$\Delta 133p53/FLp53$ predicts poor clinical outcome in esophageal squamous cell carcinoma

Qimin Tu (✉ tuqimin75@sina.com)

Enshi clinical college of wuhan univerisity <https://orcid.org/0000-0003-0525-4010>

Hongjian Gong

Wuhan Women and Children Medical Care Center

Chunhui Yuan

Wuhan Women and Children Medical Care Center

Gao Liu

Enshi clinical college of Wuhan University

Jinqi Huang

Enshi clinical college of Wuhan University

Zhichao Li

Wuhan Women and Children Medical Care Center

Jianfei Luo

Wuhan University Renmin Hospital

Research article

Keywords: Esophageal squamous cell carcinoma, p53, $\Delta 133p53$, Prognosis, Recurrence

Posted Date: September 25th, 2019

DOI: <https://doi.org/10.21203/rs.2.15278/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Cancer Management and Research on August 1st, 2020. See the published version at <https://doi.org/10.2147/CMAR.S263559>.

Abstract

Background p53 isoform $\Delta 133p53$ is directly transactivated by p53 and antagonizes p53 activities in cancer progression. However, its correlation with prognosis and cancer recurrence in esophageal squamous cell carcinoma (ESCC) is still unclear. Methods Expression of $\Delta 133p53$ and $\Delta 133p53$ /full-length p53 (FLp53) in tissues and serums of 180 ESCC patients was evaluated using qRT-PCR. Results Tissue $\Delta 133p53$ expression and $\Delta 133p53$ /FLp53 ratio were significantly increased in ESCC tissue compared with adjacent normal tissue. Pre-operative $\Delta 133p53$ expression and $\Delta 133p53$ /FLp53 ratio in tissue or serum samples were positively associated with TNM stage and post-operative recurrence. Kaplan-Meier curve and multivariate cox regression analyses revealed that the tissue and serum $\Delta 133p53$ /FLp53 ratio (cutoff value: 2.9160) were independent prognostic factors for overall survival (OS) and progression-free survival (PFS) in ESCC patients, and showed no statistical difference in receiver-operating characteristic (ROC) curve analysis, while serum $\Delta 133p53$ showed no significant prognostic value. More importantly, the serum $\Delta 133p53$ /FLp53 ratio in ESCC patients was significantly decreased within 72 h post tumor resection and patients with a consistently high serum $\Delta 133p53$ /FLp53 ratio (≥ 2.9160) had higher recurrence rates than those with consistently low ratio values. In addition, dynamic detection in each follow-up timepoint showed that serum $\Delta 133p53$ /FLp53 ratios were higher than 2.9160 upon recurrence, and they even increased prior to radiologic progression. Conclusion The serum $\Delta 133p53$ /FLp53 ratio can be a novel predictor for survival outcome and may serve as a real-time parameter for monitoring recurrence in ESCC patients after surgery.

Background

Esophageal cancer is the fourth leading cause of cancer-related death in both men and women in China [1], and approximately 90% of Chinese patients with esophageal cancer were diagnosed with esophageal squamous cell carcinoma (ESCC) [2]. Recently, multiple approaches have been developed to improve the outcome of ESCC, including esophagectomy combined with radiotherapy or chemotherapy. However, the 5-year overall survival rate is still inferior and ranges from 15 to 25% in all patients [3]. This is thought to be due to a combination of late stage of diagnosis, rapid development of ESCC and lack of effective therapies [4]. Moreover, molecular biomarkers capable of predicting recurrence and metastasis, which frequently developed in ESCC patients diagnosed with localized disease, have not yet been developed and thus treatment decisions regarding systemic therapy have been delayed.

Events involved in driving cancer evolution affect tumor aggressiveness, response to treatment and patient prognosis [5]. To identify prognostic biomarkers, several recent large-scale genomic studies have characterized hundreds of somatic mutations, copy number alterations (CNAs) and structural variants (SVs) in ESCC [4, 6-10]. Mutations in SLC39A6 [4], EP300 (10%) [9, 10], and FAM135B (6.8%) [7], as well as amplification or overexpression of EGFR [5] were associated with a poor prognosis of ESCC. Moreover, many genes involved in cell cycle regulation, DNA repair and apoptosis were mutated or amplified in 99% of cases, including TP53 (93%), CCND1 (33%), CDK4/CDK6 (23.6%), CDKN2A (20%), NFE2L2 (10%) and RB1 (9%) [7, 9]. As the highest frequency of mutated gene, mutation of TP53 in the DNA binding domain

has been regarded as the main cause for p53 inactivation and proposed as a key factor in driving ESCC progression [9]. Two compounds (i.e. APR-246, COTI-2) can reactivate missense-mutant p53 protein have being tested in clinical Phase I/II trials [11]. However, a growing body of evidence has suggested that abundance of p53 isoforms in cancer tissues, due to alternative splicing or usage of alternative promoters, also modulates the biological processes regulated by full-length p53 (FLp53) [12]. Importantly, the origin of the tumor-promoting functions of p53 mutations also depend on the shorter p53 isoforms from the mutated FLp53 in some specific conditions, like missense mutations [13].

