STXBP4 Drives Tumor Growth and Is Associated with Poor Prognosis through PDGF Receptor Signaling in Lung Squamous Cell Carcinoma

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Abstract

Purpose: Expression of the ΔN isoform of p63 (ΔNp63) is a diagnostic marker highly specific for lung squamous cell carcinoma (SCC). We previously found that Syntaxin Binding Protein 4 (STXBP4) regulates ΔNp63 ubiquitination, suggesting that STXBP4 may also be an SCC biomarker. To address this issue, we investigated the role of STXBP4 expression in SCC biology and the impact of STXBP4 expression on SCC prognosis.

Experimental Design: We carried out a clinicopathologic analysis of STXBP4 expression in 87 lung SCC patients. Whole transcriptome analysis using RNA-seq was performed in STXBP4-positive and STXBP4-negative tumors of lung SCC. Soft-agar assay and xenograft assay were performed using overexpressing or knockdown SCC cells.

Results: Significantly higher levels of STXBP4 expression were correlated with accumulations of ΔNp63 in clinical lung SCC specimens (Spearman rank correlation ρ = 0.219). Notably, STXBP4-positive tumors correlated with three important clinical parameters: T factor (P < 0.001), disease stage (P = 0.030), and pleural involvement (P = 0.028). Whole transcriptome sequencing followed by pathway analysis indicated that STXBP4 is involved in functional gene networks that regulate cell growth, proliferation, cell death, and survival in cancer. Platelet-derived growth factor receptor alpha (PDGFRα) was a key downstream mediator of STXBP4 function. In line with this, shRNA mediated STXBP4 and PDGFRα knockdown suppressed tumor growth in soft-agar and xenograft assays.

Conclusions: STXBP4 plays a crucial role in driving SCC growth and is an independent prognostic factor for predicting worse outcome in lung SCC. These data suggest that STXBP4 is a relevant therapeutic target for patients with lung SCC.

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Introduction

Non–small cell lung cancer (NSCLC) accounts for approximately 85% of all cases of lung cancer and is mainly subclassified into adenocarcinoma (AC) and squamous cell carcinoma (SCC; ref. 1). Current treatment strategies for NSCLC include chemotherapy, depending on the histological tumor type, and targeted agents for patients whose tumors carry a specific targetable genomic alteration. Although there have been significant advances in the treatment of lung SCC, further improvements in prognosis are dependent upon the identification of SCC-specific molecules or genomic alterations that can be used as therapeutic biomarkers and/or targets (2).

Several immunohistochemical markers have been investigated for their utility in distinguishing lung SCC from lung AC, including TTF-1, napsin A, and CK5/6, and the ΔN isoform of p63 (ΔNp63; refs. 3–5). The latter is a highly specific marker for lung SCC, and genomic regions containing the TP63 gene are frequently amplified in a variety of SCCs, including lung, head, and neck, bladder, and cervical cancers (4, 6–8). Although these findings suggest that ΔNp63 is a lung SCC oncogene, the pathologic relevance of p63 in tumorgenesis remains unclear (9, 10).

Alternative splicing of the TP63 gene generates transcripts encoding two opposing classes of proteins: one containing the transactivation domain (TAp63) and the other lacking the domain (ΔNp63; refs. 11–13). Early studies showed that ΔNp63 acts as a dominant-negative transcriptional repressor to inhibit p53- or TAp63-mediated transcription in vitro and in vivo, consistent with a potential oncogenic role for the ΔNp63 isoform (12, 14). However, the ΔNp63 isoform also has transcriptional activity that is independent of the second transactivation domain (15). ΔNp63 is regulated in a coordinated manner by two scaffold proteins, syntaxin binding protein 4 (STXBP4) and receptor of...
activated kinase C1 (RACK1; encoded by the GNB2L1), which bind to ΔNp63 (16, 17). STXB4, originally identified as a glucose transporter, is localized on human chromosome 17q22 and plays a role in the translocation of transport vesicles from the cytoplasm to the plasma membrane (18, 19). While ΔNp63 plays a role in maintaining the viability and proliferative capacity of basal epithelial cells, STXB4 is a positive regulator of ΔNp63 stability and is also crucial for keratinocyte proliferation (16, 20).

