TPX2 Promotes Metastasis and Serves as a Marker of Poor Prognosis in Non-Small Cell Lung Cancer

Background: Metastasis contributes to the high mortality rate of non-small cell lung cancer (NSCLC), and gaining a better understanding of its metastatic mechanisms would aid in initiating effective clinical treatment.

Material/Method: In this study, bioinformatics analyses of the GEO database and TCGA-LUAD were first used to identify the key node gene regulating NSCLC malignant progression. Further in vitro experiments, including wound healing assay, invasion assay, Western blot assay, and luciferase report assay, were used to clarify the functions and mechanism of TPX2 in NSCLC.

Results: Results of the TCGA analysis showed that TPX2 was significantly positively correlated with tumor metastasis and growth and the clinical stage of NSCLC. In addition, high levels of TPX2 significantly indicated a poor survival rate. In vitro experimental results also revealed that the upregulation of TPX2 significantly promoted NSCLC cell migration and invasion and could affect cell replasticity. Further results indicated that TPX2 significantly activated the epithelial-mesenchymal transition process and promoted the expression and activities of matrix metalloproteinase (MMP)2 and MMP9.

Conclusions: This study demonstrated that TPX2 promotes the metastasis and malignant progression of NSCLC and could thus serve as a marker of poor prognosis in NSCLC.

MeSH Keywords: Carcinoma • Carcinoma, Non-Small-Cell Lung • Neoplasm Metastasis

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Background

Lung cancer ranks as the leading cause of cancer-related deaths worldwide. Non-small cell lung cancer (NSCLC) is one type of lung cancer, and it has a high incidence rate, accounting for about 85% of all lung cancers [1,2]. Patients with NSCLC usually have a poor prognosis due to diagnosis at an advanced stage [3]. More than 1.5 million deaths are caused by metastatic NSCLC each year, and these place a heavy burden on social health [1]. Only a small number of early-stage NSCLC patients can be treated with surgery and achieve a high 5-year survival rate. For more advanced NSCLC, chemotherapy and radiotherapy are suggested, but the 5-year survival rate is only ~23%. Immunological, radiotherapy, and targeted therapies are associated with some success in treating advanced NSCLC, but there are still limitations precluding their uses in some cases [4–8]. For immunological therapies, targeting activity is the key to successful treatment, but a high treatment cost restricts the further promotion of this therapy [9]. Thus, a better understanding of the mechanisms of NSCLC metastasis is urgently needed to advance clinical treatments for patients with the disease.

Circulating tumor cells (CTCs) represent a pattern of blood-borne metastasis, which is a necessary way for tumors to become established at distant sites [10]. Gene expression profile data can reflect the changes of functions and signaling pathways in cells and thus reveal the pathological mechanisms underlying various diseases [11–13]. In the present study, the GEO database of GSE50991 containing mRNA expression profiles of circulating and non-metastatic lung tumor cells was used [14]. By investigating differential gene expression and function and conducting signaling pathway enrichment analyses [15], we identified TPX2 as the key regulatory gene in NSCLC metastasis and malignant progression. TPX2 overexpression has been found in a wide range of tumor types, including bladder, cervical, gastric, and hepatocellular carcinoma cancers [16–19]. Although several studies have shown that TPX2 contributes to cell proliferation and apoptosis of lung cancer [20], whether it plays an important role in the metastasis and malignant progression of NSCLC and what the underlying mechanism might be remain unclear.

In this study, we aimed to identify the key node gene in regulating the metastasis and malignant progression of NSCLC and then to verify the relationship between TPX2 and the clinicopathological features of NSCLC. We also aimed to further clarify the molecular mechanisms of TPX2 on cell metastasis in NSCLC.

Material and Methods

Analysis of mRNA expression profiles

The mRNA expression profile of GSE50991 was downloaded from the GEO database for use in this study [14]. Nine independent experiments were conducted to compare the mRNA expression profiles of circulating and nonmetastatic NSCLC tumor cells separately. We set the cutoff limit of \(|\log_{2}\text{fold change}| \geq 2\) for the differentially expressed genes (DEGs). The R packages pheatmap and limma were used to analyze the DEGs among these 18 groups. The DEGs were further subjected to function and pathway analyses. The protein-protein interaction (PPI) annotation and visualization were retrieved using the STRING database and Cytoscape software. The Cytoscape apps CentiScape and MCODE were used for further topological analysis. The GO and KEGG pathway analyses were conducted by using the Metascape website (https://metascape.org), while the enriched functions and pathways were visualized through the R package of ggplot2. The gene set enrichment analysis (GSEA) of total mRNA expression profiles was conducted using the R packages of clusterProfiler, enrichplot, and ggplot2 [21].

Analysis of the TCGA data

The RNA-seq gene expression data of 594 samples (535 LUAD samples and 59 normal tissue samples) generated by Illumina HiSeq were obtained from The Cancer Genome Atlas (TCGA). The clinical information associated with samples was downloaded from the GDC data portal. The immunochemistry results of TPX2 in NSCLC were obtained from the open website of The Human Protein Atlas (https://www.proteinatlas.org/). The clinical analysis of TPX2 in NSCLC was conducted by combining the TCGA and Protein Atlas-downloaded data (Supplementary Table 1). We set the median value as the dividing point for high expression and low expression of TPX2 in the clinical analyses.