The human p53 gene encodes 12 natural p53 isoforms, including $\Delta 133\text{p53}$, an N-terminally truncated isoform (lacking the first 132 amino acids) [14]. $\Delta 133\text{p53}$ is directly transactivated by FLp53 in response to DNA-damaging signals and antagonizes p53 apoptotic activity [15]. Furthermore, $\Delta 133\text{p53}$ also stimulates angiogenesis, metastasis and generation of cancer stem cells regardless of p53 mutations, and its mRNA expression is associated with increased risk of recurrence and poor outcome in various cancers [16-20]. Recently, several studies further clarified that elevated $\Delta 133\text{p53}$ promotes an immunosuppressive and chemoresistant environment by inducing secretion of CCL-2, IL-6 and the recruitment of tumor-associated macrophages [21, 22]. Thus, $\Delta 133\text{p53}$ isoform serves as an important oncogene by antagonistically modulating p53 functions [23, 24]. Previously, we have demonstrated that $\Delta 133\text{p53}$ promotes DNA double-strand break (DSB) repair and tumorigenesis by coordinating with p73 in a p53-independent manner [12, 25], however the expression level of $\Delta 133\text{p53}$ and its prognostic performance in ESCC remains unknown.

In the present study, we first measured the levels of $\Delta 133\text{p53}$ and p53 and calculated their ratio ($\Delta 133\text{p53}/\text{FLp53}$) in cancerous tissue compared to adjacent normal tissues; subsequently, we analyzed their prognostic value in ESCC patients undergoing tumor resection. Circulating p53 mutations were significantly associated with cancer recurrence and overall survival of patients with breast cancer [26]. Based on promising results in the tissue, we further investigated dynamic changes and prognostic significance of the serum $\Delta 133\text{p53}/\text{FLp53}$ ratio in ESCC patients. It was found that a preoperative high serum or tissue $\Delta 133\text{p53}/\text{FLp53}$ ratio were found to be independent prognostic factors for overall survival (OS) or progression-free survival (PFS) in ESCC patients undergoing curative resection. More importantly, patients with a consistently high serum $\Delta 133\text{p53}/\text{FLp53}$ ratio (≥ 2.9160) (pre- and post-operative) had higher recurrence rates than those with consistently low ratio values. Dynamic detection at each follow-up timepoint indicated that serum $\Delta 133\text{p53}/\text{FLp53}$ ratios increased prior to radiologically detected progression. Based on these results, monitoring dynamic changes of serum $\Delta 133\text{p53}/\text{FLp53}$ ratio may be a promising predictor of treatment response and recurrence risk in ESCC patients.

Methods

Patients and samples

FFPE cancerous tissue samples and parallel serum samples of 180 ESCC patients with overall survival information were collected from the Central Hospital of Enshi Autonomous Prefecture (China) between

January 2009 to December 2012. All cases were pathologically confirmed as ESCC and did not receive chemotherapy and radiotherapy before tumor resection. Serum samples were obtained before and within 72 h following surgery, and then stored at -80°C for further use. FFPE tissue samples were obtained at the time of surgery and adjacent normal tissue samples were also collected from 42 of 180 ESCC patients. Due to the fact that 31 patients had no regular follow-up checks for recurrence or progression after surgery (confirmed by CT scan), 149 in 180 ESCC patients with recurrence information were included for PFS analysis. Patient clinical information, including age, gender, BMI, smoking, clinicopathological features (tumor location, TNM and differentiation stage) were obtained from the clinical or pathologic records. All cases of ESCC were staged based on the 7th edition of the AJCC/UICC TNM classification system. This study was approved by the Ethics Committee of Central Hospital of Enshi Autonomous Prefecture. All participants were informed about the purpose, procedures, benefits and potential risks of the study, and their written or oral consents were obtained.

RNA isolation and cDNA synthesis

Five 8- μm -thick FFPE tissue samples were cut from each block and placed in sterile 1.5-ml centrifuge tubes ready for extraction. Total RNA was extracted using an RNAprep Pure FFPE Kit (TIANGEN Biotech, Beijing, China) according to the manufacturer's instructions. Total RNA of 300 μL serum was isolated by using a Liquid Total RNA Isolation Kit (BioTeke, Beijing, China) as we described previously [27] and treated with DNase I prior to reverse transcription. The concentration of RNA was measured by Nanodrop 2000 spectrophotometer (Thermo Fisher) following purification with RNeasy mini kit (QIAGEN). First strand cDNA was synthesized using M-MLV Reverse Transcriptase (Invitrogen) and conditions were as follows: 65°C for 5 min, and then 37°C for 50 min, 70°C for 15 min.

