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Association of Topoisomerase II (TOP2A) and Dual-Specificity Phosphatase 6 (DUSP6) Single Nucleotide Polymorphisms with Radiation Treatment Response and Prognosis of Lung Cancer in Han Chinese

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Background: Mutations of DNA topoisomerase II (TOP2A) are associated with chemotherapy resistance, whereas dual-specificity phosphatase 6 (DUSP6) negatively regulates members of the mitogen-activated protein (MAP) kinase superfamily to control cell proliferation. This study assessed *TOP2A* and *DUSP6* single nucleotide polymorphisms (SNPs) in non-small cell lung cancer (NSCLC) patients for association with chemoradiotherapy responses and prognosis.

Material/Methods: A total of 140 Chinese patients with histologically confirmed NSCLC were enrolled and subjected to genotyping of *TOP2A* rs471692 and *DUSP6* rs2279574 using Taqman PCR. An independent sample *t* test was used to analyze differences in tumor regression after radiotherapy versus SNP risk factors. Kaplan-Meier curves analyzed overall survival, followed by the log-rank test and Cox proportional hazard models.

Results: There were no significant associations of *TOP2A* rs471692 and *DUSP6* rs2279574 polymorphisms or clinicopathological variables with response to chemoradiotherapy ($p>0.05$). Comparing overall survival of 87 patients with stage I–III NSCLC treated with radiotherapy or chemoradiotherapy to clinicopathological variables, the data showed that tumor regression, weight loss, clinical stage, and cigarette smoking were independent prognostic predictors ($p=0.009$, 0.043, 0.004, and 0.025, respectively). Tumor regression rate >0.34 was associated with patent survival versus tumor regression rate ≤0.34 ($p=0.007$).

Conclusions: *TOP2A* rs471692 and *DUSP6* rs2279574 SNPs were not associated with chemoradiotherapy response, whereas tumor regression, weight loss, clinical stage, and cigarette smoking were independent prognostic predictors for these Chinese patients with NSCLC.

MeSH Keywords: Carcinoma, Non-Small-Cell Lung • Chemoradiotherapy • Polymorphism, Single Nucleotide

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Background

Lung cancer is prevalent worldwide, accounting for 1.6 million new cases and 1.4 cancer-related deaths in 2008 [1]. Histologically, lung cancer can be divided into non-small cell lung cancer (NSLCL) and small cell lung cancer, and the former accounts for approximately 80% of all lung cancer cases [1]. NSCLC is most often diagnosed at an advanced stage, when surgical resection of the tumor lesion is no longer an option. Therefore, patients are likely to receive chemoradiotherapy, although more recently targeted therapies have provided a novel strategy for the control of advanced NSCLC [2]. Ionizing radiotherapy is used to treat advanced non-resectable NSCLC in an estimated 64.3% of patients [3,4], but the outcome of this treatment varies significantly. Ionizing radiation induces cell death mainly through generation of short-lived but highly reactive DNA radicals that evolve into stable, long-lived DNA lesions, such as DNA double strand breaks [5] or through interactions with the cell membrane [6]. However, dramatic differences in survival outcomes have been reported even in NSCLC patients with tumors of a similar pathological or clinical stage that received identical treatments, suggesting that the patient's sensitivity to radiotherapy could play an important role in NSCLC prognosis [7,8]. Biomarkers for the prediction and monitoring of treatment responses and survival are urgently sought in order to appropriately tailor treatment regimens to patients.