Cell culture and transfection

The NSCLC cells of A549, H1975, H1299, H522, and H1650 were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (HyClone) and penicillin (50 μg/mL) plus streptomycin (50 μg/mL). All cultures were maintained under a humidified atmosphere containing 5% CO₂. When cells reached an acceptable density, the TPX2 overexpression plasmid and shTPX2 knockdown plasmid were transfected into NSCLC cells with the transfection reagent (Roche, Switzerland), separately. The images of cells were visualized using a microscope (Nikon, Japan).
**Wound healing assays**

Treated NSCLC cells were seeded into 24-well culture plates at a density of 5×10³ cells/well. After about 12 h of incubation, a 200 μL pipette tip was used to scratch a wound into the surface. After incubation with serum-free medium for another 48 h, images of the healing were taken. All experiments were performed at least in triplicate, and mean±SD values are presented.

**Invasion assays**

Treated NSCLC cells were diluted with serum-free medium at a concentration of 1×10⁵ cells/mL, and 200 μL of the diluted medium was then added to a Matrigel-coated top chamber. After 48 h of invasion in a humidified atmosphere containing 5% CO₂, the passed cells were fixed in 4% paraformaldehyde and stained with crystal violet solution, and images of the passed NSCLC cells were then taken. All experiments were performed at least in triplicate, and the mean ± SD values are presented.

**Western blotting**

The total proteins in treated cells were isolated by using the RIPA lysis buffer, and the protein concentrations were measured by bicinchoninic acid (BCA) protein assay kit (Thermo, USA). Western blotting was performed through sodium dodecyl sulfate polyacrylamide gel electrophoresis and subsequent transfer to polyvinylidene difluoride membranes. The membranes were then probed with primary antibodies of TPX2, E-cad, claudin 3, vimentin, fibronectin, or GAPDH, followed by incubation with a corresponding second antibody (1: 10 000, Santa Cruz Biotechnology, USA). All primary antibodies were purchased from Abcam and incubated with 1: 1000 dilutions. Densitometric analysis was performed by using the ImageJ software. All experiments were performed at least in triplicate, and the mean±SD values are presented.

**Luciferase reporter assays**

The luciferase reporter plasmids (GeneCopoeia, China) were designed by inserting the promoter clones for the matrix metalloproteinase 2 (MMP2) or MMP9 gene to the vector of pEZX-PG04.1 with the tracking gene of secreted alkaline phosphatase. After cotransfection of the MMP2 or MMP9 luciferase reporter plasmid and TPX2 overexpression or knockingdown plasmid, the NSCLC cells were incubated for another 48 h in a humidified atmosphere containing 5% CO₂. Fluorescence detection was performed by using the Dual-Luciferase Assays System (Promega) with a Luminescent reader system. All experiments were performed at least in triplicate, and the mean±SD values are presented.

**MMP activity assays**

The MMP activity assays were performed by using the MMP activity assay kit (Abcam, USA). The media collected from treated NSCLC cells were incubated with the APMA working solution for about 15 min at room temperature. Then, the MMP Green Substrate solution was added and incubated for another 1 h at 37°C. Fluorescence detection was performed by using a microplate reader system at wavelengths of 490 and 595 nm. All experiments were performed at least in triplicate, and the mean±SD values are presented.

**Statistical analysis**

IBM SPSS Statistics 22.0 software (Chicago, IL, USA) was used to perform statistical analyses. Gap closure was analyzed by one-way ANOVA followed by pairwise comparison with the control groups by paired t test. All data are presented as mean±SD. All experiments were performed at least in triplicate, and P<0.05 was considered statistically significant.

**Results**

**Selection of the key candidate gene in regulating the malignant progression of NSCLC**

mRNA expression profile data downloaded from the GEO database were used to screen for the key candidate gene. The heatmap analysis results showed very different expressed gene profiles between the circulating tumor cells and non-metastatic tumor cells of NSCLC (Figure 1A). The volcano plot further described the DEGs between these 2 groups, in which many genes were significantly upregulated, whereas others were significantly downregulated (Figure 1B). To screen for the key candidate gene regulating the malignant progression of NSCLC, the upregulated genes were subjected to PPI analysis. The constructed PPI network considered 601 nodes and 2416 edges (Figure 1C, left). By using the Cytoscape APPs of CentiScape and MCODE, we further screened the topological network with features of Degree and K-core, and a hub network was identified with the MCODE-score of 21.478 (Figure 1C, right). As a result, the potential hub node of TPX2 was identified as a candidate gene in regulating the malignant progression of NSCLC.

**TPX2 promotes metastasis and malignant progression of NSCLC**

To explore the correlation between TPX2 expression and NSCLC, the LUAD dataset from TCGA was analyzed. The TPX2 immunohistochemistry results showed a remarkable increase of TPX2 expression in metastatic lung cancer tissues compared with non-metastatic lung cancer tissues (Figure 2A). Statistical results
Figure 1. TPX2 was selected as the key candidate gene in regulating the malignant progression of NSCLC. (A) Heatmap analysis of DEGs revealed a substantial difference between circulating and nonmetastatic NSCLC tumor cells. (B) Volcano plot for the DEGs between circulating and nonmetastatic NSCLC tumor cells, where the red and green plots indicating the upregulated and downregulated genes, respectively. (C) PPI network analysis of DEGs revealed NSCLC-related PPI hubs, and topological PPI network indicated TPX2 as a key node gene. DEGs – differentially expressed genes; CTCs – circulating tumor cells; Ctrl – nonmetastatic NSCLC tumor cells; PPI – protein–protein interaction.
Figure 2. Correlation of TPX2 with NSCLC tumor characteristics in clinical samples. (A) TPX2 staining was weakly positive and strongly positive in nonmetastatic and metastatic lung cancer tissues, separately. (B) TPX2 expression was significantly upregulated in the metastatic NSCLC samples compared with the nonmetastatic NSCLC samples. (C) TPX2 expression was significantly increased with the NSCLC tumor size. (D) TPX2 expression was significantly increased with the NSCLC clinical stage. (E) Overall survival analysis of TPX2 on patients with NSCLC (mean±SD; ** P<0.01).
showed that TPX2 expression was significantly increased when lung cancer cells metastasized (Figure 2B). Similarly, TPX2 expression had significantly positive correlations with tumor size (Figure 2C) and clinical stage (Figure 2D). Further analysis revealed that NSCLC patients with high TPX2 expression had a significantly lower survival rate compared with those with low TPX2 expression (P=0.0025, Figure 2E).

**TPX2 affects migration, invasion, and cell replasticity in NSCLC**

To clarify the exact role of TPX2 in NSCLC, the mRNA expression profile data were subjected to GSEA analysis. The GSEA results showed that when metastasis occurred, the junction between cell and cell or that between cell and matrix decreased; the regulation of apoptosis decreased, while the malignant progression of NSCLC increased; and the epithelial-mesenchymal transition (EMT) process and ATP generation increased (Figure 3A). The DEGs were further subjected to GO and KEGG analyses of the upregulated genes. The GSEA and KEGG analyses of the downregulated genes. TPX2 expression had significantly positive correlations with tumor size (Figure 2C) and clinical stage (Figure 2D). Meanwhile, the pathways and functions associated with the downregulated genes included the regulation of tube diameter, the EMT process and ATP generation increased (Figure 3A). The DEGs were further subjected to GO and KEGG analyses of the upregulated genes. The GSEA and KEGG analyses of the downregulated genes. Therefore, we designed in vitro experiments for verification. First, 5 NSCLC cell lines were chosen to detect the background expression levels of TPX2. A549 and H1650 were separately

**Figure 3.** Function and pathway analyses of the mRNA expression profiles. (A) GSEA results of total mRNA showed differential regulation among cell adhesion, EMT, and ATP metabolism. (B) GO and KEGG analyses of the upregulated genes. (C) GO and KEGG analyses of the downregulated genes. GSEA – Gene Set Enrichment Analysis; GO – Gene Ontology; KEGG – Kyoto Encyclopedia of Genes and Genomes.
Figure 4. TPX2 affected NSCLC migration, invasion, and cell replasticity. (A) Relative TPX2 expression analysis in 5 NSCLC cell lines; the ratio of densitometry value to the corresponding GAPDH value was used to reveal the relative protein expression. (B) The upregulation of TPX2 promoted the migration of the A549 cells, whereas its downregulation inhibited the migration of the H1650 cells. (C) The upregulation of TPX2 promoted the invasion of the A549 cells, whereas its downregulation inhibited the invasion of the H1650 cells. (D) Morphological observations of different treated A549 cells in pseudopod and cell rounding (mean±SD; n=3 in triplicate; ** P<0.01).
screened as TPX2 low-expression and high-expression models for subsequent experiments (Figure 4A). The wound healing assay showed that after 48 h of treatment, scratch wounds were significantly thinner in the high TPX2 expression groups (Figure 4B). In the Matrigel invasion assay, the passed cells were significantly increased when TPX2 was upregulated in the A549 cells and significantly decreased when TPX2 was knocked down in the H1650 cells (Figure 4C). The morphological results showed notable differences between the control and TPX2 overexpression groups, including pseudopod disappearance and cell rounding (Figure 4D).

**TPX2 promotes the EMT process and the expression and activity of MMP2 and MMP9**

During the omics analysis, we found significant differences in the regulation of the EMT process and extracellular matrix organization, and we suspected that TPX2 promoted the
metastasis and malignant progression of NSCLC through EMT and MMPs. The Western blot results showed that when TPX2 was upregulated in the A549 cells, the expression of the epithelial marker proteins E-cad and claudin 3 was significantly decreased, whereas the expression of the mesenchymal marker proteins vimentin and fibronectin was significantly increased. Similarly, when TPX2 was knocked down in the H1650 cells, the expression of the epithelial marker proteins was significantly upregulated and that of the mesenchymal marker proteins was significantly downregulated (Figure 5A). In addition, MMP2 and MMP9 transcription was significantly increased after overexpression of TPX2 in A549 cells and significantly decreased when TPX2 was knocked down in H1650 cells (Figure 5B). The results of the MMP activity assays further revealed that the enzyme activities of MMP2 and MMP9 significantly increased with the upregulation of TPX2 in A549 cells and significantly decreased with the downregulation of TPX2 in H1650 cells (Figure 5C).

Discussion

Bioinformatics analysis allows the identification of tumor-associated node genes from large data repositories to assist in the clarification of mechanisms and the prognostic assessment of cancer [15,22]. In the present study, we chose 9 samples of circulating NSCLC tumor cells and 9 samples of nonmetastatic NSCLC tumor cells in a GEO database to identify DEGs. Through further topological network analysis, we screened the node gene TPX2. TPX2 is a microtubule-associated protein whose expression is strictly controlled within the cell cycle; it appears during the G1/S stage and vanishes after the completion of cytokinesis [23,24]. Several studies have revealed that overexpression of TPX2 is correlated with tumor proliferation, stage, grade, and survival rate in some malignant tumors [25]. Thus, we speculated that TPX2 might play an important role in the metastasis and malignant progression of NSCLC. Further study verified our hypothesis through the TCGA database, and the results showed that the high expression of TPX2 was significantly positively correlated with metastasis, clinical stage, and poor survival prognosis of NSCLC.

As a microtubule-associated protein, TPX2 has been reported to mediate spindle filament assembly during mitosis and to be related to cell proliferation [23]. Although a number of studies have clarified the relationship between TPX2 and tumor apoptosis and metastasis in liver and gastric cancer [16,19], the detailed mechanism of TPX2 in NSCLC remains unclear. In this study, results from omics data analysis showed that the cell adhesion function and EMT process were significantly dysregulated after NSCLC metastasized. Further in vitro experiments demonstrated that TPX2 indeed promoted the EMT process and the degradation of extracellular matrix. Meanwhile, morphological observations of TPX2-overexpressed NSCLC cells showed that TPX2 changed the cell shape from a clawed epithelial morphology to one with pseudopod disappearance and cell rounding, with the near-suspended cells remaining viable. We speculated that the regulation of microtubules by TPX2 likely mediated the cytoskeleton remodeling of NSCLC cells, which in turn activated the mesenchymal transition of tumor cells and expression of MMPs and enabled NSCLC cells to metastasize. However, it remained unclear how cytoskeleton remodeling affected the EMT process and MMP-mediated degradation of extracellular matrix, and further research is required.

Conclusions

In summary, this study demonstrated that TPX2 promoted NSCLC metastasis and malignant progression. The effects of TPX2 in NSCLC may have been mediated through regulation of cytoskeleton remodeling as well as the further activation of the EMT process and MMP expression and enzyme activities (Figure 6). TPX2 might serve as a marker of metastasis and poor prognosis in NSCLC, and it could potentially serve as a target for the clinical treatment of NSCLC.

Conflict of interest

None.
## Supplementary Data

### Supplementary Table 1. The TCGA and Protein Atlas – downloaded data.

| Sample          | FPKM | TPX2 | AJCC pathologic m | AJCC pathologic n |
|-----------------|------|------|-------------------|-------------------|
| TCGA-78-7167    | 0.8  | M1   | N0                |                   |
| TCGA-49-4510    | 1.1  | M0   | N0                |                   |
| TCGA-55-8512    | 1.2  | M1b  | N1                |                   |
| TCGA-86-A4P8    | 1.2  | MX   | N2                |                   |
| TCGA-78-7156    | 1.5  | M1   | N1                |                   |
| TCGA-91-6849    | 1.7  | MX   | N2                |                   |
| TCGA-67-6217    | 1.9  | M0   | N1                |                   |
| TCGA-91-A4BD    | 2.1  | MX   | N1                |                   |
| TCGA-NI-A7XG    | 2.2  | M0   | N1                |                   |
| TCGA-05-4384    | 2.6  | M0   | N2                |                   |
| TCGA-MP-A4T6    | 3.2  | MX   | N2                |                   |
| TCGA-05-5423    | 3.3  | M0   | N1                |                   |
| TCGA-64-1680    | 3.4  | M1   | N2                |                   |
| TCGA-44-2659    | 3.6  | M0   | N1                |                   |
| TCGA-55-A50W    | 3.9  | MX   | N2                |                   |
| TCGA-49-4505    | 3.7  | M0   | N1                |                   |
| TCGA-69-8253    | 4    | MX   | N1                |                   |
| TCGA-NI-A4VI    | 4    | M0   | N1                |                   |
| TCGA-50-5068    | 4.3  | MX   | N1                |                   |
| TCGA-86-7714    | 4.4  | M0   | N2                |                   |
| TCGA-66-8212    | 4.5  | M0   | N2                |                   |
| TCGA-49-4490    | 4.8  | M0   | N2                |                   |
| TCGA-44-2665    | 5.1  | M0   | N1                |                   |
| TCGA-97-8192    | 5.5  | M0   | N1                |                   |
| TCGA-55-A5SO    | 5.3  | M0   | N1                |                   |
| TCGA-38-4627    | 5.4  | M0   | N1                |                   |
| TCGA-55-7227    | 5.7  | M0   | N1                |                   |
| TCGA-73-4675    | 5.7  | M0   | N1                |                   |
| TCGA-49-4512    | 6.8  | M0   | N1                |                   |
| TCGA-50-5045    | 7.5  | M0   | N1                |                   |
| TCGA-49-4506    | 7.8  | M0   | N1                |                   |
| TCGA-62-A46Y    | 7.8  | M0   | N2                |                   |
| TCGA-97-8547    | 8.2  | MX   | N2                |                   |
| TCGA-38-4628    | 8.3  | M0   | N1                |                   |
| TCGA-05-4396    | 8.6  | M0   | N1                |                   |
| TCGA-55-7283    | 8.6  | MX   | N2                |                   |
| TCGA-MP-A4TD    | 8.6  | M0   | N2                |                   |
| TCGA-53-7626    | 9.3  | M0   | N1                |                   |
| TCGA-55-6984    | 9.3  | M0   | N1                |                   |
| TCGA-95-A4VK    | 9.3  | M0   | N2                |                   |
| TCGA-49-4512    | 9.3  | MX   | N1                |                   |
| TCGA-55-9114    | 9.4  | MX   | N1                |                   |
| TCGA-55-A48X    | 9.5  | M0   | N1                |                   |
| TCGA-05-5420    | 9.5  | M0   | N1                |                   |
| TCGA-55-8094    | 10.2 | M1b  | N0                |                   |
| TCGA-MP-A4T9    | 10.2 | MX   | N2                |                   |
| TCGA-78-7160    | 10.3 | M0   | N1                |                   |
| TCGA-91-6830    | 10.7 | MX   | N1                |                   |
| TCGA-05-4402    | 10.9 | M1   | NX                |                   |
| TCGA-05-4425    | 11.1 | M1   | N0                |                   |
| TCGA-86-8279    | 11.1 | M0   | N1                |                   |
| TCGA-93-A4JP    | 11.3 | M1b  | NX                |                   |
| TCGA-55-8615    | 11.3 | MX   | N2                |                   |
| TCGA-44-6774    | 11.6 | M0   | N2                |                   |
| TCGA-97-7554    | 11.6 | M0   | N2                |                   |
| TCGA-05-4418    | 12   | M0   | N2                |                   |
| TCGA-50-5051    | 12   | M0   | N2                |                   |
| TCGA-55-8508    | 12   | MX   | N1                |                   |
| TCGA-50-5429    | 12.3 | M0   | N2                |                   |
| TCGA-50-5930    | 12.8 | M0   | N2                |                   |
| TCGA-64-5815    | 13   | M0   | N1                |                   |
| TCGA-97-7148    | 13.1 | M0   | N1                |                   |
| TCGA-55-7907    | 13.3 | MX   | N1                |                   |
| TCGA-86-6851    | 13.4 | M0   | N1                |                   |
| TCGA-MP-A4SY    | 13.5 | M0   | N1                |                   |
| TCGA-MP-A4T8    | 13.5 | M0   | N2                |                   |
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### DATABASE ANALYSIS