Quantitative real-time PCR (qRT-PCR)

The expressions of $\Delta 133\text{p}53$ and full-length p53 (FLp53) were determined on the ABI-7300 (Thermo Fisher) using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific) according to the manufacturer's instructions. The primers used were as follows: $\Delta 133\text{p}53$ [15] (Forward: 5'-TGGGTTGCAGGAGGTGCTTAC-3', Reverse: 5'-CCACTCGGATAAGATGCTGAGG-3'), FLp53 (Forward: 5'-GCTCAAGACTGGCGCTAAA-3', Reverse: 5'-GTTTTTCAGGAAGTAGTTTCCATAG G-3'), and GAPDH (Forward: 5'-GAGTCAACGGATTTGGTCGTAT-3', Reverse: 5'-ATGGGTGGA ATCATATTGGAAC-3') was used as the endogenous control [28]. The reactions were started at 95°C for 5 min, followed by 42 cycles of 95°C for 30 s, 59.3°C for 30 s, and 72°C for 30 s. All experiments were carried out in triplicate for each data point. Relative quantification of $\Delta 133\text{p}53$ and FLp53 expression was calculated with the $2^{-\Delta\text{Ct}}$ method.

Statistical analysis

SPSS software (version 17.0; USA) and Graphpad Prism software (version V.5.00, USA) were used to perform the statistical analyses in this study. Differences in the $\Delta 133p53/FLp53$ ratio between ESCC and paired adjacent normal tissues were compared using a paired Student's *t*-test. Patients were divided into relative high- and low-expression groups according to the cutoff value determined by X-tile 3.6.1 software (Yale University, New Haven, CT, USA) [29] in serum and tissue samples for survival analysis. The χ^2 test or Fisher's exact test were used to determine the correlations of $\Delta 133p53/FLp53$ in tissue or serum samples with the clinicopathological factors of ESCC patients. Pearson's correlation coefficient analysis was used to determine the association of the $\Delta 133p53/FLp53$ ratio in the tissue and in the serum. Survival analysis was carried out by the Kaplan-Meier method, and subjected to the log rank test. Univariate and multivariate Cox survival (Forward Stepwise Likelihood Ratio method) analyses were applied to assess the hazard ratios (HRs). $P < 0.05$ was considered statistically significant. Receiver operating characteristic (ROC) curve analysis was conducted by MedCalc statistical software (Version 18.11.6, USA) to determine the predictive value of the parameters.

Results

$\Delta 133p53$ and $\Delta 133p53/FLp53$ ratio significantly increased in ESCC tissues

As there is no specific antibody for the $\Delta 133p53$ isoform [30], we first examined the mRNA abundance of $\Delta 133p53$ in 42 pairs of tumor and adjacent normal tissues to determine whether $\Delta 133p53$ was associated with ESCC progression. $\Delta 133p53$ isoform significantly increased in cancerous tissues (0.10 ± 0.09 vs 0.05 ± 0.03 , $P = 0.0019$) (Fig. 1A). We simultaneously detected mRNA expression of FLp53 in these paired samples and confirmed that FLp53 is significantly decreased in cancerous tissues of ESCC patients (0.06 ± 0.04 vs 0.17 ± 0.14 , $P < 0.0001$) (Fig. 1B). In addition, we also noticed that the expression ratio of $\Delta 133p53$ to FLp53 significantly increased in cancerous tissues (2.40 ± 3.16 vs 0.50 ± 0.64 , $P = 0.0006$) (Fig. 1C).

Elevated tissue $\Delta 133p53/FLp53$ ratio predicts poor outcome of ESCC patients

To confirm the association of $\Delta 133p53$ and FLp53 with clinical outcomes of ESCC patients, we then measured $\Delta 133p53$ and FLp53 expression in 180 FFPE ESCC tissue samples. The optimal cut-off values of $\Delta 133p53$, FLp53 and $\Delta 133p53/FLp53$ ratio for predicting the prognosis of ESCC were estimated by X-tile 3.6.1 software (Fig. S1). ESCC patients were divided into two groups ($\Delta 133p53 < 0.0425$ and ≥ 0.0425 ; FLp53 < 0.0195 and ≥ 0.0195 ; $\Delta 133p53/FLp53$ ratio < 2.6470 and ≥ 2.6740). The clinicopathological characteristics of the ESCC patients divided by the cut-off values of $\Delta 133p53$, FLp53 and $\Delta 133p53/FLp53$ ratio are shown in Table 1. Our results indicated that the elevated $\Delta 133p53$ was significantly associated with increased TNM stage ($P = 0.0011$), differentiation grade ($P = 0.0030$) and recurrence ($P = 0.0371$), although it had no significant correlations with other clinicopathological features