Aberrant expression or single nucleotide polymorphisms (SNPs) in certain genes may influence host responses to chemoradiotherapy. For example, altered expression of DNA repair proteins and their polymorphisms has been reported to modulate sensitivity to radiotherapy [8–11]. Other studies reported that aberrant expression of other genes, including cell proliferation-related genes, could also influence sensitivity to radiotherapy [11]. To this end, we have focused on the influence of TOP2A and DUSP6 on radiotherapy outcomes. Kodiakov et al. [12] demonstrated that TOP2A expression was associated with clinicopathological parameters and tumor cell proliferation in lung adenocarcinoma. DUSP6 is a putative negative feedback regulator of the RAS-ERK pathway and plays a physiologically important role in maintenance of cell homeostasis in response to growth factors [13–15]. Disruption of this feedback loop could result in neoplastic, and even malignant, transformation. Okudela et al. reported that levels of DUSP6 expression decreased as both growth activity and histological grade of lung cancer increased [16]. Moreover, DUSP6 is highly polymorphic, and functional *DUSP6* SNPs have been identified and demonstrated to regulate DUSP6 expression [16,17]. Given the important role of TOP2A and DUSP6 in carcinogen metabolism and cell proliferation, it is conceivable that genetic variations in TOP2A and DUSP6 that alter gene expression and/or protein production may act as markers of radiotherapy response. Thus, in this study, we assessed the frequency of *TOP2A* and *DUSP6* SNPs in a cohort of 140 Chinese NSCLC patients, and probed the association between *TOP2A* and *DUSP6* SNPs with radiotherapy response and overall survival. We aimed to identify *TOP2A* and *DUSP6* SNP biomarkers that predict treatment response and survival of patients with advanced stage NSCLC.

Material and Methods

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of our university and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from each patient before participation in this study.

Study population

A total of 140 consecutive patients with histologically confirmed NSCLC were recruited from the First Affiliated Hospital of China Medical University between June 2009 and December 2012. The inclusion criteria were: i) patients were pathologically diagnosed with NSCLC and computed tomography (CT) scan revealed progressive lesions; ii) patients were not eligible for surgery and NSCLC was defined as non-resectable; iii) patients had a WHO-Eastern Cooperative Oncology Group (ECOG) performance status ≤2; iv) patients did not show evidence of pleural effusion; and v) patients could be encompassed within a tolerable radiotherapy treatment volume.

Thirty-four patients had intrathoracic recurrent disease (recurrence after previous surgery), and 106 patients had previously untreated primary NSCLC. Treatments were selected based on physical examination, medical history, complete blood test, routine blood chemistry, and CT scan of the chest and abdomen. All demographic and clinical data, including gender, age, clinical stage, ECOG performance status, tobacco smoking status, treatment management, and weight loss within six months after the initial hospital visit, were collected from patient's medical records. Patients were categorized as never-smokers if they had smoked <100 cigarettes in a lifetime. Former smokers were those that had quit tobacco smoking at least one year before study enrollment, and current smokers were those that continued smoking or quit smoking less than one year before study enrollment. The patients were followed up on regularly, and the last follow-up was conducted in January 2014. The median duration of follow-up was 26 months, and ranged from two to 62 months.

Chemoradiotherapy and response evaluation

Radiotherapy was administered to each patient using three-dimensional conformal radiotherapy (3D-CRT). Specifically,

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patients were placed in the radiotherapy position on a dedicated CT-simulator with both arms above the head, using a dedicated immobilization and patient laser marker system. CT images were retrieved and saved in a Pinnacle(3) radiation treatment planning system (Philips, Best, The Netherlands). The pulmonary gross tumor volume (GTV) was set as the primary tumor in the lung window (width 1,600 Hu, length –800 Hu) and defined by radiation oncologists. The volumes were generated for each phase within the treatment planning system. Only the primary tumor volume was evaluated, unless a nodal volume was the dominant or only lesion. The clinical target volume (CTV) was routinely created by expanding both the GTV and the metastatic mediastinal lymph nodes (GTV-n) by 0.6–0.8 cm. Where necessary, the planning target volume (PTV) was also created by expanding the CTV by 0.5 cm to compensate for any setup errors and respiratory motion. The radiotherapy protocol was designed to deliver a prescribed dose of 40~65 Gy in 20–30 fractions to the PTV. The treatments were delivered with a PRIMUS 6-MV X-ray linear accelerator (Siemens Corporation, Munich, Germany). All patients were treated for five days per week with 2 Gy daily fractions. After receiving a total dose of 40 Gy/20 fractions in four weeks, each patient was repeatedly scanned in the radiotherapy position with the dedicated CT-simulator, and the new CT data set was compared to the previous CT data set. However, the target volume was again contoured on the basis of the new CT data set according to indications of tumor regression. The new 3D-CRT plan was then calculated using a prescribed dose of 7.5~25 Gy administered to the new PTV in 3~10 fractions of 2.5 Gy/fraction.

