The Role of TOP2A Protein Expression and Aneuploidy in NSCLC

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Research Article

Keywords: Non-small cell lung cancer, TOP2A, aneuploidy, IHC, FISH, Prognosis

Posted Date: December 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1142263/v1

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Abstract

Purpose

DNA topoisomerase II alpha (TOP2A) is a cell-cycle dependent protein associated with cell proliferation and division. Abnormal regulation of TOP2A and aneuploidy induces tumorigenesis and poor prognosis. Data related to TOP2A protein in non-small cell lung cancer (NSCLC) is limited to few studies on gene status. The present study aimed to investigate the consistency between aneuploidy and expression of TOP2A gene and protein, respectively, and the role of aneuploidy in the prognosis of NSCLC patients.

Methods

Clinical data and lung cancer tissues were collected from 244 patients with NSCLC. TOP2A protein and gene expression were detected using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH), respectively. Nonparametric Spearman's rank sum test was used to analyze clinicopathological data. Kaplan–Meier and multivariate Cox regression methods were used for survival analysis.

Results

Enhanced expression and amplification rate of TOP2A protein and gene were 29.9% (73/244) and 0.4% (1/244), respectively. The aneuploidy rate was 31.6% (77/244). In NSCLC, patients with enhanced expression of TOP2A protein and aneuploidy correlated with clinical stages \((p < 0.001)\). Enhanced expression of TOP2A protein was consistent with aneuploidy as detected by Kappa test \((K = 0.307)\) and this correlation was confirmed by chi-square test \((p < 0.001)\). The overall survival of patients with aneuploidy was shorter than diploidy \((p < 0.001)\). Clinically advanced patients with aneuploidy together with TOP2A overexpression had poor prognosis \((p < 0.001)\).

Conclusion

Aneuploidy and overexpression of TOP2A is a predictor of poor prognosis in patients with advanced NSCLC.

Introduction

According to the Global Cancer Report 2020 published by the World Health Organization, lung cancer remains the second most prevalent malignancy next to breast cancer. It is considered the deadliest of all cancers worldwide (IARC 2020). Non-small cell lung cancer (NSCLC) is an extremely proliferative, aggressive, and metastatic malignant tumor that accounts for approximately 80–85% of all lung cancers (Molina et al. 2008). Majority of the patients with NSCLC have advanced disease at the time of diagnosis, with 75% of them having progressed to distant metastases (Zhang B et al. 2018). Hence, early differential diagnosis is of great significance in improving the survival, quality of life, and prognosis of patients with NSCLC.

Chromosomal gene amplifications or genomic DNA copy number aberrations, frequently observed in several solid tumors are known to play an important role in tumor progression (Klein and Klein 1986; Albertson et al. 2003). Chromosome 17 harbors majority of these significant gene amplifications (Matsui et al. 2013). DNA topoisomerase II alpha (TOP2A) gene is located on chromosome 17 at 17q21-q22. It encodes a 170 kDa nuclear
enzyme associated with a number of cellular processes, including DNA replication, chromatin condensation, chromosome segregation, and structural maintenance of chromosomes (Champoux 2001).

TOP2A is a cell-cycle-dependent protein involved in cell division and proliferation (Lee and Berger 2019). Abnormal regulation of TOP2A induces tumorigenesis and accordingly, several studies have reported aberrant expression of TOP2A protein in diverse malignancies (Fritz et al. 2005; Grenda et al. 2020; Hooks et al. 2018; Reddy et al. 2018). Altered TOP2A is associated with the development of multiple tumors. In patients with breast cancer, TOP2A gene amplification augments sensitivity to anthracyclines (Press et al. 2019). In addition, gene amplification increases the number of independent or synergistic oncogenic factors (Matsui et al. 2013). Enhanced expression of TOP2A is associated with poor prognosis (Yang et al. 2018) and lower expression with drug resistance in patients with NSCLC (Sakurai et al. 2020). However, the pre- or post-drug resistance dependent occurrence of this phenotype needs to be elucidated (Burgess et al. 2008).

The present study aimed to investigate the consistency of TOP2A gene and protein expression, and the regulatory mechanisms associated with TOP2A protein expression. Tissue samples and complete clinical data were collected from 244 patients with NSCLC. The relationship between TOP2A gene status, the clinicopathological characteristics, and prognosis of NSCLC were analyzed. In addition, the clinical significance of TOP2A gene status was explored in NSCLC patients. The results provide valuable insights for the discovery of potential prognostic biomarker candidates and novel bio-targets for the treatment of NSCLC.