such as age, gender, and BMI index (Table 1). Decreased FLp53 was significantly associated with male gender ($P = 0.0166$), higher BMI ($P = 0.0078$), lower tumor location ($P = 0.0295$), increased TNM stage ($P < 0.0001$), differentiation grade ($P < 0.0001$) and recurrence $P < 0.0001$) (Table 1). Increased $\Delta 133p53/FLp53$ ratio was significantly associated with Lower tumor location ($P = 0.0091$), increased TNM stage ($P < 0.0001$), differentiation grade ($P < 0.0001$) and recurrence $P < 0.0001$) (Table 1).

The median survival time of all patients was 25.47 months (range 0.3–60 months), 106 patients (58.89%) were censored and 74 patients (41.11%) died during our follow-up period. Kaplan-Meier analysis revealed that increased tissue abundance of $\Delta 133p53$ ($\chi^2 = 4.83$, $P = 0.0279$) (Fig. 1D) and decreased p53 expression ($\chi^2 = 27.76$, $P < 0.0001$) (Fig. 1E), as well as increased $\Delta 133p53/FLp53$ ratio ($\chi^2 = 42.34$, $P < 0.0001$) (Fig. 1F) were significantly related to worse OS. By the time of analysis, 149 of 180 ESCC patients had regular follow-up checks for recurrence after surgery. Recurrence occurred in 61 of 149 patients, with a median follow-up time of 13.4 months (range 0.8–42 months), and 88 patients had clear evidence of no recurrence. Tissue $\Delta 133p53$ and $\Delta 133p53/FLp53$ ratio were significantly increased in recurrent patients compared with non-recurrent ones ($P = 0.0032$ and $P < 0.0001$, respectively), and FLp53 was decreased in recurrent patients ($P = 0.0023$) (Fig. S2). Kaplan-Meier analysis revealed that increased $\Delta 133p53$ ($\chi^2 = 4.69$, $P = 0.0304$) (Fig. 1G) showed a weak correlation with poor PFS, while decreased p53 expression ($\chi^2 = 15.53$, $P < 0.0001$) (Fig. 1H) and increased $\Delta 133p53/FLp53$ ratio ($\chi^2 = 39.18$, $P < 0.0001$) (Fig. 1I) were significantly related to worse PFS. Compared with tissue $\Delta 133p53$ and FLp53 expression, tissue $\Delta 133p53/FLp53$ ratio showed the strongest correlation with OS and PFS of ESCC patients.

Serum $\Delta 133p53/FLp53$ ratio shows prognostic significance in ESCC patients

p53 could exist as circulating DNA or mRNA in cancers [28, 31], we next further evaluated $\Delta 133p53$ and FLp53 mRNA expression in serum of 180 ESCC patients. Pearson's correlation coefficient analysis revealed that serum $\Delta 133p53$, FLp53 and $\Delta 133p53/FLp53$ ratio were positively correlated with their tissue expression, and Pearson's correlation coefficients were 0.7019 ($P < 0.0001$) (Fig. 2A), 0.4030 ($P < 0.0001$) (Fig. 2B) and 0.4137 ($P < 0.0001$) (Fig. 2C), respectively. ESCC patients were divided into two groups ($\Delta 133p53 < 0.0870$ and ≥ 0.0870 ; FLp53 < 0.0300 and ≥ 0.0300 ; $\Delta 133p53/FLp53$ ratio < 2.9160 and ≥ 2.9160) by X-tile 3.6.1 software (Fig. S3). Correlation analysis revealed that the elevated serum $\Delta 133p53$ was significantly associated with male gender ($P = 0.0105$), increased M stage ($P = 0.0086$), N stage ($P = 0.0184$) and TNM stage ($P = 0.0008$), as well as recurrence ($P = 0.0005$) (Table 2). Increased $\Delta 133p53/FLp53$ ratio was significantly associated with high BMI index ($P = 0.0287$), increased M stage ($P < 0.0001$), N stage ($P = 0.0133$), TNM stage ($P < 0.0001$), differentiation grade ($P = 0.0131$) and recurrence ($P < 0.0001$) (Table 2). Whereas decreased serum FLp53 was only associated with increased TNM stage ($P = 0.0276$) and recurrence ($P = 0.0007$) (Table 2).