Tumor size, in terms of GTV of radiotherapy, was calculated by the radiotherapy plan system before radiotherapy was initiated and in the middle of the radiotherapy period (40 Gy/20 fraction/4 weeks). The formula $R = \frac{GTV - GTVs}{GTV}$ was used to calculate the ratio of alterations in tumor size after radiotherapy, where R was the relative decrease in size of the tumor, GTV was the tumor size pre-radiotherapy, and GTVs was the tumor size after 40 Gy/20 fraction/4 weeks of radiotherapy.

Meanwhile, chemotherapy, including cisplatin (or carboplatin) in combination with a third-generation drug (e.g., etoposide, gemcitabine, vinorelbine, or taxanes), was given to most patients. Specifically, platinum-based doublet chemotherapy was administered for 2–6 cycles. Overall, 15 (10.7%) patients received radiotherapy alone, 53 (37.9%) patients received sequential chemoradiotherapy and 72 (51.4%) concurrent chemoradiotherapy.

SNP selection and genotyping

To select candidate gene SNPs for genotyping, we searched the international HapMap database (*http://hapmap.ncbi.nlm.nih.gov/*) and identified sequences that included 1 kb upstream and downstream of each gene in SNP selection. We then downloaded genotype data restricted to those of the Han population from Beijing, China (CHB), and identified two SNPs (rs471692 in *TOP2A* and rs2279574 in *DUSP6)* with minor allele frequencies (MAF) above 1% and with a minimum value of 0.8 for the r^2 parameter using the tagger algorithm implemented in Haploview Ver. 4.2 [18,19]. In particular, *DUSP6* RS2279574 is localized in exon 1, and causes an amino acid change from Val to Leu, while *TOP2A* RS471692 is localized in an intron, which does not alter any amino acids of the TOP2A protein.

To genotype these two SNPs, we collected 5 mL of peripheral blood from each participant into a sodium citrate tube, then extracted patient genomic DNA using a standard proteinase K digestion, followed by phenol-chloroform extraction and ethanol precipitation. These DNA samples were then genotyped using a TaqMan SNP genotyping assay kit (Affymatrix Inc., Cleveland, OH, USA) in an Applied Biosystems 7500 FAST Real-Time PCR System (Foster City, CA, USA). The primers and probes used for *TOP2A* rs471692 were 5'-ATC ACA AAC CTA AAA AAG AAA TCC A-3' and 5'-AAT TAT TTA CCT GGG TCC ATG TTC T-3'; for *DUSP6* RS2279574, 5'-AGC TTC TTG AGC AGCAGC CCG AGC A-3' and 5'-CGA CTC GCC GCC CGT ATT CTC GTT C-3'. The TaqMan universal PCR master mix and predesigned SNP-genotyping assay mixture containing PCR primers and probes were purchased from ABI. The PCR amplification contained 25 µL master mix (Applied Biosystems), 10 µL of DNA, 2.5 µL of probe, and 12.5 µL of ddH_2Or which was subjected to an initial denaturing step at 95°C for 10 minutes, followed by 47 cycles of 92°C for 30 seconds, 60°C for one minute, and a final extension at 60°C for one minute. To ensure accuracy of PCR amplification, we included three positive controls and two negative controls in each 96-well plate and randomly repeated 10% of the samples for quality control purposes. The concordance rate was 100% of the repeated analyses. A further 60 samples were randomly selected for direct DNA sequencing to confirm the TaqMan results, and DNA sequence data indicated 100% concordance.