Patients And Methods

Patients and study design

Tissue samples of 244 patients diagnosed with NSCLC and 55 normal control tissues were collected from the Shanxi Tumor Hospital (Taiyuan, China) in 2017. This was an observational study. The Research Ethics Committee of the Shanxi Tumor Hospital has confirmed that no ethical approval is required. The study was conducted in accordance with the guidelines of the Declaration of Helsinki, and written informed consent was obtained from all patients. The histology of the samples were reassessed and confirmed by two experienced pathologists according to the criteria listed in the new International Association for the Study of Lung Cancer (IASLC) classification (2017). Patients receiving radiation or chemotherapy were excluded from the study. Patient’s complete clinicopathological and follow-up data, including sex, age, Lymph node metastasis, clinical stage, pathological classification, pathological grade, family history of malignant tumor, and smoking history were collected and documented. The collected specimens were handled and made anonymous according to the accepted ethical and legal standards. All patients were followed-up until the set deadline of November 2021.

Immunohistochemistry (IHC)

TOP2A protein expression level in tissue microarrays was detected by IHC using rabbit monoclonal anti-Topo-IIA antibody (1:8000 dilution; ab180393; Abcam, Cambridge, UK). Cells were considered positive for TOP2A if staining was present in the nuclei. The proportion of Top2A positive cells was evaluated using a semi-quantitative scoring method based on the proportion of positive cells. The median expression value of TOP2A was used as the cut-off value. With more than 50% being considered a marker of overexpression based on previous studies.

Fluorescence in situ hybridization (FISH)
FISH amplification probes were purchased from Bipin (F.01368-01, Am Bipin, Guangzhou, China). Chromosome 17 mitogen labeled with the green fluorescent probe was used as an internal control. The TOP2A gene locus was labeled with the red fluorescent probe. For analysis, the nuclei present in the tumor-infiltrated area scanned in the field of view were counted. According to the Chinese HER2 detection guidelines for breast cancer (2019), TOP2A gene amplification was defined as follows: (1) ratio $\geq 2.0$, average gene/average centromere or average value of the red signal/average value of the green signal; (2) ratio < 2.0, but average copy number of $TOP2A \geq 6.0$, or average red signal number $\geq 6.0$, or several red signals connected in clusters). At a ratio < 0.8, $TOP2A$ was considered absent. No $TOP2A$ gene amplification was indicated if the ratio was < 2.0, and the average red signal number was < 4.0. In informative cases (> 90% of nuclei showing hybridization signals), greater than two red and green signals in each tumor cell with a ratio of red to green signals > 1 was considered as an aneuploidy. The reliability of the FISH data was confirmed by additional cell counts and repeated tests.

**Statistical analysis**

Fisher's exact test was used to assess the correlation between immunohistochemical expression and clinicopathological features. Correlation between variables was analyzed using the nonparametric Spearman rank-sum test. Survival curves were plotted using the Kaplan–Meier method and the log-rank test was used to compare survival curves. Multivariate analysis was performed using the Cox proportional risk model to assess the dependence of Topo-II expression.

**Results**

**Clinicopathological features**

Complete follow-up data of 244 patients with NSCLC included in the study were documented. The male to female ratio was 3.9:1. 155 patients were pathologically classified as squamous carcinoma and 89 as adenocarcinoma. The mean age and survival were 60.7 years and 40.6 months, respectively. As of 1 November, 2021, 108 patients passed away. The annual and 3-year survival rates were 93% and 66%, respectively. Of the analyzed 177 patients with NSCLC, 77% (134/177) had a history of smoking and 14.4% (25/177) had a family history of malignant tumors.

**IHC findings**

The expression of TOP2A was detected in the nucleus. The positive expression rate of TOP2A was higher in NSCLC tissues (29.9%, 73/244) compared to that in normal tissues (9%, 5/55) with significant statistical difference ($p < 0.05$). The expression of TOP2A protein indicated two main distribution patterns: either solely in cancer cells at the tumor margin and rarely in the central region of the tumor, or homogeneously in the entire infiltrating tumor region (Fig. 1). In addition, TOP2A overexpression closely correlated with the clinical stage of the disease (Table 1).