Serum $\Delta 133p53$ and $\Delta 133p53/FLp53$ ratio were significantly increased in recurrent patients compared with non-recurrent ones ($P = 0.0002$ and $P < 0.0001$, respectively) (Figs. S4A and S4C), FLp53 was

decreased in recurrent patients ($P = 0.0001$) (Fig. S4B). However, survival analysis indicated that increased serum $\Delta 133p53$ showed no association with OS ($\chi^2 = 2.89$, $P = 0.0890$) (Fig. 2D) and PFS ($\chi^2 = 2.93$, $P = 0.0871$) (Fig. 2E). Decreased p53 expression correlated with decreased OS ($\chi^2 = 5.80$, $P = 0.0160$) (Fig. 2F) and PFS ($\chi^2 = 10.27$, $P = 0.0014$) (Fig. 2G); and increased $\Delta 133p53/FLp53$ ratio correlated with poor OS ($\chi^2 = 17.26$, $P < 0.0001$) (Fig. 2H) and PFS ($\chi^2 = 56.16$, $P < 0.0001$) (Fig. 2I). Compared with serum $\Delta 133p53$ and FLp53 expression, serum $\Delta 133p53/FLp53$ ratio showed the strongest correlation with OS and PFS of ESCC patients.

Tissue and serum $\Delta 133p53/FLp53$ ratio are poor independent prognostic factors for ESCC

The univariate survival analysis indicated that tissue and serum $\Delta 133p53/FLp53$ ratio were correlated with OS (Table 3) and PFS (Table 4) of ESCC patients. Furthermore, the multivariate analyses identified that the tissue $\Delta 133p53/FLp53$ ratio was an independent prognostic factor for OS (HR = 3.864; 95% CI = 2.293–6.511, $P < 0.0001$) (Table 3) and PFS (HR = 2.283; 95% CI = 1.387–3.760, $P < 0.0001$) (Table 4) of ESCC patients. The serum $\Delta 133p53/FLp53$ ratio was also an independent prognostic factor for OS (HR = 2.503; 95% CI = 1.465–4.276, $P = 0.0011$) (Table 3) and PFS (HR = 3.230; 95% CI = 1.947–5.359, $P < 0.0001$) (Table 3) of ESCC patients. Besides, M stage and recurrence were also predictive indicators for OS and PFS. However, tissue and serum $\Delta 133p53$ or FLp53 were not independent prognostic factors for OS and PFS of ESCC patients. The one exception is tissue FLp53 (data not shown).

Tissue and serum $\Delta 133p53/FLp53$ ratios show no difference in prognostic performance for OS and PFS

We also conducted a ROC analysis of tissue and serum $\Delta 133p53/FLp53$ ratio. The AUC values of tissue and serum $\Delta 133p53/FLp53$ ratio in the Cox regression model for OS were 0.695 ($P = 0.0351$) and 0.641 ($P = 0.0360$), respectively (Fig. 3A), and 0.692 ($P = 0.0337$) and 0.649 ($P = 0.0340$) for PFS, respectively (Fig. 3B). Prognostic performance of tissue and serum $\Delta 133p53/FLp53$ ratio showed no statistical difference for OS and PFS ($P = 0.2066$ and $P = 0.3207$, respectively).

The dynamic change of serum $\Delta 133p53/FLp53$ ratio after surgery and its association with recurrence

To evaluate whether serum $\Delta 133p53/FLp53$ ratio dynamically correlates with treatment response, we further measured its postoperative level in 180 ESCC patients within 72 h after resection. Postoperative serum $\Delta 133p53/FLp53$ ratio significantly dropped after surgery (4.31 ± 7.69 vs 2.81 ± 4.12 , $P = 0.0098$) (Fig. 4A). Based on changes between preoperative and postoperative serum $\Delta 133p53/FLp53$ ratio, patients were divided into four groups: I, persistent high levels at the two time points; II, preoperative high followed by postoperative low; III, preoperative low followed by postoperative high, and IV, preoperative low followed by postoperative low, using the same cutoff value of preoperative serum $\Delta 133p53/FLp53$

ratio (2.9160). Persistent high levels of serum $\Delta 133p53/FLp53$ ratio predicted poor OS (Fig. 4B), higher recurrence rates (Fig. 4C) and worse PFS (Fig. 4D) in ESCC patients. Patients in group I showed significantly shorter time to relapse (TTR) and higher recurrence rates than group IV (median TTR of 5.57 months *vs* not reached; recurrence of 27/28 *vs* 14/88, $P < 0.0001$), as well as group III (median TTR of 5.57 months *vs* 29.4 months; recurrence of 27/28 *vs* 4/13, $P < 0.0001$), while there was a propensity of increased recurrence compared with group II (median TTR of 5.57 months *vs* 8.07 months; recurrence of 27/28 *vs* 16/20; $P = 0.0643$) (Fig. 4C). Compared with group IV, patients in groups I and II had significantly shorter TTR and higher recurrence rates (all $P < 0.0001$, Fig. 4C).