In this report, we focused on STXB4 and its oncogenic function in lung SCC, with particular emphasis on the interactions between STXB4 and p63. We also addressed the relevance of STXB4 expression to patient prognosis. Initially, we assessed the expression of STXB4 and ΔNp63 in SCC tumors by immunohistochemistry and found that positive STXB4 expression signified worse overall survival (OS) and progression-free survival (PFS). We further performed a genome-wide transcriptome analysis (RNA-seq) using next-generation sequencing (NGS) and found that platelet-derived growth factor receptor α (PDGFRα) is a key downstream mediator of STXB4 function. The data suggest that STXB4 is a new diagnostic marker in lung SCC and STXB4 might be a relevant therapeutic target for the treatment of patients with this disease.

**Translational Relevance**

ΔNp63 is a diagnostic marker highly specific for lung squamous cell carcinoma (SCC), but the regulation of p63 protein stability and the pathologic relevance of p63 in tumorigenesis remains uncertain. We report here for the first time that Syntaxin Binding Protein 4 (STXB4) expression increases the oncogenic potential of ΔNp63, and STXB4 is an independent negative prognostic marker for predicting poor outcome in lung SCC. Whole transcriptome analysis (RNA-seq) using next-generation sequencing (NGS) and immunohistochemistry and found that positive STXB4 expression signified worse overall survival (OS) and progression-free survival (PFS). The main eligibility criteria were as follows: age 20 to 85 years; performance status based on Eastern Cooperative Oncology Group ≤ 2; estimated life expectancy ≥ 3 months; adequate hepatic, cardiac, renal, and bone marrow functions. The study was conducted in compliance with the Declaration of Helsinki and was approved by the Institutional Review Board of the participating hospitals and institutions. All patients provided written informed consent before registration. Tumor samples were stored at −80°C until use.

**Immunohistochemistry**

Immunohistochemical analysis was performed on formalin-fixed and paraffin-embedded SCC sections. The sections were deparaffinized, blocked in PBS containing 5% FBS for 1 hour, and incubated overnight with diluted primary antibodies at 4°C in a humidified chamber. Staining reactions were developed using Vectorstain universal ABC Kit (Vector Laboratories) and then DAB Kit (Vector Laboratories) for immunohistochemistry. Meyer’s hematoxylin (HIC World) was used as a nuclear counterstain. STXB4, p63, and ΔNp63 levels were assessed by immunohistochemical staining and scored using a semiquantitative method: 1 ≤ 10%, 2 = 10%–25%, 3 = 25%–50%, 4 = 51%–75% and 5 ≥ 75% of positive cells. The tumors in which the stained cancer cells were scored as 3, 4, or 5 were defined as STXB4-positive; 1 and 2 were defined as STXB4-negative.

We used antibodies specific for p63 (4A4; Santa Cruz Biotechnology) and STXB4 (Abcam). Rabbit polyclonal ΔNp63 antibody was previously described (16). CD147 (Santa Cruz Biotechnology) and mTOR (Cell Signaling Technology) immunohistochemistry was performed according to the procedures described in a previous report (22). The following diluted antibodies were used: p63 (1:100 dilution), ΔNp63 (1:100 dilution), STXB4 (1:100 dilution), CD147 (1:100 dilution), and mTOR (1:80 dilution). Highly cellular areas of the sections were evaluated for Ki-67 expression. All epithelial cells with nuclear staining of any intensity were defined as high expression. Approximately 1,000 nuclei were counted on each slide. Proliferative activity was assessed as the percentage of Ki-67-stained nuclei (Ki-67 labeling index) in the sample. The median value of the Ki-67 labeling index was evaluated, and tumor cells with greater than the median value were defined as high expressors. The sections were assessed using light microscopy in a blind fashion by at least two of the authors.

**Plasmids and antisense oligonucleotides**

Human cDNAs encoding FLAG-tagged or HA-tagged ΔNp63α, STXB4, and PDGFRα were cloned into the LPCX retroviral expression vector (Takara Bio). The sequences of the above constructs were verified using DNA sequencing. For siRNA experiments, 19 nucleotide siRNA duplexes with 3′dTdT overhangs were synthesized by Dharmacon (GE Dharmacon). The siRNA oligonucleotide sequences for Luciferase control (LUC), ΔNp63, STXB4, and PDGFRα are described in the Supplementary Information.