**FISH findings**

FISH analysis revealed a single case of $TOP2A$ amplification, 8 cases where $TOP2A$ expression was absent, and 77 cases of aneuploidy (Fig. 2). Patients aneuploidy was independent and not related to the clinicopathological parameters, including age of onset, tumor size, lymph node metastasis, clinical stage, pathological stage, and pathological grade. However, a significant correlation was detected between aneuploidy and clinical stage of the disease ($p < 0.001$) (Table 1). In addition, TOP2A protein positivity was consistent with aneuploidy as indicated by
Kappa analysis ($p < 0.001, K = 0.307$). The number of amplified and missing samples was too small for statistical analysis. We will not repeat it here.
Table 1
Clinicopathological characteristics of patients with non-small cell lung cancer (NSCLC) according to TOP2A protein expression and aneuploidy.

| Variable                      | Total | TOP2A protein | 𝑥² | 𝑝   | TOP2A aneuploidy | 𝑥² | 𝑝   |
|-------------------------------|-------|---------------|-----|-----|------------------|-----|-----|
|                               |       | -             | +   |     | -                | +   |     |
|                               | 244   | 171 (70.1)    | 73  (29.9) |     | 167 (68.4)       | 77  (31.6) |     |

*TOP2A aneuploidy*

|                              |       | -             | +   |     |                                  |     |     |
|------------------------------|-------|---------------|-----|-----|----------------------------------|-----|-----|
| +                            | 113   | 62 (54.9)     | 51  (45.1) | 0.001*|                                  |     |     |
| -                            | 131   | 109 (83.2)    | 22  (16.8) |     |                                  |     |     |

Age (yrs.)

|       |       |       |     |     |       |     |     |
|-------|-------|-------|-----|-----|-------|-----|-----|
| ≥ 60  | 137   | 99 (72.3) | 38  (27.7) | 0.709 | 99 (72.3) | 38  (27.7) | 2.111 | 0.146 |
| < 60  | 107   | 72 (67.3) | 35  (32.7) |     | 68 (63.6) | 39  (36.4) |     |     |

Gender

|       |       |       |     |     |       |     |     |
|-------|-------|-------|-----|-----|-------|-----|-----|
| Male  | 195   | 133 (68.2) | 62  (31.8) | 1.631 | 130 (66.7) | 65  (33.3) | 1.418 | 0.234 |
| Female| 49    | 38 (77.6) | 11  (22.4) |     | 37 (75.5) | 12  (24.5) |     |     |

Clinical stage

|       |       |       |     |     |       |     |     |
|-------|-------|-------|-----|-----|-------|-----|-----|
| I     | 98    | 81 (82.7) | 17  (17.3) | 16.176 | 79 (80.6) | 19  (19.4) | 16.004 | 0.001*|
| II    | 65    | 45 (69.2) | 20  (30.8) |     | 45 (69.2) | 20  (30.8) |     |     |
| III   | 76    | 43 (56.6) | 33  (43.4) |     | 41 (53.9) | 35  (46.1) |     |     |
| IV    | 5     | 43 (56.6) | 33  (43.4) |     | 2 (40.0)  | 35  (46.1) |     |     |
|       |       | 2 (40.0)  | 3   (60.0)  |     | 2 (40.0)  | 3   (60.0)  |     |     |

Lymph node metastasis

|       |       |       |     |     |       |     |     |
|-------|-------|-------|-----|-----|-------|-----|-----|
| Yes   | 86    | 61 (70.9) | 25  (29.1) | 0.169 | 106 (67.1) | 52  (32.9) | 0.381 | 0.537 |
| No    | 158   | 110 (69.6) | 48  (30.4) |     | 61 (70.9) | 25  (29.1) |     |     |

Data are presented as n (%)
| Variable                              | Total | TOP2A protein | $\chi^2$ | $p$    | TOP2A aneuploidy | $\chi^2$ | $p$    |
|--------------------------------------|-------|---------------|----------|--------|-----------------|----------|--------|
|                                      |       | -             | +        |        | -               | +        |        |
| Tumor size (cm)                      |       |               |          |        |                 |          |        |
| $\geq 4\text{ cm}$                   | 97    | 66 (68.0)     | 31 (32.0)| 0.320  | 0.572           | 100 (68.0)| 47 (32.0)| 0.030  | 0.864 |
| < 4 cm                               | 147   | 105 (71.4)    | 42 (28.6)|        | 67 (69.1)       | 30 (30.9)|         |
| Histology                            |       |               |          |        |                 |          |        |
| LUSC                                 | 155   | 110 (71.0)    | 45 (29.0)| 0.159  | 0.690           | 109 (70.3)| 46 (29.7)| 0.695  | 0.404 |
| LUAD                                 | 89    | 61 (68.5)     | 28 (31.5)|        | 58 (65.2)       | 31 (34.8)|         |
| Histopathological grade              |       |               |          |        |                 |          |        |
| 1                                    | 4     | 3 (100)       | 0        | 1.742  | 0.418           | 3 (66.7) | 1 (33.3) | 0.157  | 0.924 |
| 2                                    | 101   | 70 (69.3)     | 31 (30.7)|        | 70 (68.7)       | 31 (31.3)|         |
| 3                                    | 139   | 97 (69.8)     | 42 (30.2)|        | 72 (51.8)       | 45 (32.4)|         |
| Tobacco smoking                      |       |               |          |        |                 |          |        |
| Yes                                 | 134   | 88 (65.7)     | 46 (34.3)| 0.652  | 0.419           | 95 (70.9)| 39 (29.1)| 1.692  | 0.193 |
| No                                  | 40    | 29 (72.5)     | 11 (27.5)|        | 24 (60.0)       | 16 (40.0)|         |
| Family history of cancer            |       |               |          |        |                 |          |        |
| Yes                                 | 25    | 19 (76.0)     | 6 (24.0) | 1.017  | 0.313           | 16 (64.0)| 9 (36.0) | 0.260  | 0.610 |
| No                                  | 149   | 98 (65.8)     | 51 (34.2)|        | 103 (69.1)      | 46 (30.9)|         |

Data are presented as n (%)

lung squamous cell carcinoma (LUSC), lung adenocarcinoma (LUAD)

Cox proportional risk model
Factors with significant correlation were screened using univariate Cox regression analysis. To determine independent prognostic factors, a multifactorial Cox proportional risk model was constructed using TOP2A protein expression ($p < 0.001$), aneuploidy ($p < 0.001$), and clinical stage ($p < 0.001$). The results indicated TOP2A aneuploidy ($p < 0.001$) and clinical stages ($p < 0.01$) as independent risk factors for NSCLC (Table 2). Clinically advanced patients with aneuploidy, together with enhanced expression of TOP2A protein had poor prognosis as revealed by the Survival curves ($p < 0.001$) (Fig. 3).
Table 2
Summary of clinicopathological characteristics of patients with non-small cell lung cancer (NSCLC) detected by univariate and multivariate Cox regression analysis

| Variable                  | Total | Univariate Cox regression | Multivariate Cox regression |     |
|---------------------------|-------|---------------------------|-----------------------------|-----|
|                           |       | HR (95% CI)               | HR (95% CI)                 |     |
|                           |       | x²                         | p                           | x²  |
|                           |       |                            |                             | p   |
| **TOP2A**                 |       |                            |                             |     |
| +                         |       | 3.397 (2.164-5.332)       | 28.28 0.001*                |     |
| -                         |       | 0.2944(0.1876-0.4620)     |                             |     |
| **TOP2A aneuploidy**      |       |                            |                             |     |
| +                         |       | 42.86(25.24-72.78)        | 193.4 0.001*                |     |
| -                         |       | 0.02333(0.1374-0.03962)   |                             |     |
| Age(yrs.)                 |       |                            |                             |     |
| ≥ 60                      |       | 0.8445(0.5762-1.238)      | 0.7503 0.3864               |     |
| < 60                      |       | 1.184(0.8078-1.736)       |                             |     |
| Gender                    |       |                            |                             |     |
| Male                      |       | 0.9462(0.5875-1.524)      | 0.5163 0.8202               |     |
| Female                    |       | 1.057(0.6561-1.702)       |                             |     |
| Clinical stage            |       |                            |                             |     |
| Early clinical stage      |       | 0.2810(0.1811-0.4360)     | 32.06 0.001*                |     |
| Late clinical stag (II&III)|     | 3.559(2.293-5.523)        |                             |     |

Data are presented as n (%)  
HR: hazard ratio; CI: confidence interval
| Variable                        | Total | Univariate Cox regression | Multivariate Cox regression |  |  |
|--------------------------------|-------|---------------------------|-----------------------------|---|---|
|                                |       | HR (95% CI)               | x^2             | p  | HR (95% CI)                        | x^2 | p  |
| Lymph node metastasis         |       |                           |                 |    |                                  |     |    |
| Yes                            | 86    | 0.8824(0.5955-1.308)      | 0.3884          | 0.5331 |
|                                | 158   | 1.133(0.7647-1.679)       |                 |    |                                  |     |    |
| No                             |       |                           |                 |    |                                  |     |    |
| Tumor size(cm)                 |       |                           |                 |    |                                  |     |    |
| ≥ 4cm                          | 97    | 0.8866(0.6031-1.303)      | 0.3751          | 0.5402 |
| < 4cm                          | 147   | 1.128(0.7673-1.658)       |                 |    |                                  |     |    |
| Histology                      |       |                           |                 |    |                                  |     |    |
| LUSC                           | 155   | 1.070(0.7227-1.583)       | 0.1133          | 0.7364 |
| LUAD                           | 89    | 0.9349(0.6316-1.384)      |                 |    |                                  |     |    |
| Histopathological grade       |       |                           |                 |    |                                  |     |    |
| Low grade (§&§)                | 135   | 0.8806 (0.6021-1.288)     | 0.4299          | 0.5121 |
| High grade (§)                 | 139   | 1.136 (0.7765-1.661)      |                 |    |                                  |     |    |
| Tobacco smoking               |       |                           |                 |    |                                  |     |    |
| Yes                            | 134   | 0.7128(0.4136-1.229)      | 1.486           | 0.2228 |
| No                             | 40    | 1.403(0.8140-2.418)       |                 |    |                                  |     |    |

Data are presented as n (%)  
HR: hazard ratio; CI: confidence interval
| Variable                    | Total | Univariate Cox regression | Multivariate Cox regression |
|----------------------------|-------|---------------------------|-----------------------------|
|                            |       | $x^2$ p                    | $x^2$ p                     |
| Family history of cancer   |       |                            |                             |
| Yes                        | 25    | 1.198(0.6220-2.306)        | 0.2913 0.5894               |
| No                         | 149   | 0.8349(0.4336-1.608)       |                             |

Data are presented as n (%)

HR: hazard ratio; CI: confidence interval

**Discussion**

TOP2A plays a key role in cell survival. It helps unwind an entangled double strand, introduces a break in double stranded DNA to permit another strand to cross the site, and subsequently reseals the nicked end to form a topological structure that favors DNA transcription and replication, thus making it a necessary vital enzyme for cells to maintain life activities. This process requires a conformational change in the structure of TOP2A for it to complete catalytic reactions. Enhanced expression of TOP2A protein in rapidly proliferating cells makes it an important therapeutic target for cancer therapy. The optimal defined cut-off value for TOP2A expression in various types of tumors according to previous studies is 13-30% in breast cancer (An et al. 2018; Nikolényi et al. 2012; O’Malley et al. 2011), 18% in epithelial ovarian cancer (Ghisoni et al. 2019), 15% in neuroblastoma, 15% in luminal fluid cytology (Kimura et al. 2010), and 75% in NSCLC (Sakurai et al.2020). However, such high expression levels were not detected in current samples, probably because of the differences related to patient selection, tissue processing, and fixation methods. Therefore, a median cut-off value of 50% was used in the present study.

Previous studies believed that the increase of protein expression mainly depended on gene amplification and the increase of transcription (Bagci et al. 2015). In the present study, aneuploidy was consistent with elevated TOP2A expression as detected by kappa consistency test. Thus, in the current study, gene transcription is proposed as the major regulatory process associated with the enhanced expression of TOP2A protein. An earlier published data indicated translation regulation of TOP2A in Hela cells (Subramanya et al. 2011). To increase the expression of TOP2A protein, HuR promotes the translation of TOP2A by enhancing the binding and not by altering the level of TOP2A mRNA (Hitti et al. 2016). The results of IHC and FISH obtained in the present study are inconsistent with the above-published in vitro studies. In addition, the mRNA of zinc finger protein 148 (ZFN148) and TOP2A regulate each other through ceRNA in colorectal cancer cells, with their target miRNAs such as miR101, miR144, miR335, and miR365 negatively regulating TOP2A mRNA (Gao et al. 2017), suggesting a novel regulatory mechanism of TOP2A. Thus, the mechanisms regulating the expression of TOP2A protein are diverse and needs to be elucidated.