Statistical analysis

The Hardy-Weinberg equilibrium was determined using a goodness-of-fit χ^2 test. The two-sided χ^2 test was performed to assess statistically significant differences between the demographic and clinical features of patients with recurrent and primary NSCLC. Overall survival was calculated as the length of time between histological diagnosis and death from any cause, or the date of the last follow-up. Differences in mean relative tumor size between pairs of groups were analyzed using an independent sample *t*-test. Association between the genotype and survival rate was estimated by the Kaplan-Meier curve and then the log-rank test. The median survival time (MST) was also calculated accordingly. A multivariate Cox proportional hazards model was used to estimate the hazard ratios (HRs) and their 95% confidential intervals (CIs) for overall survival.

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All statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) and a $p<0.05$ of a two-side test was considered statistically significant.

Results

Characteristics of patients

Clinicopathological data from these 140 consecutive patients with histologically confirmed NSCLC are summarized in Tables 1 and 2. In brief, of these patients, 105 (75.0%) were male, and 35 (25.0%) were female, and the median age was 60 years (range 31 to 82 years); 51 (36.4%) had adenocarcinoma, 89 (63.6%) squamous cell carcinoma, while 106 (75.7%) patients had primary NSCLC and 34 (24.3%) patients had an intrathoracic recurrent tumor after previous surgery. According to the AJCC 6th edition stage grouping criteria [20], six patients had stage I, 36 patients stage II, 76 patients stage III, and 22 patients stage IV disease, while 28 patients scored 0 on the ECOG performance status according to the World Health Organization (WHO) recommendations (Zubrod ECOG/WHO scores) and 112 patients scored 1.

Furthermore, there were no statistically significant differences in the gender, weight loss, tobacco smoking status, ECOG, location of tumor, histology, clinical stage, tumor stage, and lymph node metastasis of patients with primary tumors and recurrent tumors ($p>0.05$). Radiotherapy was delivered to both primary and recurrent NSCLC patients for a median dose of 56.0 (ranged from 47.5~65 Gy), while chemotherapy included cisplatin (or carboplatin) in combination with a third-generation drug (e.g., etoposide, gemcitabine, vinorelbine, or taxanes), administered for 2–6 cycles to 53 (37.9%) patients as sequential chemoradiotherapy and 72 (51.4%) as concurrent chemoradiotherapy; 15 (10.7%) patients only received radiotherapy.

Responses of patients to chemoradiotherapy

After a median radiation dose of 56.0 Gy and/or 2–6 cycles of platinum-based doublet chemotherapy, the mean tumor regression was 0.34 cm^3 during the course of radiotherapy, while the GTVs after 20 fractions of radiotherapy were slightly higher than the basal GTV in three patients. In comparison to the 15 patients treated with radiotherapy only, chemoradiotherapy achieved similar treatment responses.

Association of tumor regression with clinicopathological characteristics and allele frequency of *TOP2A* and *DUSP6* SNPs

There was no statistically significant difference between radiotherapy response and clinicopathological characteristics of NSCLC patients (Table 2). The genotype frequency of *TOP2A* rs471692 was 47.9% for C/C, 45.7% for C/T, and 6.4% for T/T, and the genotype frequency of *DUSP6* RS2279574 was 10.7% for C/C, 42.9% for C/A and 46.4% for A/A. However, the genotype frequency of *DUSP6* rs2279574 or *TOP2A* rs471692 was not significantly associated with clinicopathological characteristics, such as gender, age, weight loss, ECOG grade, location of tumor, clinical stages, lymph node metastasis, pathological type, or tobacco smoking status ($p>0.05$; Table 3).