| Sample          | FPKM TPX2 | AJCC pathologic m | AJCC pathologic n |
|-----------------|-----------|-------------------|-------------------|
| TCGA-55-8505    | 13.7      | MX                | N2                |
| TCGA-99-8025    | 13.8      | M0                | N1                |
| TCGA-73-4659    | 13.9      | M0                | N2                |
| TCGA-50-6593    | 14        | M0                | N1                |
| TCGA-55-8055    | 14.2      | M0                | N1                |
| TCGA-78-7166    | 14.4      | M0                | N1                |
| TCGA-L9-A7SV    | 14.4      | M0                | N1                |
| TCGA-55-6981    | 14.5      | M0                | N2                |
| TCGA-95-A4WP    | 14.7      | M0                | N2                |
| TCGA-L9-A743    | 14.7      | M0                | N1                |
| TCGA-44-3396    | 14.8      | M0                | N2                |
| TCGA-78-7147    | 15        | M0                | N1                |
| TCGA-95-7567    | 15        | M0                | N1                |
| TCGA-55-6970    | 15.3      | MX                | N2                |
| TCGA-55-6982    | 15.4      | M0                | N1                |
| TCGA-MP-A4T7    | 15.5      | M0                | N2                |
| TCGA-49-AAR4    | 16.2      | MX                | N2                |
| TCGA-MP-A4T5    | 17.4      | MX                | N1                |
| TCGA-55-6712    | 17.8      | MX                | N1                |
| TCGA-64-1679    | 18        | M0                | N2                |
| TCGA-49-6745    | 18.3      | M0                | N2                |
| TCGA-62-8398    | 18.3      | M0                | N2                |
| TCGA-49-4494    | 18.6      | M0                | N2                |
| TCGA-78-8640    | 18.8      | M0                | N1                |
| TCGA-86-8074    | 18.8      | M0                | N1                |
| TCGA-78-7145    | 19.3      | M1                | N1                |
| TCGA-55-8032    | 19.4      | M1                | NX                |
| TCGA-49-6797    | 19.5      | M0                | N1                |
| TCGA-64-1679    | 19.5      | M0                | N2                |
| TCGA-TG-8245    | 19.7      | M0                | N1                |
| TCGA-05-4434    | 20        | M1                | N1                |
| TCGA-49-6761    | 20.7      | MX                | N2                |
| TCGA-14-4045    | 21.1      | MX                | N2                |
| TCGA-MP-A4TK    | 21.2      | MX                | N1                |
| TCGA-38-4632    | 21.4      | M1                | N1                |
| TCGA-64-5779    | 21.6      | M0                | N2                |
| TCGA-50-5941    | 21.8      | M0                | N2                |
| TCGA-73-4676    | 21.8      | M0                | N1                |
| TCGA-62-8399    | 21.9      | M0                | N2                |
| TCGA-MN-A4N1    | 22        | M0                | N1                |
| TCGA-35-5375    | 22.5      | M0                | N2                |
| TCGA-95-7562    | 22.5      | M0                | N1                |