In the present study, although TOP2A amplification was not significant in the analyzed 244 NSCLC samples, patients with enhanced expression of TOP2A protein showed varied degree of heteroploidy in tumor cells as determined by IHC and FISH. The presence of heteroploid karyotype is related to the physiological mechanism associated with the action of TOP2A (cell cycle progression and chromosome segregation in mammalian cells cultured in the presence of the topoisomerase II inhibitors ICRF-187 [(+)-1,2-bis(3,5-dioxopiperazinyl-1-yl) propane; ADR-529] and ICRF-159 (razoxane); Daniloski et al. 1994). The primary action of TOP2A during meiosis is when the
DNA structure fails to successfully deconvolve, leading to abnormal cell division and chromosomal segregation, resulting in a heteroploid karyotype (Lee and Berger 2019). The mechanism associated with increasing the aneuploidy has been partially elucidated in recent years. A previous study identified the role of osteoprotegerin (OPG) in inducing an increase in the TOP2A copy number (Goswami 2015). In relapsed diffuse large B cell lymphoma (DLBCL) patients, an increase in polyplody 17 reflected the genetic compensation of tumor cells in response to TOP2 inhibition (Pedersen et al. 2015). Several studies have suggested the involvement of aneuploidy in tumor development and drug resistance (Replogle et al. 2020). Aneuploidy predicted shorter overall survival (OS) and progression free survival (PFS) in patients with DLBCL (Chen et al. 2012) and response to polyethylene glycol liposomal adriamycin in epithelial ovarian cancer (Erriquez et al. 2015). The significant correlation between heteroploidy and poor patient prognosis warrants the need for alternate novel methods to replace FISH in detecting chromosomal concordance. Several studies have shown the applicability of quantitative polymerase chain reaction (qPCR) in screening patients with aneuploid tumors. The method was used to detect expression markers related to chromosomal instability, such as AURKA, FOXM1, TOP2A, and TPX2, to classify patients with grade II breast tumors into two groups with good and poor prognosis (Szászet al. 2013). Compared with immunohistochemistry and FISH to assess the karyotype of patients, qPCR is undoubtedly inexpensive and rapid; however, screening for tumor-associated markers associated with chromosomal instability-dependent expression needs to be further explored. As a retrospective study, we were unable to collect blood specimens from patients for screening biochemical markers, which limits the expansion of the research direction. Future research could aim at analyzing blood specimens for TOP2A mRNA-related data, to not only elucidate TOP2A protein regulatory mechanisms, but also to decipher the ability of TOP2A to screen NSCLC patients with aneuploid chromosomes using qPCR.

In the present study, the 16th case was a patient with squamous lung cancer with haploid tumor cells exhibiting a total deletion of chromosome 17 and a single signal each for TOP2A and gsp17, and with normal diploid precancerous cells in the surroundings. Tumor cells of diverse haplogroups have different mutations to resist the action of targeted drugs (Real and Marsiglia 2020). Accordingly, haploinsufficiency in acute lymphoblastic leukemia was found to be associated with poor patient prognosis (Safavi et al. 2013; Safavi and Paulsson 2017). The present study was limited in analyzing the role of haplogroups in NSCLC because of the small sample size.

Currently two major types of antitumor drugs targeting TOP2A are available. The first type converts the topoisomerase into a toxin that induces DNA double-strand breaks to inhibit DNA replication, resulting in the death of tumor cells expressing high levels of TOP2A. However, its use is severely limited by the dose, as it tends to cause cardiotoxicity and secondary malignancies. The second type is less commonly used in clinical practice and comprises of a catalytic inhibitor of TOP2A, which uses an enzymatic reaction to inhibit the ATPase activity to block the conformational conversion of TOP2A enzyme (Delgado et al. 2018; Hasan et al. 2008). Anthracyclines are a type of TOP2A toxins, whose cumulative dose-induced side effects include cardiotoxicity, myelosuppression, and gastrointestinal reactions (Qiu et al. 2021). The exact mechanism of anthracycline-induced cardiotoxicity is unknown, however, several theories are proposed to explain the same, such as oxygen radical damage theory, iron ion metabolism disorder theory, calcium overload theory, and anthracycline-induced apoptosis (Hurvitz, 2021). Assessment of the risk of the toxic effects of anthracyclines remains limited to real-time monitoring of the patient's physical status during administration using ECG scanners, cardiac enzyme profiles, echocardiography, and calcium-regulated tetraphosphate tests (Alexandre et al. 2020). In addition, secondary tumors and drug resistance related to the use of anthracyclines remains inevitable. The issue of adverse effects and safety of drug therapy remains a challenge in systemic oncology treatment. In drug therapy, control of drug safety together with ensuring
effectiveness remains an issue to be explored. Since current medical research does not support "de-anthracycline," adjuvant combination of anthracyclines with targeted drugs has become a routine treatment for patients with advanced cancer. Accordingly, the use of anthracyclines is continued in oncology treatment regimens because of their "cost-effectiveness." Anthracyclines are widely used in the treatment of lung cancer. However, the clinical evaluation of anthracyclines is not routinely based on the IHC results of TOP2A. Patients with enhanced expression of TOP2A show higher sensitivity to anthracyclines. Cell lines resistant to anthracyclines exhibit low expression of TOP2A. However, the occurrence of this phenotype pre- or post-drug resistance remains uncertain. The ability of IHC to predict sensitivity to anthracyclines remains to be studied (Burgess et al. 2008).

