) in non-small cell lung cancer, 9 cases of TNS1 fusion to ALK were
9 found²¹. A fusion of ALK to TNS1 has also been described in a patient with a uterine
10 mesenchymal tumor originally diagnosed as a leiomyosarcoma²². In 4T1 triple negative breast
11 cancer cells, expression of TNS1 can be positively regulated by the long non-coding RNA
12 MaTAR25²³.

13 We found, by mining multiple independently published microarray datasets, that TNS1
14 was among the genes most differentially expressed in the primary tumors of patients with non-
15 small cell lung cancer; TNS1 was expressed at significantly lower levels in NSCLC tumors
16 as compared to the lung, and decreased expression of TNS1 was significantly associated with
17 worse overall survival in NSCLC patients. TNS1 may be of relevance to the biology of tumor
18 initiation or progression in patients with NSCLC of the adenocarcinoma type, the most common
19 type of the leading cause of cancer death in the United States and worldwide.

References

1. Siegel, R.L., Miller, K.D. and Jemal, A., 2019. Cancer statistics, 2019. *CA: a cancer journal for clinicians*, 69(1), pp.7-34.
2. 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.
3. Meister, M., Belousov, A., Xu, E.C., Schnabel, P., Warth, A. and Hoofmann, H., 2014. Intra-tumor heterogeneity of gene expression profiles in early stage non-small cell lung cancer. *J Bioinf Res Stud*, 1, p.1.
4. 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 TGFβ pseudoreceptor BAMBI in non-small cell lung cancer enhances TGFβ signaling and invasion. *Cancer research*, 76(13), pp.3785-3801.
5. Lung Cancer - Non-Small Cell: Statistics. <https://www.cancer.net/cancer-types/lung-cancer-non-small-cell/statistics>.
6. Györfy, B., Surowiak, P., Budczies, J. and Lanczky, A., 2013. Online survival analysis software to assess the prognostic value of biomarkers using transcriptomic data in non-small-cell lung cancer. *PloS one*, 8(12), pp.e82241-e82241.
7. Davis, S., Lu, M.L., Lo, S.H., Lin, S., Butler, J.A., Druker, B.J., Roberts, T.M., An, Q. and Chen, L.B., 1991. Presence of an SH2 domain in the actin-binding protein tensin. *Science*, 252(5006), pp.712-715.
8. CHEN, H., ISHII, A., WONG, W.K., CHEN, L.B. and Lo, S.H., 2000. Molecular characterization of human tensin. *Biochemical Journal*, 351(2), pp.403-411.
9. Lo, S.H., Janmey, P.A., Hartwig, J.H. and Chen, L.B., 1994. Interactions of tensin with actin and identification of its three distinct actin-binding domains. *The Journal of cell biology*, 125(5), pp.1067-1075.
10. Lo, S.H., Yu, Q.C., Degenstein, L., Chen, L.B. and Fuchs, E., 1997. Progressive kidney degeneration in mice lacking tensin. *The Journal of cell biology*, 136(6), pp.1349-1361.

- 11.Hall, E.H., Balsbaugh, J.L., Rose, K.L., Shabanowitz, J., Hunt, D.F. and Brautigan, D.L., 2010. Comprehensive analysis of phosphorylation sites in Tensin1 reveals regulation by p38MAPK. *Molecular & Cellular Proteomics*, 9(12), pp.2853-2863.
- 12.McCleverty, C.J., Lin, D.C. and Liddington, R.C., 2007. Structure of the PTB domain of tensin1 and a model for its recruitment to fibrillar adhesions. *Protein science*, 16(6), pp. 1223-1229.
- 13.Georgiadou, M., Lilja, J., Jacquemet, G., Guzmán, C., Rafaeva, M., Alibert, C., Yan, Y., Sahgal, P., Lerche, M., Manneville, J.B. and Mäkelä, T.P., 2017. AMPK negatively regulates tensin-dependent integrin activity. *Journal of Cell Biology*, 216(4), pp.1107-1121.
- 14.Zhao, Z., Tan, S.H., Machiyama, H., Kawauchi, K., Araki, K., Hirata, H. and Sawada, Y., 2016. Association between tensin 1 and p130Cas at focal adhesions links actin inward flux to cell migration. *Biology open*, 5(4), pp.499-506.
- 15.Chen, H., Duncan, I.C., Bozorgchami, H. and Lo, S.H., 2002. Tensin1 and a previously undocumented family member, tensin2, positively regulate cell migration. *Proceedings of the National Academy of Sciences*, 99(2), pp.733-738.
- 16.Qian, X., Li, G., Asmussen, H.K., Asnaghi, L., Vass, W.C., Braverman, R., Yamada, K.M., Popescu, N.C., Papageorge, A.G. and Lowy, D.R., 2007. Oncogenic inhibition by a deleted in liver cancer gene requires cooperation between tensin binding and Rho-specific GTPase-activating protein activities. *Proceedings of the National Academy of Sciences*, 104(21), pp. 9012-9017.
- 17.Repapi, E., Sayers, I., Wain, L.V., Burton, P.R., Johnson, T., Obeidat, M.E., Zhao, J.H., Ramasamy, A., Zhai, G., Vitart, V. and Huffman, J.E., 2010. Genome-wide association study identifies five loci associated with lung function. *Nature genetics*, 42(1), pp.36-44.
- 18.Bernau, K., Torr, E.E., Evans, M.D., Aoki, J.K., Ngam, C.R. and Sandbo, N., 2017. Tensin 1 is essential for myofibroblast differentiation and extracellular matrix formation. *American journal of respiratory cell and molecular biology*, 56(4), pp.465-476.
- 19.Zhou, H., Zhang, Y., Wu, L., Xie, W., Li, L., Yuan, Y., Chen, Y., Lin, Y. and He, X., 2018. Elevated transgelin/TNS1 expression is a potential biomarker in human colorectal cancer. *Oncotarget*, 9(1), p.1107.
- 20.Gamerith, G., Rainer, J., Huber, J.M., Hackl, H., Trajanoski, Z., Koeck, S., Lorenz, E., Kern, J., Kofler, R., Kelm, J.M. and Zwierzina, H., 2017. 3D-cultivation of NSCLC cell lines induce gene expression alterations of key cancer-associated pathways and mimic in-vivo conditions. *Oncotarget*, 8(68), p.112647.

- 1
2
3 21. Ross, J.S., Ali, S.M., Fasan, O., Block, J., Pal, S., Elvin, J.A., Schrock, A.B., Suh, J., Nozad,
4 S., Kim, S. and Lee, H.J., 2017. ALK fusions in a wide variety of tumor types respond to
anti-ALK targeted therapy. *The oncologist*, 22(12), p.1444.
- 5 22. Lee, J., Singh, A., Ali, S.M., Lin, D.I. and Klempner, S.J., 2019. TNS1-ALK Fusion in a
6 Recurrent, Metastatic Uterine Mesenchymal Tumor Originally Diagnosed as
7 Leiomyosarcoma. *Acta medica academica*, 48(1), pp.116-120.
- 8 23. Chang, K.C., Diermeier, S.D., Allen, T.Y., Brine, L.D., Russo, S., Bhatia, S., Alsudani, H.,
9 Kostroff, K., Bhuiya, T., Brogi, E. and Bennett, C.F., 2020. MaTAR25 LncRNA Regulates the
10 Tensin1 Gene to Impact Breast Cancer Progression. *bioRxiv*.
- 11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

Rank	ID	p-value	t	B	FC	Gene	Gene name
11	221748_s_at	2.67E-18	-18.450614	31.6508359	0.8378 ± 0.0235	TNS1	tensin 1

Table 1: TNS1 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 TNS1 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
234	8058869	3.44E-20	-11.303582	35.393624	0.8788 ± 0.0576	TNS1	tensin 1

Table 2: TNS1 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 TNS1 expression in NSCLC tumors as compared to the lung, and gene are listed in this chart.

Rank	ID	p-value	t	B	Gene
60	A_33_P3209491	3.28E-13	-1.25E+01	20.1813704	TNS1

Table 3: TNS1 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, gene and gene name are listed in this chart.

Figure 1: TNS1 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of TNS1 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 TNS1 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 2: TNS1 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of TNS1 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 TNS1 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 3: TNS1 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of TNS1 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 TNS1 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 4: TNS1 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 TNS1 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 TNS1 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)
45.6	89

Table 4: TNS1 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 TNS1 in tumors is listed in this chart.