**Materials and Methods**

**Cell culture**

The human lung SCC cell lines, RERF-LC-Sq1 and EBC-1, were obtained from the Japanese Collection of Research Bioresources (JCRB). The cell lines were last authenticated by short tandem repeat (STR) analysis on December 22, 2015 (RERF-LC-Sq1), or on June 10, 2016 (EBC-1). RERF-LC-Sq1 cells were cultured in RPMI1640 with 10% fetal bovine serum (FBS), and EBC-1 cells were cultured in Eagle’s minimal essential medium (EMEM) with 10% FBS at 37°C in a 5% CO2 incubator.

**Patients**

Human tissue specimens were surgically resected from 87 lung SCC patients at Gunma University Hospital and its affiliated hospitals between August 2003 and December 2010 (21). The main eligibility criteria were as follows: age 20 to 85 years; performance status based on Eastern Cooperative Oncology Group ≤ 2; estimated life expectancy ≥ 3 months; adequate hepatic, cardiac, renal, and bone marrow functions. The study was conducted in compliance with the Declaration of Helsinki and was approved by the Institutional Review Board of the participating hospitals and institutions. All patients provided written informed consent before registration. Tumor samples were stored at −80°C until use.
sequences of the shRNA oligonucleotides are described in Supplementary Information.

Immunoblotting analysis
Immunoblotting analysis was performed as previously described (23). In short, cells were solubilized with lysis buffer (20 mmol/L sodium phosphate [pH 7.0], 125 mmol/L NaCl, 30 mmol/L sodium pyrophosphate, 0.1% NP-40, 5 mmol/L EDTA, 10 mmol/L sodium fluoride, 0.1 mmol/L NaF, 1 mmol/L phenylmethylsulfonyl fluoride) supplemented with Complete protease inhibitor cocktail (Roche), and homogenized by passage through a 20G needle. The eluates were then concentrated and separated by SDS-PAGE. Transfer to nitrocellulose membranes and screening using rabbit polyclonal antibodies for ΔNp63 and STXBP4 were carried out as previously described (24). We used antibodies specific for p63 (4A4), ΔNp63α, STXBP4, Phospho-PDGFβR(Thr849; Cell Signaling Technology), PDGFRα (Abcam), phospho-p38MAPK (Thr180/Tyr182; Cell Signaling Technology), p38MAPK (Cell Signaling Technology), and β-Actin (Sigma-Aldrich).

Genome-wide transcriptome analysis (RNA-seq) and real-time RT-PCR
Total RNA was prepared from surgically resected samples using a RNeasy Mini kit (Qiagen). RNA quality was assessed using an Agilent Bioanalyzer (Agilent Technologies). High-quality RNA (RNA integrity numbers > 7.0) from six STXBP4-positive and six STXBP4-negative samples were used for genome-wide transcriptome analysis (RNA-seq experiments). mRNAs were captured using a Dynabeads mRNA DIRECT Micro Purification Kit (Thermo Fisher Scientific). The mRNA was then used to generate sequencing libraries of barcoded fragments using the Ion Total RNA-Seq Kit v2 (Thermo Fisher Scientific) following the manufacturer’s instructions. Libraries were sequenced on an Ion Proton System using four libraries per Ion PI Chip v2, Ion PI Template OT2 200 kit v3 and Ion PI Sequencing 200 kit v3 (Thermo Fisher Scientific). BAM files generated by the Ion Proton System were converted to FASTQ files using bam2fastq software (v1.1.0, https://github.com/qbiomics/bam2fastq), and reads shorter than 21 nucleotides were removed. Quantification of each gene was undertaken as previously described (25). Briefly, the reads were aligned to the UCSC reference human genome 19 (hg19) using a combination of TopHat2 (v2.0.11, http://ccb.jhu.edu/software/tophat/index.shtml), and the Bowtie2 (2.2.0.0, http://bowtie-bio.sourceforge.net/index.shtml) pipelines. The read counts were obtained using Partek Genomics Suite software (http://www.partek.com/). Differentially expressed genes were detected using edgeR software (26) and genes with a false discovery rate (FDR) < 0.50 (P < 0.01) were analyzed by Ingenuity Pathway Analysis (Qiagen).