Twelve patients with no history of radio- or chemo-therapy after tumor resection were selected for measurement of real-time changes of serum $\Delta 133p53/FLp53$ ratio in each time of follow-up (tumor recurrence was monitored by CT scans every 4 months in the first two years). Six patients were confirmed to have lung, brain or lymph node metastasis (Fig. 4E), and the other 6 showed no evidence of recurrence (Fig. 4F). The serum $\Delta 133p53/FLp53$ ratios were higher than 2.9160 at the time of detected recurrence in 6 patients and even increased prior to radiologic progression in 2/6 patients, while they were lower than 2.9160 in 4/6 patients with no recurrence (Fig. 4G). In addition, healthy donors exhibited significantly lower serum $\Delta 133p53/FLp53$ ratios than did ESCC patients preoperatively, mostly lower than 2.9160 (Fig. S4D). The best cutoff value of the serum $\Delta 133p53/FLp53$ ratio to distinguish ESCC patients from healthy donors was 0.6798 (Sensitivity: 76.11%; Specificity: 77.55%, AUC = 0.8376), which was also less than 2.9160 (Fig. S4E). These results indicate the potential usefulness of serum $\Delta 133p53/FLp53$ ratio as a prognostic predictor for ESCC, and may also reflect recurrence risk in a real-time manner.

Discussion

This investigation provides the novel finding that the pre-operative tissue abundance of $\Delta 133p53$ as well as the $\Delta 133p53/FLp53$ ratio were significantly elevated in recurrent ESCC patients compared with non-recurrent patients. This finding is consistent with previous reports that $\Delta 133p53$ increases risk of cancer recurrence in breast cancer [18] and rectal cancer [32]. Furthermore, clinicopathological factor distribution analyses also revealed that $\Delta 133p53$ and FLp53 expression, as well as the $\Delta 133p53/FLp53$ ratio correlated significantly with TNM stage or recurrence state. Most importantly, the Kaplan-Meier curve revealed that increased tissue $\Delta 133p53$ and $\Delta 133p53/FLp53$ ratio and decreased FLp53 were correlated to worse OS and PFS in ESCC patients. Thus, $\Delta 133p53$ may also play an oncogenic role in ESCC progression.

$\Delta 133p53$ constituted an independent prognostic marker in patients with advanced serous ovarian cancer [33]. In our study, tissue FLp53 and $\Delta 133p53/FLp53$ ratio were shown to serve as independent prognostic factors for OS and PFS, while $\Delta 133p53$ was not in a further univariate and multivariate analysis. Although we have previously confirmed that $\Delta 133p53$ can act synergistically with p73 to upregulate the transcription of rad51, lig4 and rad52 by joining together to bind to a novel type of $\Delta 133p53$ -responsive element in their promoters [12, 25], the detailed molecular mechanism of $\Delta 133p53$ function is still unclear, the possibility of this inconsistency may result from the fact that $\Delta 133p53$ has three isoforms (α ,

β and γ), which have been shown to have specific functions in a cancer or disease-specific manner and could be regulated by different factors [18]. As in breast cancer, $\Delta 133p53\beta$ promotes cancer cell invasion and associates with cancer progression, while $\Delta 133p53\alpha$ and γ are not [18]. In human glioblastoma, the $\Delta 133p53$ angiogenic effect is due to $\Delta 133p53\alpha$ and γ , but not the β isoform [16]. SRSF1, an essential splicing factor, promotes vascular smooth muscle cell (VSMC) proliferation and injury-induced neointima formation via inducing $\Delta 133p53\alpha$, but not the γ and β isoforms [34]. It is worth noting that the expression level of SRSF1 differs among 33 cancer types as confirmed by Cancer Genome Atlas (TCGA) Program. The detailed roles of individual $\Delta 133p53$ isoforms (α , β and γ) and their functional molecular mechanisms should be investigated in future studies.

Circulating DNA/RNAs are not affected by tissue sampling timing and conditions, and thus are optimal diagnostic or prognostic biomarkers for various cancers, including ESCC [35, 36]. We previously reported that circulating RNAs could be used as diagnostic biomarkers for non-small cell lung carcinoma [27]. In this investigation, we then further measured $\Delta 133p53$ and FLp53 expression in serum samples by using GAPDH [28] as an internal control. The Pearson's correlation coefficient of $\Delta 133p53$ was 0.7019 between serum and tissue, significantly higher than that of FLp53 ($r = 0.4030$) and $\Delta 133p53$ /FLp53 ratio ($r = 0.4137$), which indicated that cancer cells may be the main source of $\Delta 133p53$. $\Delta 133p53$ is significantly increased during the progression from premalignant adenoma to malignant carcinoma [37]. Similar to results obtained from tissue samples, the serum abundance of $\Delta 133p53$ as well as the $\Delta 133p53$ /FLp53 ratio were significantly increased in recurrent ESCC patients, and were related to TNM stage and recurrence state. Although serum $\Delta 133p53$ showed no prognostic value in ESCC patients, the $\Delta 133p53$ /FLp53 ratio showed a better prognostic performance than FLp53 alone for OS (χ^2 value, 17.26 vs 5.80) and PFS (χ^2 value, 56.16 vs 10.27) prediction. Furthermore, ROC analysis revealed that prognostic performance of the serum $\Delta 133p53$ /FLp53 ratio showed no statistical difference from the tissue ratio, both of which were confirmed to be independent prognostic biomarkers for OS and PFS in multivariate analysis.

The clinical utility of monitoring circulating DNA/RNA changes with treatment and recurrence has been reported in various types of cancers [38, 39]. In this study, we reported for the first time that a significant decrease of serum $\Delta 133p53$ /FLp53 ratio was observed soon after resection, which may well be attributed to surgical resection of the primary tumor. Patients with consistently high serum $\Delta 133p53$ /FLp53 ratio (higher than 2.9160 before and after surgery) showed increased recurrence rate and shorter TTR than those with postoperatively or consistently low levels. Furthermore, serum $\Delta 133p53$ /FLp53 ratios were higher than 2.9160 and even increased prior to the time of radiologically detected recurrence. Based on these results, we propose that serum $\Delta 133p53$ /FLp53 ratio may be a real-time surrogate indicator for treatment response and recurrence risk following the ESCC curative resection.

Conclusions

In summary, the results of this study demonstrate that preoperative high serum or tissue $\Delta 133p53$ /FLp53 ratio are independent prognostic factors for OS or PFS in ESCC patients undergoing curative resection.

Serum $\Delta 133p53/FLp53$ ratio in ESCC patients was significantly decreased within 72 h post tumor resection and patients with a consistently high serum $\Delta 133p53/FLp53$ ratio (≥ 2.9160) (pre- and post-operative) had higher recurrence rates than those with consistently low ratio values. In addition, dynamic detection at each follow-up timepoint showed that serum $\Delta 133p53/FLp53$ ratios were increased upon radiologically detected progression. Thus, the serum $\Delta 133p53/FLp53$ ratio can be a novel predictor for survival outcome and may serve as a real-time parameter for monitoring recurrence in ESCC patients after surgery.

Abbreviations

ESCC: esophageal squamous cell carcinoma; FLp53: full-length p53; OS: overall survival; PFS: progression-free survival; ROC: receiver-operating characteristic curve; CNAs: copy number alterations; TTR: time to relapse; VSMC: vascular smooth muscle cell

Declarations

Acknowledgements

We kindly thank the editor and reviewers for careful review and valuable comments, which have led to a significant improvement of the manuscript.

Author contributions

J.L. and Z.L. conceived and designed the study; Q.T., G.L. and J.H. acquired the samples; H.G. and C.Y. performed the experiments; C.Y. analyzed the data; Z.L. drafted the manuscript; all authors critically reviewed, edited and approved the manuscript. J.L. and Z.L. supervised the study. All authors contributed substantially to the study and final manuscript.

Funding

This work was supported by National Natural Science Foundation of China (grant No. 81760540). This work was also funded by Natural Science Foundation of Hubei Province, China (2018CFB164), Natural Science Foundation of Health and Family Planning Commission of Wuhan Municipality (WX18Q03, WX18Q27).

Availability of data and material

We are willing to share anonymized data after required ethical approval by the Ethics Committee of Central Hospital of Enshi Autonomous Prefecture.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Central Hospital of Enshi Autonomous Prefecture. All participants were informed about the purpose, procedures, benefits and potential risks of the study, and their written or oral consents were obtained. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

References

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. *CA Cancer J Clin.* 2016; 66(2): 115-32.
2. Deng J, Chen H, Zhou D, Zhang J, Chen Y, Liu Q, Ai D, Zhu H, Chu L, Ren W, et al. Comparative genomic analysis of esophageal squamous cell carcinoma between Asian and Caucasian patient populations. *Nature Communications.* 2017; 8(1): 1533.
3. Mariette C, Markar SR, Dabakuyo-Yonli TS, Meunier B, Pezet D, Collet D, D'Journo XB, Brigand C, Perniceni T, Carrère N, et al. Hybrid Minimally Invasive Esophagectomy for Esophageal Cancer. *N. Engl. J. Med.* 2019; 380(2): 152-62.
4. Wu C, Li D, Jia W, Hu Z, Zhou Y, Yu DK, Tong T, Wang M, Lin DM, Qiao Y, et al. Genome-wide association study identifies common variants in SLC39A6 associated with length of survival in esophageal squamous-cell carcinoma. *Nat Genet.* 2013; 45(6): 632–8.
5. Ohashi S, Miyamoto S, Kikuchi O, Goto T, Amanuma Y, Muto M. Recent Advances from Basic and Clinical Studies of Esophageal Squamous Cell Carcinoma. *Gastroenterology.* 2015; 149(7): 1700-15.
6. Hao JJ, Lin DC, Dinh HQ, Mayakonda A, Jiang YY, Chang C, Jiang Y, Lu CC, Shi ZZ, Xu X, et al. Spatial intratumoral heterogeneity and temporal clonal evolution in esophageal squamous cell carcinoma.

- Nat. Genet. 2016; 48(12): 1500-7.
7. Song Y, Li L, Ou Y, Gao Z, Li E, Li X, Zhang W, Wang J, Xu L, Zhou Y, et al. Identification of genomic alterations in oesophageal squamous cell cancer. *Nature*. 2014; 509(7498): 91-5.
 8. Lin DC, Hao JJ, Nagata Y, Xu L, Shang L, Meng X, Sato Y, Okuno Y, Varela AM, Ding LW, et al. Genomic and molecular characterization of esophageal squamous cell carcinoma. *Nat. Genet.* 2014; 46(5): 467-73.
 9. Gao YB, Chen ZL, Li JG, Hu XD, Shi XJ, Sun ZM, Zhang F, Zhao ZR, Li ZT, Liu ZY, et al. Genetic landscape of esophageal squamous cell carcinoma. *Nat. Genet.* 2014; 46(10): 1097-102.
 10. Sawada G, Niida A, Uchi R, Hirata H, Shimamura T, Suzuki Y, Shiraishi Y, Chiba K, Imoto S, Takahashi Y, et al. Genomic Landscape of Esophageal Squamous Cell Carcinoma in a Japanese Population. *Gastroenterology*. 2016; 150(5): 1171-82.
 11. Bykov VJN, Eriksson SE, Bianchi J, Wiman KG. Targeting mutant p53 for efficient cancer therapy. *Nature Reviews Cancer*. 2017; 18: 89.
 12. Gong L, Gong H, Pan X, Chang C, Ou Z, Ye S, Yin L, Yang L, Tao T, Zhang Z, et al. p53 isoform $\Delta 113p53/\Delta 133p53$ promotes DNA double-strand break repair to protect cell from death and senescence in response to DNA damage. *Cell Res*. 2015; 25(3): 351–69.
 13. Candeias MM, Hagiwara M, Matsuda M. Cancer-specific mutations in p53 induce the translation of $\Delta 160p53$ promoting tumorigenesis. *EMBO reports*. 2016; 17(11): 1542–51.
 14. Mondal AM, Zhou H, Horikawa I, Suprynowicz FA, Li G, Dakic A, Rosenthal B, Ye L, Harris CC, Schlegel R, et al. $\Delta 133p53\alpha$, a natural p53 isoform, contributes to conditional reprogramming and long-term proliferation of primary epithelial cells. *Cell Death Dis*. 2018; 9(7): 750.
 15. Gong L, Pan X, Chen H, Rao L, Zeng Y, Hang H, Peng J, Xiao L, Chen J. p53 isoform $\Delta 133p53$ promotes efficiency of induced pluripotent stem cells and ensures genomic integrity during reprogramming. *Sci Rep*. 2016; 6: 37281.
 16. Bernard H, Garmy-Susini B, Ainaoui N, Van Den Berghe L, Peurichard A, Javerzat S, Bikfalvi A, Lane DP, Bourdon JC, Prats AC. The p53 isoform, $\Delta 133p53\alpha$, stimulates angiogenesis and tumour progression. *Oncogene*. 2013; 32(17): 2150–60.
 17. Roth I, Campbell H, Rubio C, Vennin C, Wilson M, Wiles A, Williams G, Woolley A, Timpson P, Berridge MV, et al. The $\Delta 133p53$ isoform and its mouse analogue Delta122p53 promote invasion and metastasis involving pro-inflammatory molecules interleukin-6 and CCL2. *Oncogene*. 2016; 35(38): 4981–9.
 18. Gadea G, Arsic N, Fernandes K, Diot A, Jorruiz SM, Abdallah S, Meuray V, Vinot S, Anguille C, Remenyi J, et al. TP53 drives invasion through expression of its $\Delta 133p53\beta$ variant. *Elife*. 2016; 5: e14734.
 19. Lei J, Qi R, Tang Y, Wang W, Wei G, Nussinov R, Ma B. Conformational stability and dynamics of the cancer-associated isoform $\Delta 133p53\beta$ are modulated by p53 peptides and p53-specific DNA. *Faseb j*. 2019; 33(3): 4225–35.
 20. Arsic N, