The type III TGF beta receptor 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 the gene encoding the type III transforming growth factor (TGF)- β receptor, TGFBR3, was among the genes whose expression was most quantitatively different in tumors from patients with NSCLC as compared to the lung. TGFBR3 expression was significantly decreased in NSCLC tumors as compared to the lung, and lower expression of TGFBR3 in patient tumors was significantly associated with worse overall survival. We previously reported differential and decreased expression of LTBP4⁵ which associated with patient survival in NSCLC. TGFBR3, and the TGF- β signaling pathway may be important for initiation or progression of non-small cell lung cancer in humans.

Keywords: TGFBR3, NSCLC, non-small cell lung cancer, TGF-beta signaling pathway in NSCLC, 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 years⁶. 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 years⁶. 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 TGFBR3
10 in adenocarcinoma tumors from patients with NSCLC, suggesting TGFBR3 may be important
11 for NSCLC tumor initiation or progression.
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16 **Methods**

17 We utilized microarray datasets GSE33532², GSE43458³ and GSE74706⁴ for this
18 differential gene expression analysis of NSCLC tumors in conjunction with GEO2R. GSE33532
19 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology; for this
20 analysis, we used $n=20$ control lung tissue and $n=10$ NSCLC tumors, and the analysis was
21 performed using platform GPL570. GSE43458 was generated using Affymetrix Human Gene
22 1.0 ST Array technology; for this analysis, we used with $n=30$ control lung tissue and $n=80$
23 NSCLC tumors, and the analysis was performed using platform GPL6244. GSE74706 was
24 generated using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology; for
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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.

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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 TGFBR3 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 tool⁷ for correlation of
13 TGFBR3 mRNA expression levels with overall survival in $n=1144$ non-small cell lung cancer
14 patients.
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20 **Results**

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22 We harnessed the power of multiple, independently published microarray datasets^{2,3,4} to
23 discover in an unbiased fashion and at the transcriptome-level the most striking gene expression
24 features of NSCLC tumors.
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26 TGFBR3 is differentially expressed in non-small cell lung cancers.

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28 We found significant differential expression of the gene encoding the type III

transforming growth factor (TGF)- β receptor, TGFBR3 (TGFBRIII), in NSCLC tumors when compared to the lung² (Table 1). When sorting each of the transcripts measured based on significance of difference in expression of TGFBR3 between NSCLC tumors and the normal lung, TGFBR3 ranked 90 out of 25906 total transcripts (Table 1). Differential expression of TGFBR3 in NSCLC tumors was statistically significant (Table 1; $p=4.3E-15$).

We queried a second microarray dataset³ to determine if we could validate differential expression of TGFBR3 in non-small cell lung cancers (Table 2). We again found significant differential expression of TGFBR3 in NSCLC tumors of the adenocarcinoma type when compared to the normal lung (Table 2). When sorting each of the transcripts measured based on significance of difference in expression of TGFBR3 between NSCLC tumors and the normal lung, TGFBR3 ranked 98 out of 33252 total transcripts (Table 2). Differential expression of TGFBR3 in NSCLC tumors was statistically significant (Table 2; $p=4.17E-24$).

Analysis of a third microarray dataset⁴ revealed significant differential expression of TGFBR3 in NSCLC tumors of the adenocarcinoma type. When sorting each of the transcripts measured based on significance of difference in expression of TGFBR3 between NSCLC tumors and the normal lung, in this dataset, TGFBR3 ranked 54 out of 34183 total transcripts (Table 3). Differential expression of TGFBR3 in NSCLC tumors was statistically significant (Table 3; $p=2.74E-13$).

TGFBR3 is expressed at significantly lower levels in NSCLC tumors as compared to the lung.

We obtained exact mRNA levels for TGFBR3 from NSCLC tumors and from the lung to

1 directly compare TGFBR3 expression between tumor and control lung tissue and assess for
2 statistical significance. TGFBR3 was expressed at significantly lower levels in NSCLC tumors
3 as compared to normal lung in each dataset queried (Figure 1: $p<0.0001$, Figure 2: $p<0.0001$,
4 Figure 3: $p<0.0001$). We calculated a mean fold change of 0.6638 ± 0.0827 and 0.7817 ± 0.0900
5 in TGFBR3 expression when comparing NSCLC tumors to the lung, in one dataset (Table 1 and
6 Table 2, respectively).

7 TGFBR3 expression in NSCLC tumors correlates with overall survival.

8 We performed Kaplan-Meier survival analysis using TGFBR3 mRNA expression in
9 NSCLC tumors coupled with paired overall survival data from each patient, in 1144 NSCLC
10 patients in total, to determine whether TGFBR3 tumor expression was correlated with survival
11 outcomes in NSCLC. We found that patients whose tumors expressed lower levels of TGFBR3
12 possessed significantly shorter overall survival than patients with high tumor expression of
13 TGFBR3 (Figure 4). Median overall survival (OS) of patients in the low expression cohort was
14 54 months, while median OS in patients in the high TGFBR3 expression cohort was 125 months
15 (Table 4); this difference in median OS based on TGFBR3 tumor expression in NSCLC was
16 statistically significant (Figure 4; logrank p -value: $1.4e-13$; hazard ratio: 0.46 (0.37 - 0.57); false
17 discovery rate=0.01).