| Sample          | FPKM TPX2 | AJCC pathologic m | AJCC pathologic n |
|-----------------|-----------|-------------------|-------------------|
| TCGA-69-7978    | 22.6      | MX                | N1                |
| TCGA-55-6979    | 23.1      | M0                | N1                |
| TCGA-MP-A4T4    | 23.3      | M0                | N1                |
| TCGA-64-1677    | 23.5      | M0                | N2                |
| TCGA-53-A4EZ    | 23.7      | MX                | N1                |
| TCGA-05-4432    | 24.1      | M0                | N1                |
| TCGA-49-4507    | 24.4      | M0                | N1                |
| TCGA-55-1596    | 24.4      | M0                | N1                |
| TCGA-95-A4VN    | 24.4      | M0                | N1                |
| TCGA-50-5044    | 24.6      | M0                | N1                |
| TCGA-97-8176    | 24.7      | M0                | N1                |
| TCGA-05-4398    | 25.3      | M0                | N3                |
| TCGA-73-4670    | 25.5      | M1                | N0                |
| TCGA-62-8402    | 25.9      | M0                | N2                |
| TCGA-55-7727    | 26.2      | MX                | N2                |
| TCGA-50-5936    | 26.3      | M0                | N2                |
| TCGA-50-5933    | 26.6      | M0                | N2                |
| TCGA-62-A471    | 26.7      | M0                | N1                |
| TCGA-50-6594    | 27.1      | M0                | N2                |
| TCGA-86-8054    | 27.8      | M0                | N1                |
| TCGA-86-7711    | 28.4      | M0                | N1                |
| TCGA-78-7150    | 29.3      | M0                | N1                |
| TCGA-05-5425    | 29.6      | M0                | N1                |
| TCGA-93-A4IN    | 29.7      | M1a               | N0                |
| TCGA-MP-A4TC    | 29.8      | M0                | N2                |
| TCGA-MP-A4TI    | 30.2      | M0                | N1                |
| TCGA-95-8494    | 30.9      | M0                | N1                |
| TCGA-49-6742    | 31        | M0                | N1                |
| TCGA-86-7701    | 31.2      | M1                | N0                |
| TCGA-78-7220    | 34.3      | M0                | N2                |
| TCGA-62-8394    | 34.4      | M0                | N2                |
| TCGA-55-6975    | 34.5      | M0                | N1                |
| TCGA-05-4250    | 36.7      | M0                | N1                |
| TCGA-78-7154    | 36.7      | M0                | N2                |
| TCGA-05-4415    | 36.9      | M0                | N2                |
| TCGA-50-5072    | 37.2      | M0                | N2                |
| TCGA-44-5643    | 38.9      | M0                | N2                |
| TCGA-05-4427    | 40        | M0                | N1                |
| TCGA-55-5899    | 40.1      | M0                | N1                |
| TCGA-50-6591    | 41        | M0                | N1                |
| TCGA-49-6743    | 41.8      | MX                | N2                |
### DATABASE ANALYSIS

| Sample          | FPKM | TPX2 | AJCC pathologic m | AJCC pathologic n |
|-----------------|------|------|-------------------|-------------------|
| TCGA-49-AAR3    | 42   | MX   | N1                |                   |
| TCGA-78-8560    | 42   | MX   | N1                |                   |
| TCGA-44-7669    | 42   | MX   | N1                |                   |
| TCGA-75-5125    | 45.1 | M0   | N1                |                   |
| TCGA-44-6595    | 46.6 | M0   | N2                |                   |
| TCGA-75-6214    | 46.9 | M0   | N2                |                   |
| TCGA-44-6779    | 49.2 | MX   | N1                |                   |
| TCGA-73-4666    | 53.1 | M1   | N0                |                   |
| TCGA-55-8620    | 58.5 | M1b  | N1                |                   |
| TCGA-78-7183    | 62.8 | M0   | N2                |                   |
| TCGA-78-7167    | 63.6 | M0   | N1                |                   |
| TCGA-05-4397    | 64.5 | M0   | N1                |                   |
| TCGA-91-6848    | 64.6 | MX   | N2                |                   |
| TCGA-55-6968    | 65.9 | M1   | N0                |                   |
| TCGA-78-7146    | 74.3 | M0   | N2                |                   |
| TCGA-78-7624    | 75.6 | M1   | N0                |                   |
| TCGA-44-6148    | 75.6 | M0   | N0                |                   |
| TCGA-67-3773    | 1.1  | M0   | N0                |                   |
| TCGA-49-4486    | 1.4  | M0   | N0                |                   |
| TCGA-50-5942    | 1.4  | M0   | N0                |                   |
| TCGA-35-3615    | 1.5  | M0   | N0                |                   |
| TCGA-50-8457    | 1.5  | M0   | N0                |                   |
| TCGA-75-6206    | 1.5  | M0   | N0                |                   |
| TCGA-78-7537    | 1.6  | M0   | N0                |                   |
| TCGA-97-A4M1    | 1.6  | M0   | N0                |                   |
| TCGA-44-4A66    | 1.7  | M0   | N0                |                   |
| TCGA-73-7498    | 1.8  | M0   | N0                |                   |
| TCGA-75-5146    | 1.9  | M0   | N0                |                   |
| TCGA-78-7183    | 1.9  | M0   | N0                |                   |
| TCGA-62-8397    | 2    | M0   | N0                |                   |
| TCGA-97-A4M2    | 2    | M0   | N0                |                   |
| TCGA-78-7183    | 2    | M0   | N0                |                   |
| TCGA-97-8174    | 2.2  | M0   | N0                |                   |
| TCGA-67-4679    | 2.3  | M0   | N0                |                   |
| TCGA-55-8206    | 2.4  | M0   | N0                |                   |
| TCGA-73-4677    | 2.4  | M0   | N0                |                   |
| TCGA-44-2655    | 2.5  | M0   | N0                |                   |
| TCGA-67-3770    | 2.5  | M0   | N0                |                   |
| TCGA-38-4A4F    | 2.6  | M0   | N0                |                   |
| TCGA-55-6980    | 2.6  | M0   | N0                |                   |
| TCGA-62-8395    | 2.6  | M0   | N0                |                   |

