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# Mutations in *TP53* increase the risk of *SOX2* copy number alterations and silencing of *TP53* reduces *SOX2* expression in non-small cell lung cancer

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## Abstract

**Background:** Amplifications of the transcription factor, SRY (sex determining region Y)-box 2 (*SOX2*), are common in non-small cell lung cancer (NSCLC). *SOX2* signaling is important in maintaining the stem cell-like phenotype of cancer cells and contributes to the pathogenesis of lung cancer. *TP53* is known to inhibit gene amplifications and to repress many stem cell-associated genes following DNA damage. The aim of this study was to investigate if *TP53* mutational status affected *SOX2* copy number variation and gene expression in early-stage NSCLC patients; moreover, to assess if *TP53* regulates *SOX2* expression in human lung cancer cells.

**Methods:** 258 early-stage lung cancer patients were included in the study. Exons 4–9 in the *TP53* gene were sequenced for mutations in tumor tissues. *SOX2* copy number as well as *TP53* and *SOX2* gene expression were analyzed in tumor and in adjacent non-tumorous tissues by qPCR. *TP53* and *SOX2* were silenced using gene-specific siRNAs in human lung adenocarcinoma A427 cells, and the expression of *TP53*, *SOX2* and subset of selected miRNAs was analyzed by qPCR. The odds ratios (ORs) for associations between copy number variation and lung cancer were estimated by conditional logistic regression, and the correlation between gene status and clinicopathological characteristics was assessed by Chi-square or Fisher's exact test. Gene expression data was analyzed using nonparametric Mann–Whitney test.

**Results:** *TP53* mutations were associated with an increased risk of acquiring a *SOX2* copy number alteration (OR = 2.08, 95 % CI: 1.14–3.79,  $p = 0.017$ ), which was more frequently occurring in tumor tissues (34 %) than in adjacent non-tumorous tissues (3 %). Moreover, *SOX2* and *TP53* expression levels were strongly correlated in tumor tissues. *In vitro* studies showed that a reduction in *TP53* was associated with decreased *SOX2* expression in A427 cells. Furthermore, *TP53* knockdown reduced the miRNA hsa-miR-145, which has previously been shown to regulate *SOX2* expression.

**Conclusions:** *TP53* signaling may be important in the regulation of *SOX2* copy number and expression in NSCLC tumors, and the miRNA hsa-miR-145-5p may be one potential driver. This prompts for further studies on the mechanisms behind the *TP53*-induced regulation of *SOX2* expression and the possible importance of hsa-miR-145 in lung cancer.

**Keywords:** SRY (sex determining region Y)-box 2, NSCLC, *TP53* mutation, Copy number alteration, Hsa-miR-14

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## Background

Lung cancer is the most frequent cause of cancer-related mortality worldwide, leading to an estimated 1.4 million deaths in 2010 [1]. Smoking, occupational and environmental exposures to chemicals are major causes of lung cancer. Lung cancer constitutes a heterogeneous disease in regard to clinical presentation, pathological features and biological behavior. The majority of cases are non-small cell lung carcinomas (NSCLC), which comprises adenocarcinoma, squamous cell carcinoma and large cell carcinoma. The genomic alterations occurring in lung carcinomas are very complex [2–5]. However, alterations in the *TP53* gene are among the most significant genetic events in lung cancers [6], often occurring as a response to DNA damage caused by exposure to a variety of genotoxic agents such as polycyclic aromatic hydrocarbons (PAHs) [7]. Mutations in the *TP53* gene increase the risk for chromosomal rearrangements, such as copy number alterations, which are involved in the development and progression of many human malignancies including lung cancer [8]. Amplifications or deletions in the fragile sites harboring important transcription factors may further advance the process of carcinogenesis [9].

The transcription factor SRY (sex determining region Y)-box 2, encoded by the *SOX2* gene located at the 3q25-27 region, is often altered in NSCLC [10, 11]. *SOX2* has a crucial role in maintaining the stem cell-like phenotype in cancer cells [12, 13] and contributes to the pathogenesis of lung cancer by controlling cell proliferation and malignant transformation [11]. In lung cancer, *SOX2* gene amplification and consequent increased expression occur most frequently in squamous cell carcinoma [14, 15] and to a lesser extent in adenocarcinoma [14, 16]. Interestingly, *TP53* has been reported to regulate *SOX2* expression in embryonic stem cells [17] and recently also in the H1299 lung carcinoma cell line [18]. miRNAs are important mediators of *TP53*-signaling and in embryonic stem cells *TP53* represses *SOX2* expression through the activation of hsa-miR-145 [19–21]. This is of interest as a recent study showed that low levels of hsa-miR-145 are associated with unfavorable prognosis in NSCLC [22].