Final analyses were performed using a Universal Probe Library set (Roche) with KAPA Master mix (KAPA Biosystems) on a StepOne real-time PCR system (Thermo Fisher Scientific). The Universal Probe Library Human ACTB Gene Assay (Roche) was used for an endogenous normalization control. Sequence detection software was utilized for data analysis, and relative fold induction was determined by the comparative threshold cycle method using standard curves, which were generated by plotting the observed Ct values against the standard dilutions of a positive control sample. In all experiments, the average of three independent reactions is shown with error bars indicating standard deviation. Gene expression data were downloaded from the Gene Expression Omnibus database (GSE84339).

Subcutaneous xenografts
A total of 5 × 10^6 lentivirally transduced or retrovirally expressed cells were injected subcutaneously into nude mice (BALB/c-nu, nu, CLEA Japan), and tumor size was measured after 20 days (RERF-LC-Sq1) or 14 days (EBC-1). All animal procedures were performed with the approval of the Animal Ethics Committee of Gunma University.

Anchorage-independent growth
RERF-LC-Sq1 cells were transduced with lentiviruses carrying shRNAs for Luciferase (LUC), ΔNp63, STXBP4, or PDGFRα. For soft-agar assays, the cells were grown in triplicate for 12 days, after which anchorage-independent growth was quantified with a CytoSelect-96 kit (Cell Biolabs).

Statistical analysis
Probability values (P value) < 0.05 indicated a statistically significant difference. The Fisher exact test was used to examine the association of two categorical variables. The correlation between different variables was analyzed using the nonparametric Spearman rank test. Follow-up for the 87 patients was conducted using the patient medical records. The Kaplan–Meier method was used to estimate survival as a function of time, and survival differences were analyzed by the log-rank test. The day of surgery was defined as the starting day for measuring postoperative survival. OS was determined as the time from tumor resection to death from any cause. PFS was defined as the time between tumor resection and first disease progression or death. Multivariate analyses were performed using a stepwise Cox proportional hazards model to identify independent prognostic factors. Statistical analysis was performed using JMP 8 (SAS) software.

Results
Survival outcomes according to STXBP4 and p63 expression
The clinicopathologic features of the 87 patients included in this study are shown in Table 1. The median age of the patients was 72 (range, 56–84), the majority of patients were male (92.0%) and former or current smokers (98.9%). All patients received radical surgery with evidence of pathologic stage IA/B in 54.0%, stage IIA/B in 26.4%, and stage IIIA in 18.4% of patients. Pleural involvement, lymphatic permeation, and venous invasion were observed in 41 patients (47.1%), 47 patients (54.0%), and 40 patients (46.0%), respectively.

Frequently, lung SCCs exhibit simultaneous upregulation of both TAp63 and ΔNp63, and ΔNp63 in particular, is a putative diagnostic marker for pulmonary SCC (10). To address the clinical significance of STXBP4 expression, we investigated whether high expression of this gene correlates with ΔNp63 status. We found that 59.8% (52/87) of all patients were STXBP4-positive, and STXBP4 expression was detected in those tumors that showed an accumulation of p63 (Fig. 1A).

Statistical correlation analysis between STXBP4 expression and clinicopathologic features revealed that pathologic local tumor factor stage (disease stage), pathologic tumor–node–metastasis
We observed significantly higher levels of STXBP4 expression in those tumors that showed an accumulation of ∆Np63 (Spearman $\rho = 0.219; P < 0.05$), while among all p63 isoforms, no significant correlations were observed ($P > 0.5$; Fig. 1D). Interestingly, mTOR, a major controller of growth and is often deregulated in cancer (27), was significantly correlated with STXBP4 positivity (Spearman $\rho = 0.220; P < 0.05$), while other tumor markers, including CD147, a member of the immunoglobulin superfamily involved in angiogenesis ($P < 0.5$), and Ki-67, a general marker for cell division ($P < 0.1$), were not significantly correlated (Fig. 1D).

