The T-box transcription factor TBX3 is differentially expressed in non-small cell lung adenocarcinomas.

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Non-small cell lung adenocarcinoma (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 transcription factor TBX3 in tumors from patients with NSCLC. TBX3 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, TBX3, systems biology of NSCLC, targeted therapeutics in NSCLC.

Over 80% of patients diagnosed with non-small cell lung cancer (NSCLC) will expire within 5 years⁶. 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 independent published microarray datasets^{3,4,5}. We observed significant differential expression of TBX3 in NSCLC tumors, suggesting TBX3 may be important to the biology of non-small cell lung adenocarcinomas.

Methods

We utilized microarray datasets GSE74706³, GSE33532⁴, and GSE43458⁵ 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. GSE33532 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology (platform: GPL570) with $n=20$ 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.

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 TBX3 expression was significantly different when comparing normal lung tissue from control subjects to tumor tissue from NSCLC patients, 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 harnessed the power of multiple, 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.

TBX3 is differentially expressed in NSCLC.

We observed significant differential expression of TBX3 in human NSCLC tumors when compared to the lung³. When sorting each gene by significance of difference in expression between NSCLC tumor and the normal lung, TBX3 ranked 99 out of 34183 total transcripts. Differential expression of TBX3 was statistically significant (Table 1; $p=1.4E-12$).

We queried a second dataset⁴ to determine if we could validate TBX3 differential expression in independent microarray data. We again observed significant differential expression of TBX3 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, TBX3 ranked 117 out of 25906 total transcripts. Differential expression of TBX3 in this second dataset was also statistically significant (Table 2; $p=1.88E-14$).

Analysis of a third microarray dataset⁵ again revealed significant differential of TBX3 in primary tumors from patients diagnosed with NSCLC. When sorting each gene by significance

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of difference in expression between NSCLC tumor and the normal lung, TBX3 ranked 109 out of 33252 total transcripts. Differential expression of TBX3 in this third dataset was also statistically significant (Table 3; $p=1.17\text{E-}23$).

TBX3 expression is significantly decreased in NSCLC tumors.

We obtained exact mRNA expression levels for the differentially expressed TBX3 transcript in each of three 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 TBX3 was statistically significant when comparing NSCLC tumors to the normal lung. TBX3 was expressed at lower levels across each dataset (Figures 1-3); decreased expression of TBX3 was statistically significant in each case (Figure 1: $p<0.0001$; Figure 2: $p<0.0001$; Figure 3: $p<0.0001$). We calculated a mean fold change in TBX3 expression of 0.6463 ± 0.0492 (Figure 2) and 0.8747 ± 0.0471 (Figure 3) respectively, when comparing NSCLC tumor to normal lung.

Thus, we identified TBX3 as a differentially expressed gene in human non-small cell lung adenocarcinoma and found that TBX3 was expressed at significantly lower levels in NSCLC tumors as compared to the normal lung.

Discussion

TBX3 is essential for developmental processes across tissues⁷. The TBX3 gene is mutated in human ulnar mammary syndrome, a condition affecting development of the limbs, apocrine glands, and the genitals⁸. Tbx3-deficient mice (Tbx3 ^{-/-}) possess defects in the mammary glands, the limbs and the yolk sac; these are defects similar to those found in patients with human ulnar mammary syndrome⁹. In the heart, rate and rhythm in the atria are controlled by the sinoatrial node; TBX3 is expressed in the sinoatrial node region and deficiency of Tbx3 results in partial loss of sinoatrial node-specific gene expression¹⁰. Similarly, Tbx3 over-expression results in repression and loss of the atrial phenotype, with cells of the atria assuming a pacemaker phenotype¹⁰.

Tbx3 is co-expressed with Tbx15 in the mammary gland-forming region¹¹. Tbx3 and Bmp4 can control mammary gland formation, as well as establishment of the dorsoventral boundary early in embryogenesis. In chicken, Tbx3 can control specification of digit III in the chick leg¹². Tbx3, in conjunction with Tbx2, can also control specification of digit IV¹². Together, Tbx3 can influence development of mammary glands, the heart, of digits and of limbs.

The *Xenopus* ortholog of TBX3, ET, is capable of both transcriptional activation and repression¹³. The T-box protein T domain functions in DNA-binding for transactivation; ET contains a carboxy-terminal repressor domain, conserved in human TBX3 where it can also function in transcriptional repression¹³. TBX3 has can also control pluripotency in stem cells. In induced pluripotent stem (iPS) cells, TBX3 can support induction of pluripotency through the transcription factors Oct4, Sox2, and Nanog (OSK), in cells known as OSKT iPS cells¹⁴.