The results of the present study indicate the association of aneuploidy with high expression of TOP2A protein. The enhanced expression of TOP2A protein was primarily based on the increase in the number of the transcripts. In addition, the patient's aneuploid karyotype indicated an increased risk of death. Thus, TOP2A protein could be used as a potential biochemical marker for poor prognosis in patients with NSCLC.

**Conclusion**

Patients with aneuploidy had shorter OS. Overexpression of TOP2A protein was significantly consistent with aneuploidy. Aneuploidy and clinical stage are independent prognostic risk factors in patients with NSCLC.

**Declarations**

**Funding**

This work was supported by grants of the followings: Shanxi Province foundation of supporting scientific research projects of returned overseas students (Grant number: 2020-156); Natural Science Foundation of Lvliang Science Bureau (Grant number: 2020SHFZ31); High-tech Healthcare Innovation Team of Health Commission of Shanxi (Grant number:2020TD12); Natural Science Foundation of Shanxi Province (Grant number: 201801D121347).

**Competing Interests**

The authors declare no potential conflicts of interest.

**Author contributions**

All authors contributed to the study conception and design. Zhenwen Chen and Zijuan Zeng designed the study and wrote the manuscript. Fei Chai revised the manuscript. Yanfeng Xi and Zhenwen Chen interpreted the results of IHC. Qi Wang, Yirong Xu, and Ning Yang collected the clinical data and performed immunochemical staining. Fei Chai, Yuanyuan Gong, and Zijuan Zeng performed FISH. Zijuan Zeng, Siyi Qian, and Tingting Wen were responsible for statistical evaluation and data analysis. All authors read and approved the final manuscript.

**Data availability**

All data generated relevant to the results presented in this article are included in this article. Other data that were not relevant to the results presented here are available from the corresponding author (Zhenwen Chen) upon
reasonable request.

**Ethical approval**

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Institute Research Medical Ethics Committee of the Shanxi Tumor Hospital.

**Informed consent**

Informed consent was obtained from all individual participants included in the study.

**Acknowledgments**

This work was supported by grants of the followings: Shanxi Province foundation of supporting scientific research projects of returned overseas students (Grant number: 2020-156); Natural Science Foundation of Lvliang Science Bureau (Grant number: 2020SHFZ31); High-tech Healthcare Innovation Team of Health Commission of Shanxi (Grant number:2020TD12); Natural Science Foundation of Shanxi Province (Grant number: 201801D121347). We would like to thank Editage (www.editage.cn) for English language editing.

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Figures
Figure 1

Distribution of DNA topoisomerase II alpha (TOP2A) protein expression in non-small cell lung cancer (NSCLC) and para-carcinoma tissues. a TOP2A protein expression in para-carcinoma tissue. b TOP2A protein low-expression in NSCLC. c. TOP2A protein equally distributed in tumor. d. TOP2A protein distributed at the edges of the tumor. Magnification ×200; Scale bar, 100 μm
Figure 2
DNA topoisomerase II alpha (TOP2A) gene expression in non-small cell lung cancer (NSCLC)
a TOP2A absent  
b TOP2A amplification  
c TOP2A aneuploidy  
d TOP2A haploidy
Magnification × 1000; Scale bar, 20 μm.
Figure 3

Kaplan–Meier survival analysis. a OS based on TOP2A expression b OS based on TOP2A aneuploidy c OS based on clinical stages d OS based on TOP2A and aneuploidy co-expression combined with late clinical