Transcriptional profiling and functional screening to identify possible downstream mediators of STXBP4

Hierarchical cluster analysis after alignment of a total of 15,346 genes to the reference sequence showed that STXBP4-positive and STXBP4-negative tumors had distinctly different gene expression profiles (Fig. 2A). Among the differentially expressed genes ($P < 0.05$, FDR $< 0.5$), we identified a total of 172 genes that were either significantly upregulated (79 genes) or downregulated (93 genes) in STXBP4-positive samples. These candidate genes potentially represent a network involved in STXBP4-mediated biology (Fig. 2B). To address this possibility in more detail, we carried out Ingenuity Pathway Analysis (IPA), which revealed that more than 30% of the affected genes were classified in the functional class of "cell death and survival." This finding supported our experimental observations that STXBP4 could be linked to the poor prognosis of lung SCC (Fig. 2C).

Additionally, other significant functional classes identified by IPA, including "cellular movement" and "cell to cell signaling and interaction."
interaction,” may be relevant to the correlation of STXBP4 positivity with local tumor progression related to local tumor size (T factor) and disease stage (Fig. 2C). The canonical pathway analysis characterized two signaling pathways as the functional relationship of STXBP4 positivity. “Cellular movement” and “cell morphology” have been predicted as the most significantly activated canonical pathways (Supplementary Fig. S2).

IPA revealed that STXBP4 positivity was also correlated with the expression of growth factor receptors and components of downstream pathways. Among these genes listed in descending order of normalized expression, PDGFRA was a significant upregulated gene (FDR < 0.1; Fig. 2D and E), and a most relevant candidate for addressing the growth of STXBP4-positive lung SCC cells (Supplementary Fig. S3). PDGFRα is a receptor tyrosine kinase that is a critical regulator of growth and proliferation of certain cell types during embryonal development (28, 29). In subsequent experiments described later in this study, PDGFRα proved to be a key mediator of STXBP4 oncogenic activity.

STXBP4 regulates PDGF–PDGFR signaling in lung SCC
PDGF and PDGFR isoforms have important functions in the regulation of growth and survival of certain cell types (28, 29), and upregulation of PDGF-PDGFR signaling drives tumor cell growth. Indeed, the oncogenic properties of mutated or amplified PDGFRα have been studied in several tumor types, and PDGFRβ has been linked to not only tumor angiogenesis via paracrine effects but also cancer metastasis (30, 31).

We measured mRNA expression levels by real-time RT-PCR in a total of 52 available samples from lung SCC patients for which high-quality RNA was available (RIN > 2.0). The mRNA levels of STXBP4 were also correlated with ΔNp63 mRNA levels in those 52 samples (Supplementary Fig. S4A). Interestingly, we observed that PDGFRα expression was significantly upregulated in STXBP4-positive lung SCC samples compared with STXBP4-negative samples (P < 0.05; Fig. 3A and B). On the other hand, PDGFRβ, VEGFR1 (FLT1), VEGFR2 (KDR), and VEGFR3 (FLT4) were consistently, but not significantly, upregulated in STXBP4-positive lung SCC samples. STXBP4-
positive samples defined by immunohistochemistry also had high STXB4 mRNA expression levels compared with STXB4-negative samples, and, interestingly, PDGFRα mRNA levels were also significantly correlated with STXB4 mRNA levels (Fig. 3C). Additionally, analyses of The Cancer Genome Atlas (TCGA) datasets of lung SCC (n = 488) supported, in part, our finding that PDGFRα mRNA levels are significantly correlated with STXB4 mRNA levels (P = 0.015; Supplementary Fig. S4B).

To confirm the enhanced expression levels of PDGFRα in STXB4-high expressing tumors, the lung SCC cell line EBC-1 was transduced with STXB4 and ΔNp63α retroviruses. As shown in Fig. 3D and E, the STXB4 transduced stable clones showed high induction levels of both PDGFRα mRNA level (Fig. 3D) and PDGFRα protein levels (Fig. 3E), consistent with our findings in 52 resected patient lung SCC samples (Fig. 3B and C). Correspondingly, high expressing STXB4 cells had elevated ΔNp63 protein levels, but not mRNA levels. Additionally, the relative increase in PDGFRα mRNA expression is more marked in STXB4 low-expressing EBC-1 cells compared with STXB4 high-expressing RERF-LC-Sq1 cells (Supplementary Fig. S5A and S5B). The results indicate that STXB4 regulates PDGFRα expression in lung SCC, most likely in a ΔNp63-dependent manner, and also suggest that STXB4 may serve as a new SCC biomarker.