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2
3 TBX3 also has influence over cell cycle, cell proliferation and senescence programs.
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5 Expression of Tbx3, along with Myc and the oncogenic Ras variant, H-Ras^{Val17}, can transform
6 mouse embryo fibroblasts¹⁵. Oncogenic capacity of Tbx3 involves suppression of p53, as well as
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8 induction of p19^{ARF}. Tbx3 can interfere with Myc- induced apoptosis through a carboxy-
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10 terminal repression domain. In the cell cycle, TBX3 the capacity to repress transcription of
11 p21^{WAF1} by interacting with a T-box element adjacent to the p21^{WAF1} initiator, and in the
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13 SW1553 chondrosarcoma cell line, p21^{WAF1} repression by Tbx3 can support cell
14 proliferation¹⁶.

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16 TBX3 has roles in a number of human cancers. One study of breast cancers found that
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18 TBX3 could interact with histone deacetylases HDAC1, HDAC2, HDAC3 and HDAC5; histone
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20 deacetylase inhibition could antagonize TBX3 control of p14^{ARF} expression in a dose-
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22 dependent manner. In this regard, Tbx3 may function through HDAC recruitment to a T-box
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24 binding site at the p14^{ARF} promoter¹⁷. One study documented TBX3 over-expression in breast
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26 cancers¹⁷. The epithelial to mesenchyme transition described as important in metastasis involves
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28 loss of E-cadherin expression, which associates with invasive properties^{18,19}. Occupancy of
Tbx3 at the E-cadherin promoter has been described in melanomas, repressing E-cadherin
expression thereby²⁰. In melanomas, Tbx3 expression was highest in metastases relative to
dysplastic nevi and primary melanotic tumors²⁰. In liver cancer, expression of TBX3 could be

1 activated by dysregulated Wnt pathway signaling, with direct induction by beta-catenin²¹. TBX3
2 was found to be important for proliferation in the HepG2 liver cancer cell line *in vivo*; expression
3 of TBX3 antagonized doxorubicin-induced apoptosis²¹. Another study documented increased
4 expression of a truncated TBX3 variant in serum from patients with ovarian cancer²². In non-
5 small cell lung cancer, TBX3 was found to be over-expressed in 71 out of 93 cases of NSCLC²³,
6 while in another study, higher expression of TBX3 was associated with tumor size and TNM
7 (tumor/node/metastasis) stage²⁴. It is unclear why we observe lower rather than higher
8 expression of TBX3 in tumors from patients with NSCLC, but this pattern was consistent across
9 three independently published microarray datasets from primary tumors of humans with non-
10 small cell lung cancer.
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15 Differential expression of TBX3 in NSCLC should be validated in larger and separate
16 cohorts of patients with NSCLC. If validated, mouse models of non-small cell lung cancer^{25,26}
17 can be used to evaluate influence of TBX3 expression on NSCLC tumor cell proliferation and
18 organismal outcomes *in vivo*.
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Rank	ID	p-value	t	B	Gene
99	A_23_P13772	1.4E-12	-1.18E+01	18.7679989	TBX3

Table 1: TBX3 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
117	219682_s_at	1.88E-14	-13.394867	22.9851739	0.6463 ± 0.0492	TBX3	T-box 3

Table 2: TBX3 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 TBX3 expression when comparing tumor to lung, and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
109	7966690	1.17E-23	-12.822648	43.276199	0.8747 ± 0.0471	TBX3	T-box 3

Table 3: TBX3 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 TBX3 expression when comparing tumor to lung, and gene name are listed in this chart.

Figure 1: TBX3 expression is significantly decreased in NSCLC tumors.

Expression of TBX3 is graphically represented in control, normal lung tissue (left) and in primary tumors from patients with non-small cell adenocarcinoma (right); mean mRNA expression level is marked in each group and the result of a statistical test evaluating significance of difference in TBX3 expression between the groups, a *p*-value, is listed above.

Figure 2: TBX3 expression is significantly decreased in NSCLC tumors.

Expression of TBX3 is graphically represented in control, normal lung tissue (left) and in primary tumors from patients with non-small cell adenocarcinoma (right); mean mRNA expression level is marked in each group and the result of a statistical test evaluating significance of difference in TBX3 expression between the groups, a *p*-value, is listed above.

Figure 3: TBX3 expression is significantly decreased in NSCLC tumors.

Expression of TBX3 is graphically represented in control, normal lung tissue (left) and in primary tumors from patients with non-small cell adenocarcinoma (right); mean mRNA expression level is marked in each group and the result of a statistical test evaluating significance of difference in TBX3 expression between the groups, a *p*-value, is listed above.