There was no significant difference in the tumor regression observed in the *TOP2A* rs471692 C/C, C/T, and T/T genotypes (0.355±0.169 cm, 0.338±0.226 cm, and 0.457±0.135 cm, respectively) ($F=1.455$ $p=0.237$). Tumor regression also did not differ significantly between the CC and combined C/T and T/T genotypes of *TOP2A* ($t=-1.634$, $p=0.105$). Similarly, there was no significant difference in the tumor regression observed in the *DUSP6* rs2279574 C/C, A/C, and A/A genotypes (0.369±0.181 cm, 0.344±0.200 cm, and 0.360±0.199 cm, respectively) ($F=0.152$ $p=0.859$). Tumor regression also did not differ significantly between the CC and combined A/C and A/A genotypes ($t=-0.313$, $p=0.755$; Tables 3, 4).

Association of overall survival with clinicopathological characteristics and allele frequency of *TOP2A* and *DUSP6* SNPs

We further compared overall survival of patients with stage I–III NSCLC to assess predictors of the effect of radiotherapy and chemoradiotherapy. A total of 87 patients were analyzed, of which four were lost to follow-up (95.4% were thus included in data analysis). By the last follow-up, 53 patients had died, the majority as a result of lung cancer due to local disease progression, distant metastases, or both. One death was attributed to cardiac arrest and another to a cerebrovascular accident, but no treatment-related deaths occurred during this study. The overall one-, two-, and three-year survival rates were 74.7%, 52.6%, and 33.9%, respectively, with a median survival of 26 months (range: 2 to 62 months). Univariate analysis indicated that tumor lymph node metastasis (N stage) and clinical stage were significantly associated with overall survival of patients (Table 5), while multivariate analyses indicated the following predictors of survival after definitive radiotherapy or chemoradiotherapy: tumor regression ($p=0.009$, 95% CI 2.454 [1.256–4.796]), weight loss ($p=0.043$, 95% CI 2.791 [1.032–7.548]), clinical stage ($p=0.004$, 95% CI 1.707 [1.189–2.451]), and cigarette smoking ($p=0.025$, 95% CI 1.561 [1.056–2.306]); Table 5). However, there was no statistical significance between overall survival in *TOP2A* and *DUSP6* SNP groups ($p>0.05$).

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Table 1. Clinicopathological characteristics of NSCLC patients (n=140).

Variables	Primary tumor (n=106)		Recurrent tumor (n=34)		*P* value*
Age (years)	62	(35–82)	57	(31–78)	0.13
Gender (%)					0.82
Male	80	(57.1)	25	(17.9)	
Female	26	(18.6)	9	(6.4)	
Weight loss (%)					0.93
<5	93	(66.4)	30	(21.4)	
≥5	13	(9.3)	4	(2.9)	
Tobacco smoking (%)					0.062
Never	28	(20.0)	14	(10.0)	
Former	16	(11.4)	8	(5.7)	
Current	62	(44.3)	12	(8.6)	
ECOG(%)					0.69
0	22	(15.7)	6	(4.3)	
1	84	(60.0)	28	(20.0)	
Location of tumor (%)					0.20
Peripheral type	34	(24.3)	15	(10.7)	
Central type	72	(51.4)	19	(13.6)	
Histology (%)					0.50
Squamous cell	69	(49.3)	20	(14.3)	
Adenocarcinoma	37	(26.4)	14	(10)	
Clinical stage (%)					0.61
I	4	(2.9)	2	(1.4)	
II	27	(19.3)	9	(6.4)	
III	56	(40.0)	20	(4.3)	
IV	19	(13.6)	3	(2.1)	
Tumor stage (%)					0.33
T1–T2	63	(45.0)	17	(12.1)	
T3–T4	43	(30.7)	17	(12.1)	
Node status (%)					0.64
N0–N1	39	(27.9)	14	(10.0)	
N2–N3	67	(47.9)	20	(14.3)	
Treatment					0.48
RT alone	13	(9.3)	2	(1.4)	
Sequential	38	(27.1)	15	(10.7)	0.67
Concurrent	55	(39.3)	17	(12.1)	

* χ^2 test.