| Sample          | FPKM | TPX2 | AJCC pathologic m | AJCC pathologic n |
|-----------------|------|------|-------------------|-------------------|
| TCGA-62-A46S    | 2.6  | M0   | N0                |                   |
| TCGA-78-8560    | 2.6  | M0   | N0                |                   |
| TCGA-55-8620    | 2.6  | M0   | N0                |                   |
| TCGA-78-7183    | 2.6  | M0   | N0                |                   |
| TCGA-44-2661    | 2.9  | M0   | N0                |                   |
| TCGA-86-8056    | 2.9  | M0   | N0                |                   |
| TCGA-50-8460    | 3.1  | M0   | N0                |                   |
| TCGA-69-7763    | 3.1  | M0   | N0                |                   |
| TCGA-78-7161    | 3.1  | M0   | N0                |                   |
| TCGA-86-8076    | 3.1  | M0   | N0                |                   |
| TCGA-97-A4M3    | 3.1  | M0   | N0                |                   |
| TCGA-78-7539    | 3.2  | M0   | N0                |                   |
| TCGA-97-A4M0    | 3.2  | M0   | N0                |                   |
| TCGA-78-7633    | 3.3  | M0   | N0                |                   |
| TCGA-78-7149    | 3.5  | M0   | N0                |                   |
| TCGA-78-7540    | 3.5  | M0   | N0                |                   |
| TCGA-50-5935    | 3.7  | M0   | N0                |                   |
| TCGA-67-3772    | 3.7  | M0   | N0                |                   |
| TCGA-86-8668    | 3.7  | M0   | N0                |                   |
| TCGA-86-A456    | 3.7  | M0   | N0                |                   |
| TCGA-49-4501    | 3.9  | M0   | N0                |                   |
| TCGA-97-A4M3    | 3.9  | M0   | N0                |                   |
| TCGA-78-7162    | 3.9  | M0   | N0                |                   |
| TCGA-99-7458    | 3.9  | M0   | N0                |                   |
| TCGA-05-4249    | 4    | M0   | N0                |                   |
| TCGA-38-4626    | 4.1  | M0   | N0                |                   |
| TCGA-95-7948    | 4.1  | M0   | N0                |                   |
| TCGA-78-7539    | 4.1  | M0   | N0                |                   |
| TCGA-50-5935    | 4.2  | M0   | N0                |                   |
| TCGA-50-5944    | 4.3  | M0   | N0                |                   |
| TCGA-50-8459    | 4.4  | M0   | N0                |                   |
| TCGA-62-A46P    | 4.5  | M0   | N0                |                   |
| TCGA-55-6972    | 4.6  | M0   | N0                |                   |
| TCGA-97-A4M6    | 4.6  | M0   | N0                |                   |
| TCGA-97-A4M5    | 4.7  | M0   | N0                |                   |
| TCGA-05-4405    | 5    | M0   | N0                |                   |
| TCGA-05-4422    | 5.1  | M0   | N0                |                   |

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| Sample          | FPKM TPX2 | AJCC pathologic | AJCC pathologic |
|-----------------|-----------|-----------------|-----------------|
| TCGA-44-7671    | 5.1       | M0              | N0              |
| TCGA-97-8172    | 5.1       | M0              | N0              |
| TCGA-44-2666    | 5.3       | M0              | N0              |
| TCGA-55-4771    | 5.4       | M0              | N0              |
| TCGA-55-A57B    | 5.4       | M0              | N0              |
| TCGA-78-8655    | 5.4       | M0              | N0              |
| TCGA-73-4662    | 5.6       | M0              | N0              |
| TCGA-86-8669    | 5.6       | M0              | N0              |
| TCGA-44-8120    | 6.0       | M0              | N0              |
| TCGA-86-8280    | 6.1       | M0              | N0              |
| TCGA-97-44M7    | 6.2       | M0              | N0              |
| TCGA-44-2656    | 6.3       | M0              | N0              |
| TCGA-44-8117    | 6.3       | M0              | N0              |
| TCGA-64-1681    | 6.3       | M0              | N0              |
| TCGA-67-6216    | 6.4       | M0              | N0              |
| TCGA-86-A4P7    | 6.7       | M0              | N0              |
| TCGA-64-1676    | 6.8       | M0              | N0              |
| TCGA-55-7281    | 7.1       | M0              | N0              |
| TCGA-55-7574    | 7.5       | M0              | N0              |
| TCGA-55-8090    | 7.6       | M0              | N0              |
| TCGA-73-4658    | 7.7       | M0              | N0              |
| TCGA-71-6725    | 7.8       | M0              | N0              |
| TCGA-78-7153    | 7.9       | M0              | N0              |
| TCGA-55-8616    | 8.0       | M0              | N0              |
| TCGA-44-3918    | 8.1       | M0              | N0              |
| TCGA-67-6215    | 8.2       | M0              | N0              |
| TCGA-62-A470    | 8.4       | M0              | N0              |
| TCGA-MN-A4N4    | 8.7       | M0              | N0              |
| TCGA-05-5715    | 9.1       | M0              | N0              |
| TCGA-44-A412    | 9.2       | M0              | N0              |
| TCGA-44-A47G    | 9.4       | M0              | N0              |
| TCGA-69-7764    | 9.4       | M0              | N0              |
| TCGA-62-A46V    | 9.5       | M0              | N0              |
| TCGA-78-7152    | 9.6       | M0              | N0              |
| TCGA-05-4430    | 10.4      | M0              | N0              |
| TCGA-97-A4LX    | 10.4      | M0              | N0              |
| TCGA-MN-A4N5    | 10.5      | M0              | N0              |
| TCGA-86-8073    | 10.8      | M0              | N0              |
| TCGA-44-A47B    | 11.1      | M0              | N0              |
### DATABASE ANALYSIS

| Sample     | FPKM TPX2 | AJCC pathologic m | AJCC pathologic n |
|------------|-----------|-------------------|-------------------|
| TCGA-55-8085 | 17.9      | M0                | N0                |
| TCGA-44-3919 | 19.9      | N0                | N0                |
| TCGA-69-A59K | 18.2      | N0                | M0                |
| TCGA-86-7713 | 18.5      | N0                | M0                |
| TCGA-86-8631 | 18.7      | N0                | M0                |
| TCGA-44-7672 | 19.6      | N0                | M0                |
| TCGA-95-7944 | 19.8      | M0                | N0                |
| TCGA-64-1678 | 20.2      | M0                | N0                |
| TCGA-78-7535 | 20.2      | M0                | N0                |
| TCGA-53-7813 | 20.3      | M0                | N0                |
| TCGA-55-8208 | 20.7      | M0                | N0                |
| TCGA-86-7953 | 20.8      | M0                | N0                |
| TCGA-67-3771 | 21.4      | M0                | N0                |
| TCGA-78-7910 | 21.6      | M0                | N0                |
| TCGA-86-A4JF | 21.9      | M0                | N0                |
| TCGA-73-7499 | 22.9      | M0                | N0                |
| TCGA-73-7490 | 23.1      | M0                | N0                |
| TCGA-73-3890 | 23.5      | M0                | N0                |
| TCGA-44-3917 | 24.1      | M0                | N0                |
| TCGA-55-7576 | 25.8      | M0                | N0                |
| TCGA-64-8631 | 27.7      | M0                | N0                |
| TCGA-91-6840 | 28.1      | M0                | N0                |
| TCGA-69-7973 | 28.3      | M0                | N0                |

| Sample     | FPKM TPX2 | AJCC pathologic m | AJCC pathologic n |
|------------|-----------|-------------------|-------------------|
| TCGA-95-7947 | 28.3      | M0                | N0                |
| TCGA-78-7143 | 28.5      | M0                | N0                |
| TCGA-69-8255 | 29.0      | M0                | N0                |
| TCGA-75-7027 | 29.0      | M0                | N0                |
| TCGA-66-2662 | 30.0      | M0                | N0                |
| TCGA-MP-A4SV | 30.2      | M0                | N0                |
| TCGA-49-4514 | 31.0      | M0                | N0                |
| TCGA-55-8089 | 31.4      | M0                | N0                |
| TCGA-86-8673 | 31.9      | M0                | N0                |
| TCGA-84-7661 | 34.0      | M0                | N0                |
| TCGA-55-8089 | 35.0      | M0                | N0                |
| TCGA-86-7953 | 36.1      | M0                | N0                |
| TCGA-64-5775 | 36.3      | M0                | N0                |
| TCGA-66-44D0 | 36.4      | M0                | N0                |
| TCGA-50-5931 | 37.0      | M0                | N0                |
| TCGA-38-4631 | 37.5      | M0                | N0                |
| TCGA-44-3919 | 38.1      | M0                | N0                |
| TCGA-73-9491 | 39.9      | M0                | N0                |
| TCGA-80-5611 | 40.8      | M0                | N0                |
| TCGA-64-5774 | 43.9      | M0                | N0                |
| TCGA-50-6592 | 37.7      | M0                | N0                |
| TCGA-50-5931 | 37.0      | M0                | N0                |
| TCGA-73-9491 | 37.5      | M0                | N0                |
| TCGA-55-8205 | 52.2      | M0                | N0                |
| TCGA-78-7542 | 52.4      | M0                | N0                |

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