Given the crucial role of *TP53* and *SOX2* in lung cancer and their known association in stem cell development, we hypothesize that *TP53* may have a regulatory effect on *SOX2* in lung cancer. Thus, effects of *TP53* mutations on *SOX2* copy number alterations were studied in lung cancer tumors and correlation between the gene expression levels investigated. Furthermore, effects of *TP53* silencing on *SOX2* mRNA levels were evaluated and the possibility of miRNAs as downstream regulators was assessed.

## Methods

### Cases

Early-stage lung cancer patients ( $n = 258$ ) were Caucasians of Norwegian origin admitted to Haukeland University Hospital in Bergen between 1988 and 1994, for primary surgery. The patients were enrolled in the study, whenever practically feasible, and informed written consent covering analysis of molecular and genetic markers was signed by the patients prior to surgery. The subjects included in this manuscript are a subgroup recruited into the project “lung cancer genetics” at our institute. The project has been approved by the Regional Committee for Medical and Health Research Ethics in South Norway in accordance with the WMA Declaration of Helsinki. The ethical approval covered access to the NSCLC databank. The characteristics of the patients included in the study are summarized in Table 1. Samples of adjacent non-tumorous lung tissue, confirmed by histology, were cut from the lobectomi specimens at the time of surgery. Tumor histology was confirmed by an experienced pathologist and samples containing  $\geq 80$  % of tumor cells were analyzed in the study. After resection tumor

**Table 1** Characteristics of patients ( $n = 258$ )

<i>Age</i>	
(Mean $\pm$ SD)	63.9 $\pm$ 10.1
(Median, min-max)	66 (25–82)
<i>Gender (male: female)</i>	
	183/74
<i>Smokers/Non-smokers</i>	
	222/22
<i>No cigarettes per day</i>	
(Mean $\pm$ SD)	15.3 $\pm$ 7.9
(Median, min-max)	14 (2–50)
<i>Total years of smoking</i>	
(Mean $\pm$ SD)	41.3 $\pm$ 11.6
(Median, min-max)	43 (2–69)
<i>Total pack-years*</i>	
(Mean $\pm$ SD)	31.6 $\pm$ 18.2
(Median, min-max)	28 (1–113)
<i>Histology</i>	
Adenocarcinoma	118 (45.7 %)
Squamous cell carcinoma	103 (39.9 %)
Large cell carcinoma	36 (13.9 %)
Others	1
<i>PAH-DNA adducts<sup>†</sup></i>	
(Mean $\pm$ SD)	12.3 $\pm$ 8.9
(Median, min-max)	10 (1–46)
<i>TP53 mutated/WT</i>	134/101

\*Pack-years indicates the number of cigarettes smoked per day x number of years smoked/20. <sup>†</sup>PAH-DNA adducts per  $10^8$  nucleotides

and non-tumorous tissues were snap-frozen in liquid nitrogen and kept at  $-80^{\circ}\text{C}$  until further processing.

#### Copy number analyses by quantitative PCR

DNA was extracted from frozen lung tissue samples using standard proteinase K digestion followed by phenol–chloroform extraction and ethanol precipitation. Control DNA was isolated from blood samples by Flexigene DNA kit (Qiagen, Hilden, Germany). *SOX2* copy number alterations were evaluated by quantitative real-time PCR (qPCR) using SYBR Green I technology on an ABI PRISM<sup>®</sup> 7900HT Fast PCR System (Applied Biosystems, Thermo Scientific, Waltham, MA, USA), as described elsewhere [23, 24]. The multicopy gene *FTH1* was used as reference gene. Primer sequences were: *SOX2* forward primer, 5'-GCTCTTGGCTCCATGGGTTC-3', reverse primer, 5'-GCTGATCATGTCCCGGAGGT-3'; *FTH1* forward primer, 5'-GATGATGTGGCTTTGAAGAACTTTGCCA-3', reverse primer, 5'-CACCTCGTTGGTTCTGCAGCTTCATCA-3'. Primer specificity was determined by melting point analysis. qPCR was performed using 20 ng template DNA in a total volume of 10  $\mu\text{L}$  containing PerfeCTa SYBR Green FastMix, ROX (QuantaBioSciences, Gaithersburg, MD, USA). Cycling conditions were:  $95^{\circ}\text{C}$ , 2 min followed by 40 cycles of  $95^{\circ}\text{C}$ , 10 sec and  $60^{\circ}\text{C}$ , 45 sec. The PCR was run in duplicates using a relative standard curve approach. The standard curve was generated by performing serial dilutions of plasmid DNA containing one copy of the area of interest for each of the assayed genes. pUC57 plasmid DNA (GenScript, Piscataway, NJ, USA) was added to each standard to maintain a constant amount of total DNA per reaction tube. Only  $R^2$  values above 0.99 were accepted and data was quality controlled in accordance with previous recommendations [23]. For confirmation, analysis of  $>5\%$  of all samples was repeated. Control DNA isolated from cancer-free individuals was used to normalize for plate variability. Copy numbers below 1.5 and above 2.5 were defined as deleted and amplified, respectively.