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Table 2. Association of NSCLC clinicopathological characteristics with tumor regression (n=140).

Variables	Patients	Tumor regression (mean ±SD)	*P* value[a]
Age (yrs.)			0.87
≤60	71	0.351±0.21	
>60	69	0.356±0.18	
Gender			0.66
Male	105	0.350±0.19	
Female	35	0.366±0.19	
Weight loss			0.63
0~5%	123	0.357±0.19	
≥5%	17	0.333±0.18	
Tobacco smoking			0.091
No	42	0.311±0.22	
Yes	98	0.372±0.18	
ECOG			0.27
0	28	0.390±0.19	
1	122	0.345±0.19	
Location of tumor			0.89
Peripheral type	49	0.357±0.21	
Central type	91	0.352±0.18	
Histology			0.29
Squamous cell	89	0.367±0.19	
Adenocarcinoma	51	0.331±0.19	
Clinical stage			0.80
I–II	42	0.347±0.18	
III–IV	98	0.356±0.20	
Tumor stage			0.28
T1–T2	80	0.369±0.19	
T3–T4	60	0.333±0.19	
Node status			0.79
N0–N1	53	0.348±0.18	
N2–N3	87	0.357±0.20	
GTV* (cm^3)			0.075
≤91	72	0.325±0.19	
>91	68	0.384±0.19	
Primary or recurrent tumor			0.41
Primary	106	0.361±0.20	
Recur	34	0.329±0.18	
ChemoRT			0.10
Sequential or RT alone	68	0.326±0.19	
Concurrent	72	0.380±0.19	

* Subgroup was divided by median counts, *P* value[a] was calculated by Student *t* test.

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Table 3. Association of genotype frequency of TOP2A RS471692 and DUSP6 RS2279574 with clinicopathological characteristics of NSCLC patients (n=140).

Variables	Rs2279574			*P* value[a]	Rs471692			*P* value[b]
	C/C	C/A	A/A		C/C	C/T	T/T	
Age (yrs.)				0.54				0.53
≤60	9	32	30		34	34	3	
>60	6	28	35		33	30	6	
Gender				0.14				0.49
Male	12	40	53		53	45	7	
Female	3	20	12		14	19	2	
Weight loss				0.089				0.62
0~5%	11	56	56		57	58	8	
≥5%	4	4	9		10	6	1	
Tobacco smoking				0.45				0.89
No	3	21	18		21	18	3	
Yes	12	39	47		46	46	6	
ECOG				0.39				0.83
0	5	11	12		12	14	2	
1	10	49	53		55	50	7	
Location of tumor				0.67				0.67
Peripheral type	4	23	22		22	25	2	
Central type	11	37	43		45	39	7	
Histology				0.68				0.61
Squamous cell	8	39	42		43	39	7	
Adenocarcinoma	7	21	23		24	25	2	
Clinical stage				0.53				0.40
I–II	6	19	17		17	21	4	
III–IV	9	41	48		50	43	5	
Tumor stage				0.38				0.84
T1–T2	7	38	35		40	35	5	
T3–T4	8	22	30		27	29	4	
Node status				0.96				0.31
N0-N1	6	22	25		21	28	4	
N2-N3	9	38	40		46	36	5	
GTV* (cm^3)				0.53				**
≤91	6	30	36		33	38	1	
>91	9	30	29		34	26	8	
Primary or recurrent tumor				**				**
Primary	12	44	50		49	49	8	
Recur	3	16	15		18	15	1	
ChemoRT				0.45				0.90
Sequential or RT alone	5	30	33		32	31	5	
Concurrent	10	30	32		35	33	4	

* Subgroup was divided by median counts, *P* value[a] was calculated by Student *t* test, *P* value[b] by χ^2 test; ** expected count <5% was not calculated by χ^2 test.

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Table 4. Association of genotype frequency of *TOP2A* rs471692 and *DUSP6* rs2279574 with tumor regression of NSCLC patients.