STXB4 depletion represses lung SCC tumor growth in vivo

We next examined the oncogenic role of STXB4 in regulating the expression of PDGFRα in a lung SCC cell line using a loss-of-function approach. Two independent siRNAs against STXB4 (STXB4#1 and STXB4#2) were transfected into the lung SCC cell line RERF-LC-Sq1, and a siRNA targeting luciferase (siLUC) was used as a control. The STXB4 knockdown cells showed low expression of STXB4, which correlated with downregulation of both PDGFRA mRNA (Fig. 4A) and PDGFRα protein levels (Fig. 4B), consistent with our findings in resected patient lung SCC samples.

In order to evaluate the functional relevance of STXB4 and PDGFRα expression during tumor formation, we monitored the colony formation of RERF-LC-Sq1 cells lentivirally transduced with STXB4, ΔNp63, or PDGFRα shRNAs. As shown in Fig. 4C, PDGFRα, STXB4, or ΔNp63 knockdown in RERF-LC-Sq1 cells led to decreased anchorage-independent colony formation in soft agar. Subcutaneous transplantation of PDGFRα or STXB4 knockdown clones into immunodeficient mice resulted in suppressed tumor formation compared with control luciferase shRNA xenografts (Fig. 4D). Additionally, the knockdown effect of STXB4 and suppression of tumorigenesis are more marked in high STXB4-expressing RERF-LC-Sq1 cells compared with low STXB4-expressing EBC-1 cells (Fig. 4 and Supplementary Fig. S6). These data

**Table 2. Univariate and multivariate survival analysis in all patients**

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Figure 2.
Gene expression profiling of clinical samples from lung SCC patients. A, A cluster diagram of RNA-seq data from six pairs of STXBP4-positive and STXBP4-negative samples. The color bars represent relative expression levels: red indicates higher than average expression and blue indicates lower than average expression. B, 79 significantly differentially expressed upregulated genes and 93 downregulated genes (\(P < 0.05\) and FDR < 0.5) were identified. C, Functional analysis of differentially expressed genes was performed by Ingenuity Pathway Analysis. D, The genes are listed in descending order of normalized expression.* FDR < 0.1. E, A cluster diagram of PDGFR and VEGFR expression from RNA-seq analysis. The color bars represent relative expression levels: red indicates higher than average expression and blue indicates lower than average expression.
suggest that downregulation of STXBP4 decreases PDGFRα expression and suppresses tumor formation.

Overall, our results indicate that STXBP4 has oncogenic activity both in vitro and in vivo and further suggest that STXBP4 could be a critical driver of tumor propagation through regulating the PDGFRα pathway.

Discussion

We demonstrated that STXBP4 expression in clinical specimens was closely associated with T factor \( (P < 0.001) \), disease stage \( (P = 0.030) \), and pleural involvement \( (P = 0.028) \). Furthermore, univariate and multivariate analysis indicated that STXBP4 expression was an independent prognostic factor for OS and PFS. While p63 is essential for normal epidermal stratification and the proliferative potential of epithelial stem cells, \( \Delta \text{Np63} \) is thought to maintain the proliferative potential of basal regenerative cells, including stem cells, in skin, thymus, breast, prostate, and urothelial stratified epithelium \( (20, 32–36) \). STXBP4 can physically interact with \( \Delta \text{Np63} \) and is indispensable for stabilizing \( \Delta \text{Np63} \), which is consistent with a putative diagnostic role for STXBP4 in lung SCC.