#### Analysis of frequency of TP53 mutations and PAH-DNA adduct levels

To assess the frequency of *TP53* mutations in tumor tissues DNA was screened either by single-strand conformational polymorphism or denaturing capillary electrophoresis as previously described, covering exons 4 to 9 of the *TP53* gene, and DNA samples with alterations were sequenced [25, 26]. PAH-DNA adduct levels were determined in non-tumorous lung tissue by  $^{32}\text{P}$ -postlabelling as previously published [27]. The patients were divided into two groups on the basis of having levels greater than or less than the median (9.91) of DNA adducts per  $10^8$  nucleotides.

#### Cell culture and RNA knockdown

Lung adenocarcinoma A427 cells were maintained in RPMI-1640 medium (Thermo Scientific) with 10 % FCS (Thermo Scientific), and penicillin/streptomycin (Biowest SAS, Nuaille, France) in 5 %  $\text{CO}_2$  at  $37^{\circ}\text{C}$ . The cells were passaged every third day and passage numbers between 25 and 30 were used in all experiments. In RNA knockdown experiments 100000 cells/mL were seeded in 2 mL growth medium without penicillin/streptomycin in 6-well plates. For protein isolation 200000 cells/mL were seeded in 10 mL medium without penicillin/streptomycin in  $10\text{ cm}^2$  plates. siRNA targeting human *TP53*, *SOX2* and non-target (control) were purchased from Applied Biosystems (Thermo Scientific). Transfections were performed 24 h after seeding using 10 nM siRNA and Lipofectamin RNAiMAX reagent (Invitrogen, Thermo Scientific) according to manufacturer's instructions. The cells were harvested 48 h after transfection to analyze RNA, miRNA and protein expression.

#### Western blotting

Nuclear proteins were extracted from knockdown and control A427 cells using NE-PER Nuclear and Cytoplasmic extraction reagents (Thermo Scientific, Rockford, IL, USA). Protein concentrations were determined by BCA Protein Assay (Thermo Scientific) and 40  $\mu\text{g}$  lysate was separated by 10 % SDS-PAGE and transfer to a Immobilon PVDF membrane (Millipore, Bedford, MA, USA). To prevent non-specific background binding, the membranes were incubated with 5 % non-fat milk in Tris-buffered saline with 0.05 % Tween-20 (TBST) for 1 h at room temperature. Membranes were incubated with primary antibodies against *SOX2* and *TP53* (Cell Signaling technology Inc., Beverly, MA, USA) over night at  $4^{\circ}\text{C}$  and against *GAPDH* (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. After washing three times with TBST, the membranes were incubated with secondary anti-rabbit antibody (Cell Signaling) for 1 h at room temperature and proteins visualized by SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific). Semiquantitative densitometry analysis were performed using Image J.

#### Gene expression analysis

Total RNA was isolated from frozen lung tissue samples using standard Trizol extraction and total RNA from transfected cells was isolated using NucleoSpin miRNA (Macherey-Nagel, Duren, Germany). RNA was DNase treated with DNA-free kit (Ambion, Thermo Scientific) and its quality was assessed by 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). For gene expression analysis total RNA was reversed transcribed using qScript cDNA Supermix or qScript cDNA synthesis kit (Quanta BioSciences) for tissue samples and cells respectively.

Beta-Actin (*ACTB*) was used as reference gene. Primer sequences were: *SOX2* forward primer, 5'-GGGGAAAG-TAGTTTGCTGCC-3'; reverse primer, 5'-CGCCGCCGATGATTGTTATT-3'; *TP53* forward primer, 5'-CCATCCTCACCATCATCACA-3'; reverse primer, 5'-CACAAACACGCACCTCAAAG-3'; *ACTB* forward primer, 5'-GCGAGAAGATGACCCAGATCA-3'; reverse primer, 5'-GATAGCACAGCCTGGATAGCAA-3'. TargetScan 6.2 (<http://www.targetscan.org/>) was used to predict conserved miRNA binding sites in the *SOX2* gene. Of these five previously known targets of *TP53*; hsa-miR-145-5p, hsa-miR-145-3p, hsa-miR-200b-3p, hsa-miR-200c-3p, hsa-miR-429-1p; were selected for further analysis. Accordingly, total RNA was reversed transcribed using qScript microRNA cDNA Synthesis Kit (Quanta BioSciences) and expression was analyzed using commercial primers (Integrated DNA Technologies, Leuven, Belgium). *SNO* and *RNU6* were used as references. Expression was evaluated by qPCR using SYBR Green I technology on an ABI PRISM® 7900HT Fast Real-Time PCR System (Applied Biosystems, Thermo Scientific). Primer specificity was determined by melting point analysis.

#### Statistical analysis

Statistical analyses were carried out using IBM SPSS software version 22.0. Associations between copy number variation and lung cancer were estimated by odds ratios (ORs) and their 95 % confidence intervals (CIs) from conditional logistic regression adjusted for age, gender, total pack-years and tumor histology. The total number of patients included in the study was 258. However, for some patients, data on smoking status, gender or age was missing, and for five patients CNV data was not successfully obtained, thus giving a reduced number of patients included in the final statistical analysis. Effects of clinicopathological data on gene status were assessed by Chi-square or Fisher's exact test for categorical variables and by nonparametric tests for ordinal variables. Cancer free survival was obtained from 241 patients by Kaplan Meier analysis. Expression data was analyzed using nonparametric Mann–Whitney test or one-way ANOVA.  $p < 0.05$  was considered significant.

## Results

### *SOX2* copy number alterations are frequent in NSCLC tumors

Analyses of gene copy numbers were performed on DNA from paired non-tumorous and tumor tissue of the same patient in order to evaluate whether genomic variations in *SOX2* may be associated with lung tumor development. The *SOX2* copy number alterations were more frequent in tumors than in non-tumorous tissues of NSCLC patients (Fig. 1a). The odds for acquiring a

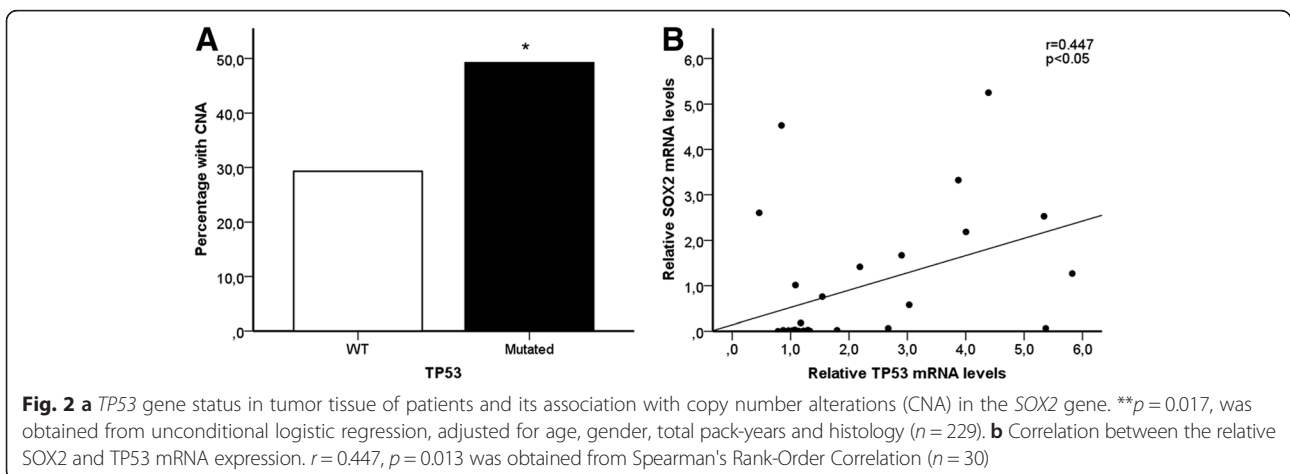
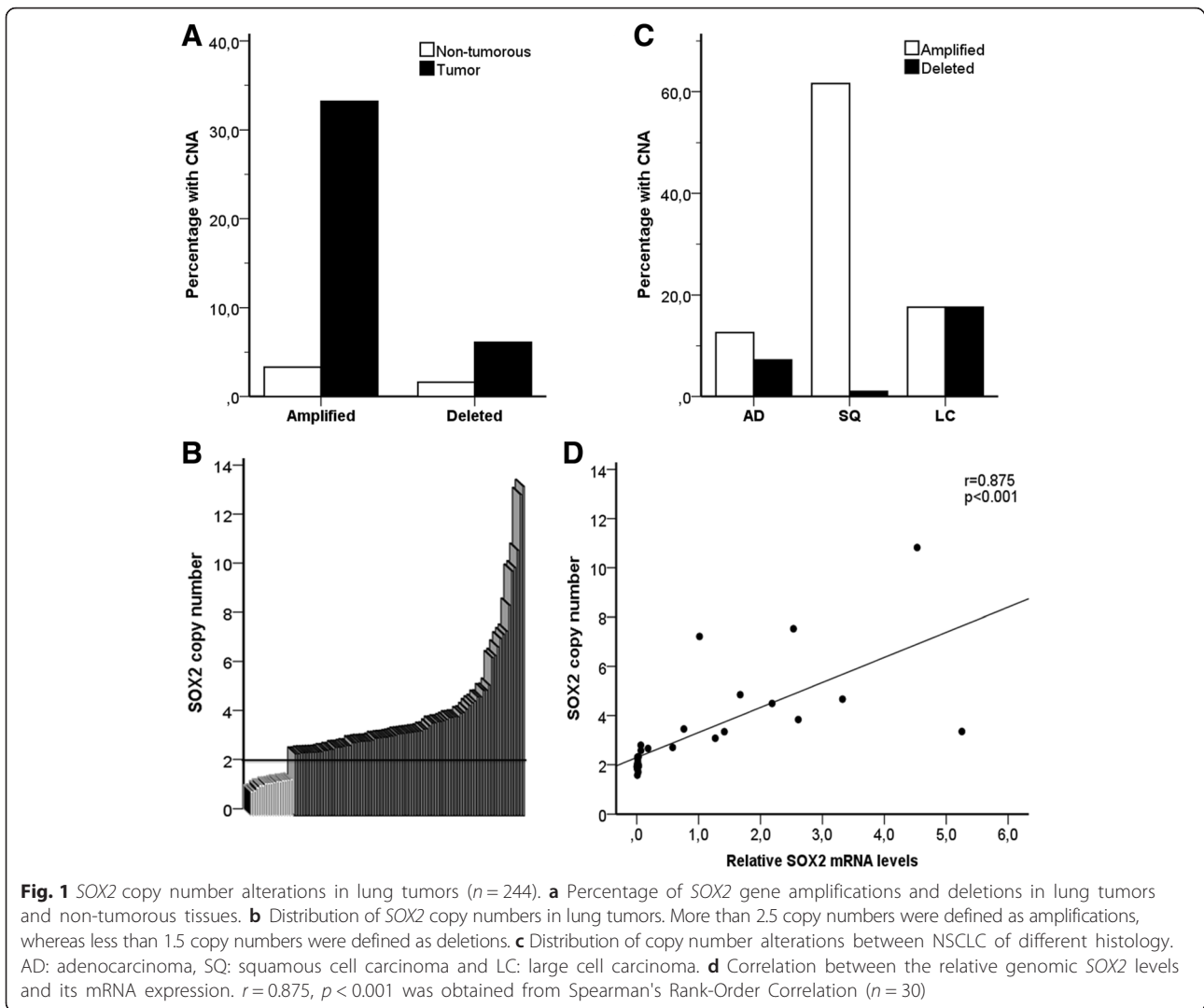
copy alteration in *SOX2* were 12.6-fold higher (95 % CI: 5.8–27.1) in tumors than in non-tumorous tissues. *SOX2* gene amplifications were observed in 33.6 % of tumors compared with 3.2 % in the adjacent non-tumorous tissues (OR: 15.7, 95 % CI: 6.3–39.1,  $p < 0.001$ ). A low occurrence of *SOX2* gene deletions was also observed in 6.1 % of tumors (OR: 6.0, 95 % CI: 1.6–23.0,  $p = 0.009$ ). Amplifications of >5 copies were observed in 15 of 81 tumors with a *SOX2* amplification (Fig. 1b), and *SOX2* copy number alterations were most frequent in squamous cell carcinoma (Fig. 1c). Moreover, changes in *SOX2* gene status were more frequent in smokers than non-smokers ( $p = 0.015$ , Additional file 1: Table S1). *SOX2* mRNA expression levels were investigated in 15 tumor and 15 non-tumorous tissue samples, and a strong correlation, ( $r = 0.875$ ,  $p = 0.001$ ), was observed between the *SOX2* copy numbers and the relative *SOX2* mRNA expression level (Fig. 1d). *SOX2* copy number alterations did not affect the survival of the patients (data not shown).

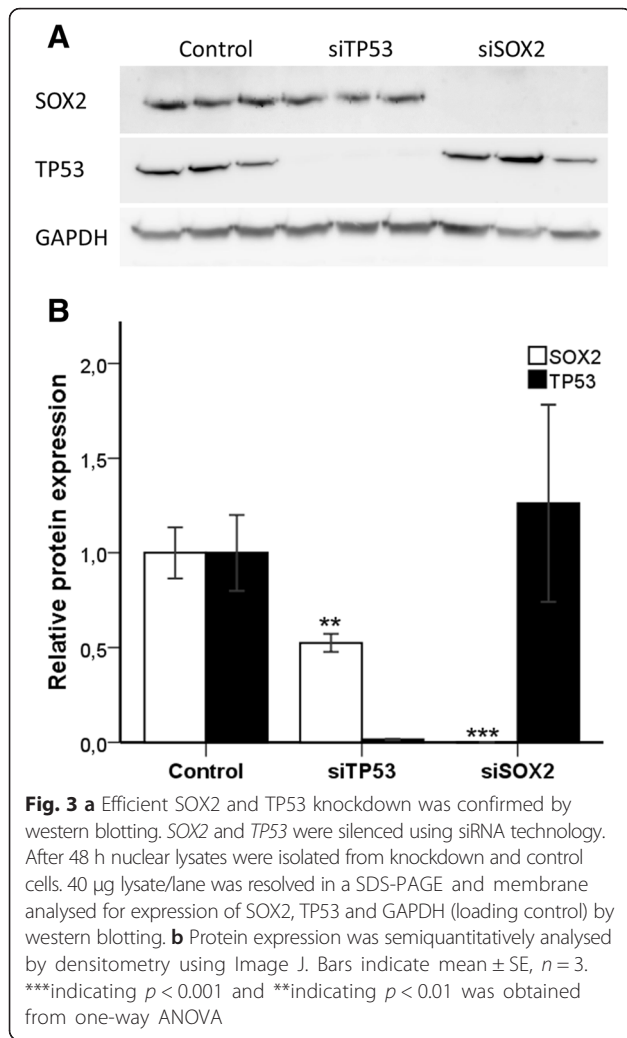
### Mutations in *TP53* gene increase frequency of *SOX2* copy number alterations

The effects of *TP53* mutations on *SOX2* copy number were investigated in 229 tumor tissues of the NSCLC patients. Of these, 56.8 % had at least one mutation in their lung tumors. Patients with mutated *TP53* had a significantly higher odds of acquiring a copy number variation in *SOX2* (OR = 2.08, 95 % CI: 1.14–3.79,  $p = 0.017$ ) than patients without *TP53* mutations (Fig. 2a). Furthermore, a positive correlation ( $r = 0.447$ ,  $p = 0.013$ ) was observed between *TP53* and *SOX2* mRNA expression levels in a subset of cancer patients ( $n = 30$ , Fig. 2b). *TP53* mutations are often occurring as a consequence of DNA-adduct formation following exposure to genotoxic carcinogens such as PAHs in cigarette smoke. However, increased PAH-adduct levels did not increase the occurrence of *SOX2* copy number alterations in our material (data not shown), despite a higher frequency of *SOX2* gene alterations in smokers compared to the non-smoking NSCLC patients.

### *TP53* affects *SOX2* expression and hsa-miRNA-145-5p levels in human lung cells in vitro

To investigate the effect of *TP53* on *SOX2* expression in NSCLC, the *TP53* gene was silenced in the lung adenocarcinoma A427 cell line using siRNA. Effective knockdown of *TP53* and *SOX2* proteins after 48 h was confirmed in three independent experiments by western blotting (Fig. 3a). In *TP53* knockdown cells a significant 40 % reduction in *SOX2* mRNA and protein expression was observed ( $p = 0.001$ , Fig. 4a and  $p = 0.01$ , Fig. 3b). *SOX2* knockdown did not affect the expression of *TP53* (Fig. 4b). Moreover, hsa-miR-145-5p expression

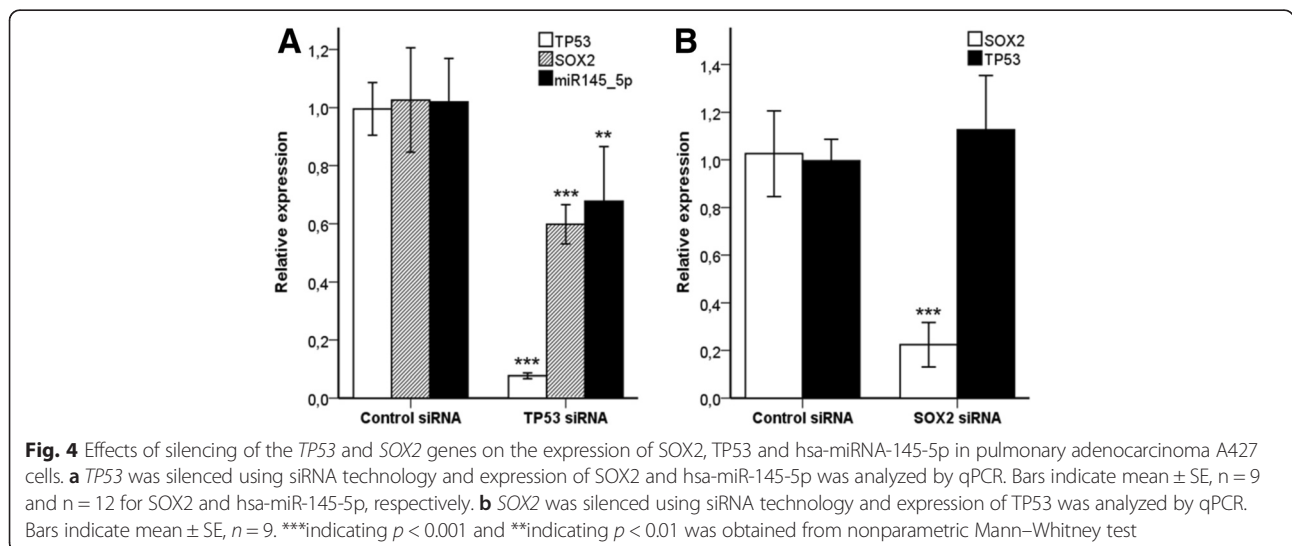




was significantly reduced in TP53 knockdown cells ( $p = 0.004$ , Fig. 4a). In contrast, the levels of hsa-miR-145-3p, hsa-miR-200b and hsa-miR-200c remained unchanged in TP53 knockdown cells, whereas hsa-miR-429 was not expressed in these cells (data not shown).