Genotype	Patients	Tumor regression (mean ±SD)	*P* value
RS2279574			0.85[a]
C/C	15	0.369±0.181	
C/A	60	0.344±0.200	
A/A	65	0.360±0.199	
C/A+A/A	125	0.352±0.199	0.75[b]
RS471692			0.23[a]
C/C	67	0.355±0.169	
C/T	64	0.338±0.226	
T/T	9	0.457±0.135	
C/T+T/T	131	0.349±0.196	0.10[b]

[a] *P* value was calculated by One-Way ANOVA; [b] *P* value was calculated by Student *t* test.

Table 5. Multivariate analysis for overall survival.

Factor	n	β	SE	*P* value	HR (95%CI)
Weight loss (≥5%/0~5%)	77/10	1.026	0.508	0.043	2.79 (1.032~7.548)
Tumor regression (≤0.34/>0.34)	44/43	0.898	0.342	0.009	2.45 (1.256~4.796)
Clinical stage (III/I–II)	31/56	0.535	0.184	0.004	1.70 (1.189~2.451)
Tobacco smoking (Current/Former/Never)	23/11/53	0.445	0.199	0.025	1.561 (1.056~2.306)

SE – standard error; HR – hazard ratio.

Table 6. Stratified analysis of tumor regression and survival of stage I–III NSCLC patients by clinical stage (n=87).

Clinical stage	Tumor regression	# Patients	MST (months)	χ^2	Log-rank p value
I/II				0.93	0.33
	≤0.34	20	62		
	>0.34	11	32		
III				7.27	0.007
	≤0.34	23	16		
	>0.34	33	34		

Stratification analysis of treatment responses and survival of patients

Clinicopathological variables may represent confounding factors affecting tumor regression and overall survival. Thus, we stratified the patients according to clinicopathological variables and analyzed tumor regression in each group (Table 6). We found that tumor regression ≤0.34 cm was associated with poor survival of stage III NSCLC patients (log-rank p=0.007; Figure 1). However, stratifying patients for weight loss, tumor histology, and ECOG performance status did not indicate any significant associations with survival (data not shown).

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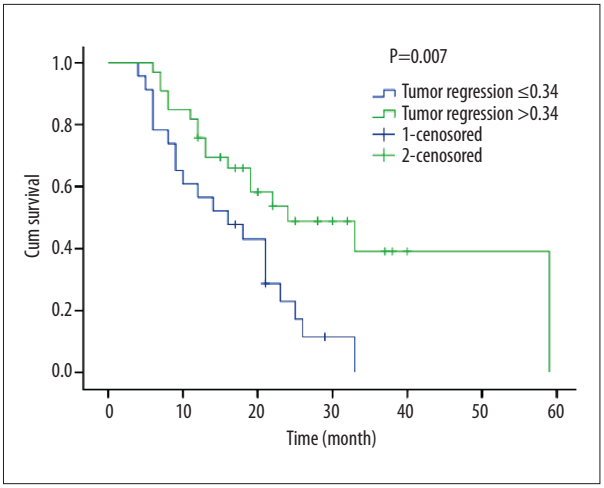

Figure 1. Kaplan-Meier curves of overall survival in patients with stage III NSCLC, stratified by ratio of tumor regression.

Discussion

The responses of NSCLC patients to conformal radiotherapy have been previously reported, but since the responses of individual patients vary dramatically, the role of conformal radiotherapy in NSCLC remains controversial [21–25].Thus, in this study, we assessed the association of SNPs in *TOP2A* and *DUSP6* with chemoradiotherapy responses and prognosis in 140 Chinese NSCLC patients. In this group we found no statistically significant differences between the response of patients with *TOP2A* rs471692 and *DUSP6* rs2279574 polymorphisms or clinicopathological variables to chemoradiotherapy. We also did not find *TOP2A* and *DUSP6* SNPs to be associated with overall survival. Multivariate analyses indicated that tumor regression, weight loss, clinical stage, and cigarette smoking were all independent prognostic predictors of response to chemoradiotherapy. Our data, thus, suggests that *TOP2A* rs471692 and *DUSP6* rs2279574 SNPs are not associated with chemoradiotherapy response, whereas tumor regression, weight loss, clinical stage, and cigarette smoking are independent prognostic predictors of chemoradiotherapy response in Chinese NSCLC patients.