18 Thus, blind comparative transcriptome analysis of non-small cell lung cancers revealed
19 differential expression of TGFBR3 as among the most significant transcriptional features of
20 NSCLC tumors, and TGFBR3 expression was significantly correlated with patient outcomes, as
21 patients with lower tumor expression of TGFBR3 possessed significantly worse overall survival.

Discussion

The type III TGF- β receptor (TGFBRIII), a membrane proteoglycan, is associated with heparin and chondroitin sulfate glycosaminoglycan chains, forms disulfide-linked complexes in 3T3 cells, and has affinity for TGF- β 1, TGF- β 2, and TGF- β 1.^{27,8}. Another name for TGFBRIII is betaglycan; betaglycan can contribute to the TGF- β signal transduction pathway by forming a ternary complex with TGF- β and the kinase subunit of the TGF- β receptor⁹. Another function for betaglycan is in serving as a co-receptor for inhibin in conjunction with the type II receptor serine kinase ActRII; betaglycan can form direct interactions with inhibin, promoting cross-linking between betaglycan and ActRII¹⁰. β -arrestins, regulators of G-protein coupled receptor kinases (GRKs), can regulate TGF- β signaling through binding of β -arrestin 2 to TGFBRIII, resulting in its endocytosis and down-regulation of TGF- β signaling¹¹.

TGFB3 is implicated in a number of human cancers. Expression of TGFB3 was found to be decreased in 18/26 non-muscle-invasive bladder cancer cases while its expression was found to be increased in 19/24 muscle-invasive bladder cancer cases¹². Depletion of TGFB3 in invasive T24 bladder cancer cells results in impairment of cell proliferation and colony formation *in vitro*¹². In patients with oral cancer, TGFB3 was expressed at lower levels and decreased TGFB3 expression was associated with worse disease-free survival¹³. Ectopic expression of TGFB3 resulted in decreased invasion and cell migration in both CAL-27 and OC-2 oral cancer cell lines¹³. While tumor volume was not significantly affected by expression of TGFB3 in CAL-27 and OC-2 cell lines grafted into nude mice, the ratio of cervical lymph nodes to which cancer metastasized was significantly altered upon expression of TGFB3¹³.

Decreased expression of TGFBR3 is associated with progression of breast cancer in human patients, and lower expression of TGFBR3 was associated with worse recurrence-free survival in patients with breast cancer¹⁴. Ectopic expression of TGFBR3 in breast cancer cell lines resulted in impairment of invasiveness in MDA-MB-231 cells and reduction of metastasis and angiogenesis *in vivo* in the 4T1 mouse model¹⁴. In the PANC-1 model of the epithelial to mesenchymal transition (EMT) in pancreatic cancer, TGFBR3 expression is lost upon induction of EMT with TGF- β and loss of TGFBR3 expression is important for motility and invasive behaviors acquired during TGF- β -induced EMT¹⁵. One report implicated TGFBR3 in lung cancer cell lines, with a long non-coding RNA lncRNA ADAMTS9-AS2 repressing microRNA miR-223-3p, thereby supporting expression of miR-223-3p target TGFBR3 with the phenotypic consequence being enhanced cell proliferation in the A549 non-small cell lung cancer cell line¹⁶.

We found, by mining multiple independently published microarray datasets, that TGFBR3 was among the genes most differentially expressed in the primary tumors of patients with non-small cell lung cancer; TGFBR3 was expressed at significantly lower levels in NSCLC tumors as compared to the lung, and decreased expression of TGFBR3 was significantly associated with worse overall survival in NSCLC patients. We previously discovered and reported statistically significant, survival-associated differential and decreased expression of LTBP4⁵, another TGF- β pathway component, in patients with non-small cell lung cancer. TGFBR3, and the TGF- β pathway, may be of relevance to the biology of tumor initiation or progression in patients with NSCLC of the adenocarcinoma type, the most common type of the leading cause of cancer death in the United States and worldwide.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
90	226625_at	4.3E-15	-14.152143	24.4423032	0.6638 ± 0.0827	TGFBR3	transforming growth factor beta receptor 3

Table 1: TGFBR3 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 TGFBR3 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
98	7917649	4.17E-24	-13.020795	44.291909	0.7817 ± 0.0900	TGFBR3	transforming growth factor beta receptor 3

Table 2: TGFBR3 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 TGFBR3 expression in NSCLC tumors as compared to the lung, and gene are listed in this chart.

Rank	ID	p-value	t	B	Gene
54	A_23_P200780	2.74E-13	-1.26E+01	20.3578741	TGFBR3

Table 3: TGFBR3 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: TGFB3 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of TGFB3 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 TGFB3 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 2: TGFB3 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of TGFB3 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 TGFB3 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 3: TGFB3 is expressed at significantly lower levels in NSCLC tumors when compared to the lung.

The mRNA expression level of TGFB3 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 TGFB3 expression between NSCLC tumors and the lung, a *p*-value, listed above.

Figure 4: TGFBR3 expression in NSCLC tumors significantly correlates with overall survival.

Depicted in this Kaplan-Meier plot is the probability of overall survival for $n=1144$ total patients stratified into two groups, based on low or high expression of TGFBR3 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 TGFBR3 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)
54	125

Table 4: TGFBR3 expression in NSCLC tumors significantly correlates with patient survival.

The median overall survival of $n=1144$ NSCLC patients based on stratification into low or high expression of TGFBR3 in tumors is listed in this chart.