**Discussion**

SOX2 has a crucial role in maintaining the stem cell-like phenotype in cancer cells [12, 13] and its overexpression is generally associated with aggressive disease and poor outcome in several different tumor types [28–32]. SOX2 has been identified as the driving gene of the 3q25-27 amplicon, which is common in most NSCLC cases and contributes to the pathogenesis of lung cancer by controlling cell proliferation and malignant transformation [11]. Comparison of tumor tissue with non-tumorous tissue obtained from the same patients confirmed that tumor tissues had significantly higher odds of acquiring a copy number alteration in the SOX2 gene. Surprisingly, SOX2 copy number deletions were detected in a subset of tumor samples in this cohort. Deletions in SOX2 copy number have not previously been reported in NSCLC and the importance of this finding remains to be elucidated. SOX2 gene amplifications were observed in 34 % of tumors in this study. SOX2 has been shown to be highly expressed in approximately 90 % of pulmonary squamous cell carcinoma and to a lesser extent in adenocarcinoma [16] and several studies have previously identified SOX2 gene amplifications in lung squamous cell carcinomas with reported frequencies from 20 % to 60 % [13, 15, 33–36]. Here, we observed higher frequency of SOX2 copy number amplifications in squamous cell carcinoma tumors (59 %) than in adeno- (20 %) or large cell carcinoma (34 %). These data are in line with previous reports indicating a role for SOX2 in carcinogenesis. Accordingly, SOX2 affects tumorigenesis and its overexpression increases cell



migration and colony formation, while its knockdown impairs cell growth and suppresses metastasis of lung cancer cells [35, 37–39]. Increased *SOX2* expression is associated with metastatic progression [40] and *SOX2* gene amplifications are more frequent in high-grade than low-grade lesions [41]. Moreover, conditional homozygous overexpression of *SOX2* in murine Clara cells induces bronchial epithelial hyperplasia with progression to lung cancer in approximately 50 % of the animals [42]. Taken together, these results support the hypothesis that *SOX2* may act as an oncogene in lung carcinogenesis.

The prognostic role of *SOX2* in NSCLC remains uncertain as conflicting data has been reported. *SOX2* amplifications and overexpression have been reported as predictors of prolonged survival in squamous cell carcinomas [15, 43]. On contrary, *SOX2* expression is suggested a poor prognosis predictor in stage I lung adenocarcinoma [44]. Wilbertz et al. investigated *SOX2* amplifications and expression in two cohorts and reported no association between *SOX2* expression and survival in lung adenocarcinoma; however, observed a decreased survival in patients with low-level amplifications in one of the cohorts [15]. Differences in biological properties of tumor-initiating cancer stem cells in squamous cell carcinoma compared to lung or breast adenocarcinoma have been suggested as an explanation to the different observed associations between *SOX2* gene amplifications and prognosis [45]. Recently, several studies have showed improved outcome as a consequence of *SOX2* gene amplification in NSCLC, independent of histological subtype [14, 46, 47]. In our study, no significant effect on survival rates could be demonstrated, but this may be due to limited number of patients investigated. Moreover, differences in patient clinical characteristics, methodology and scoring criteria might explain some of the reported discrepancies. Clearly, more studies are needed to elucidate the role of *SOX2* in lung cancer progression and prognosis.

*TP53* has been demonstrated to down-regulate several stem cell-associated genes, including *SOX2*, following DNA damage in embryonic stem cells [17]. Moreover, a recent report indicated that induction of *TP53* led to repressed *SOX2* expression in NSCLC cells [18]. Since genetic alterations in the *TP53* gene are significant events in lung cancers [6], and mutations in the *TP53* gene increase the risk for chromosomal rearrangements such as copy number alterations [8], these early reports open up the possibility of *TP53* as an important regulator of *SOX2* in NSCLC. To address this, the effects of *TP53* mutational status on *SOX2* copy number and expression levels were studied in NSCLC patients. Interestingly, patients with at least one mutation in their lung tumors possessed a 2-fold higher risk of having a copy number variation in *SOX2*. This is to our knowledge the