Indeed, Kupelian et al. previously reported that in ten NSCLC patients treated with external beam radiotherapy, tumor regression, detected by serial megavoltage CT images, occurred at a rate of 0.6% to 2.3% per day (average 1.2% per day) [21]. The smallest lesions regressed most slowly, and the largest lesions regressed most rapidly. Woodford et al. reported that in 17 NSCLC patients that received 30 fractions of radiotherapy via helical tomotherapy, a GTV change of between –12 and –87% (average –38%) was observed, and GTV change was significantly associated with patient's physical or tumor features [22]. Fox et al. evaluated sequential CT scans to quantify reduction of tumor volume after a course of conventional radiotherapy in 22 patients with stage I–III NSCLC, 15 of whom received concurrent chemotherapy [23]. The median GTV reduction did not differ significantly between patients receiving chemoradiotherapy (24.7%, range, –0.3% to 61.7%) and radiotherapy (44.3%, range 0.2% to 81.6%) [23]. Our results were consistent with these and other previous studies [21–25]. We found no patient, treatment, or tumor characteristics to be associated with tumor regression, although there was a tendency toward a greater volume reduction in patients with a GTV >91 cm^3 (GTV >91 cm^3 versus <91 cm^3) (p=0.075). The rate of tumor volume shrinkage varied during the course of radiotherapy observed in our current study, and we also observed that after four weeks of radiotherapy, the GTV of three patients had increased slightly. This phenomenon was also reported by Yee et al. [25]. Perhaps acute radiotherapy-induced cell inflammation could increase the tumor volume transiently [25,26]. In addition, accelerated repopulation with rapid proliferation and a high growth index may have contributed to this phenomenon [25].

Genetic variants have been reported to influence response to radiotherapy, and have thus improved our understanding of the effect of radiation on human tumors. Polymorphic genetic variants, such as SNPs, can alter the sensitivity of different cancers to radiotherapy [8–11]. However, to date, no cell proliferation–associated genes have been reported to affect radiotherapy responses. In this study, we identified *TOP2A* and *DUSP6* SNPs and investigated their association with NSCLC regression after chemoradiotherapy. Earlier studies [27–31] have implicated these two gene products in cell proliferation. The TOP2A enzyme controls the topologic states of DNA during gene transcription [32]. Mutations of TOP2A have been associated with chemotherapy resistance [33,34], and several anticancer agents have been developed to target TOP2A activity [33,34]. Furthermore, DUSP6 is a member of the dual specificity protein phosphatase subfamily and functions to negatively regulate the activity of the mitogen-activated protein (MAP) kinase superfamily (MAPK/ERK, SAPK/JNK, or p38), thus regulating cell proliferation and differentiation [29–31]. Previous studies have shown that lost DUSP6 expression was associated with cancer progression and resistance [17,35]. However, our current data did not reveal any association between *TOP2A* and *DUSP6* SNPs and chemoradiotherapy responses or survival of NSCLC patients. However, we did find that tumor regression, weight loss, clinical stage, and cigarette smoking were all independent predictors of chemoradiotherapy responses in these patients. Thus, future study will focus on the role of these variants in chemoradiotherapy of advanced stage NSCLC patients.

Conclusions

TOP2A rs471692 and *DUSP6* rs2279574 SNPs did not associate with chemoradiotherapy response, whereas tumor

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regression, weight loss, clinical stage, and cigarette smoking were independent prognostic predictors for these Chinese patients with NSCLC.

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Conflict of interest statement

The authors declared that there is no conflict of interest in this work.

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