Polymorphisms of STXBP4/COX11 \( (\text{rs6504950}; \text{AA}/\text{AG}-\text{genotype}) \) were associated with a significantly decreased risk of
The pathologic function of STXBP4 in human cancers remains unclear. However, STXBP4 can physically interact with p63 and is indispensable for stabilizing Np63 even in normal conditions (16). Consistent with STXBP4 localization in both the nucleus and cytoplasm, it has been suggested that nuclear STXBP4 has p63-mediated functions, and that cytoplasmic STXBP4 could facilitate other functions in a p63-independent manner (16). In fact, our data indicated that STXBP4 induction partially increased tumor growth even in the absence of elevated Np63 (Supplementary Fig. S7A), and that PDGFRα induction also partially increased tumor growth even in STXBP4 knockdown cells (Supplementary Fig. S7B). Thus, STXBP4 may contribute to the susceptibility and severity of cancer in a p63-dependent and -independent manner. Indeed, amplification and overexpression of p63 has frequently been observed in a variety of SCCs, including lung cancers and head and neck cancers (8, 38). However, p63 expression is decreased during progression to invasion and metastasis of lung, breast, and bladder cancers, and loss of p63 expression is associated with worse prognosis in some cases (35, 39, 40). It could be the balance between the TA isotype (tumor suppressive) and AN isotype (oncogenic), as well as the tissue context, which is critical for proliferation and differentiation in both epithelial stem cells and cancer stem cells.

Global transcriptome profiling using next-generation sequencing technologies has become more common for comprehensive gene expression analysis to explore novel regulators and target genes in different types of cancers. In this report, genome-wide transcriptome analysis identified mediators of STXBP4 activity, including PDGFRα, which contributes to cell growth and metastasis in a p63-dependent manner. PDGFR family proteins consist of several disulphide-bonded, dimeric isoforms (PDGF AA, PDGF AB, PDGF BB, PDGF CC, and PDGF DD) that bind in a specific pattern to two related receptor tyrosine kinases, PDGFRα and PDGFRβ.
actin reorganization, migration, and differentiation (43, 44). PDGFRα expression is observed mainly in stromal cells, but also in the sarcomatoid component of NSCLC (45). Based on recent evidence, inhibition of the p53/NF-Y complex by mutant gain-of-function p53 enhances PDGFRα expression and promotes metastasis in a subset of pancreatic cancers (31). In addition, the interaction of mutant p53 with p63 regulates the expression of p63 target genes to enhance invasion and metastasis (46). Hence, the oncogenic activity of mutant p53 is a consequence of the physical association between mutant p53 and the p53 family members p63 and p73.

Treatment strategies for lung cancer are based on the assumption that an individual patient's cancer is purely of one subtype. Because many cancers are heterogeneous and relatively resistant to chemotherapy or radiation, there is strong interest in molecular-targeted therapies based on tumor biology. In particular, targeted agents that inhibit the epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) are approved for the treatment of NSCLC harboring genetic alterations in the genes encoding these proteins (47). EGFR inhibitors, such as erlotinib and gefitinib, are only effective against NSCLCs with EGFR mutations, which occur almost exclusively in lung AC. Similarly, the recently identified EMIL4–ALK rearrangement, which predicts susceptibility to the targeted agent crizotinib, also occurs only in lung AC. Unfortunately, therapeutic advances in the treatment of lung SCC have lagged behind those for AC (48). Therefore, the capacity to distinguish between lung AC and SCC is particularly important for the effective use of novel targeted therapies to treat patients with these NSCLC subtypes.

Inhibition of the PDGFRα signaling pathway by treatment with a neutralizing PDGFRα antibody, MEDI-575, had minimal effect on tumor cell proliferation in preclinical models of NSCLC (49). Lung SCC histology also identified patients at a higher risk of bleeding during treatment with bevacizumab, a monoclonal anti-VEGF antibody (50). Thus, more studies are required to determine whether specific inhibition of PDGFR receptors, without inhibition of VEGF receptors, is of any benefit for lung cancer patients. These issues highlight the growing importance of accurate identification of NSCLC subtypes for assigning patients to appropriate histology-based therapies and the triage of tissue for appropriate molecular studies.

Disclosure of Potential Conflicts of Interest
M. Nishiyama receives commercial research support from Yakult Honsha Co. Ltd. No potential conflicts of interest were disclosed by the other authors.

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