first study to report an association between *TP53* mutations and *SOX2* copy number alterations. However, individuals carrying *TP53* mutations show generally higher levels of germline copy number variation [48]. Furthermore, smoking is known to increase the risk of acquiring *TP53* mutations [49–51] and, similarly, we and others [34, 36] have demonstrated that smokers have a higher risk of acquiring *SOX2* copy number alterations in tumors. Hence, *TP53* mutations may contribute to *SOX2* copy number alterations in lung cancer patients. Moreover, a correlation between *TP53* and *SOX2* expression levels was observed in NSCLC tumors. To further investigate this relationship, silencing of *TP53* and *SOX2* was performed in the lung adenocarcinoma cell line A427. *TP53* knockdown reduced *SOX2* mRNA and protein expression, whereas *SOX2* knockdown did not affect *TP53* expression levels, indicating a regulatory role of *TP53* on *SOX2* in NSCLC. Considering the role of *SOX2* in tumorigenesis it could be expected that *TP53* knockdown would increase *SOX2* expression. Indeed, Chen et al. have previously shown a reduction in *SOX2* expression following *TP53* induction in the *TP53*-null NSCLC cell line H1299 [18]. These discrepancies as well as reports on conflicting effects on NSCLC prognosis following *SOX2* copy number amplification indicate that the role of *SOX2* in NSCLC is far more complex than can be explained by histology or *TP53* status only.

There is a need to further investigate the mechanisms of *TP53* regulation of *SOX2*. To address this, the possible involvement of miRNAs was investigated. This is of interest as a more prominent role of miRNAs in cancer development has emerged during recent years and *TP53* regulates the expression of several genes through miRNAs [52, 53]. Hsa-miR-145 is an interesting candidate as *TP53* activates expression of hsa-miR-145 which in turn represses *SOX2* expression in embryonic stem cells [19–21]. Hsa-miR-145 expression has also been suggested as a novel marker responsible for relapse in surgically treated NSCLC [22]. Campayo et al. demonstrated that *TP53* may play a role in modulating hsa-miR-145 expression in NSCLC, and that patients with mutations in *TP53* and low hsa-miR-145 levels had lower survival in respect to patients with *TP53* wild-type or only mutations in *TP53* [22]. We here demonstrated that *TP53* knockdown repressed hsa-miR-145-5p expression in the lung adenocarcinoma cell line A427, whereas the other miRNAs investigated were unaffected. miRNAs have previously been believed to affect gene expression modulation only by negative regulation of target mRNA. Increasing evidence now indicate that miRNAs oscillate between repression and induction of gene expression in response to specific cellular conditions and cofactors [54]. Based on our findings, we suggest a possible role of hsa-miR-145 as an inducer of *SOX2* expression in NSCLC. It is known that hsa-miR-

145 exhibits opposite effects on RNA regulation in different cell types. Indeed, hsa-miR-145 mediates gene upregulation during muscle differentiation [55] and gene downregulation in osteosarcoma [56]. Other approaches to further investigate the possible regulation of TP53 on SOX2 in several different lung cancer cell lines and in different cellular states are clearly needed, as well as additional studies to fully understand the complexity of hsa-miR-145 signaling in lung cancer. Moreover, hsa-miR-638 has recently come into focus as another promising candidate, as it has been shown that hsa-miR-638 regulates SOX2 in NSCLC [57]. Interestingly, hsa-miR-638 is known as a TP53-targeting miRNA [58], further illustrating the complexity of this signaling.

## Conclusions

TP53 mutations were associated with an increased risk of acquiring a SOX2 copy number amplification in NSCLC. Furthermore, SOX2 and TP53 expression were correlated in lung tumors and reduction in TP53 resulted in decreased SOX2 and hsa-miR-145 expression in lung adenocarcinoma cells. As hsa-miR-145 has previously been shown to regulate SOX2 expression, we propose that one mechanism for the TP53 regulation of SOX2 may be through hsa-miR-145. This prompts for further studies on the mechanisms behind TP53-induced regulation of SOX2 expression and the possible importance of hsa-miR-145 in lung cancer development.

## Additional file

**Additional file 1: Table S1.** Association between clinicopathological data and SOX2 gene status in NSCLC tumors. (PDF 101 kb)

## Abbreviations

NSCLC: Non-small cell lung cancer; OR: Odds ratio; PAH: Polycyclic aromatic hydrocarbons.

## Competing interests

All authors declare no conflict of interest.

## Authors' contributions

JSE carried out molecular genetic studies, participated in statistical analysis and drafted the manuscript. VS coordinated the NSCLC databank and carried out the histopathology and survival analysis. PB and AG participated in the coordination of collection of healthy controls and commented the initial manuscript draft. AH participated in the design and coordination of the study and helped draft the manuscript. SZ led the study, was involved in TP53 mutational analysis, participated in its design and helped draft the manuscript. All authors read and approved the final manuscript.

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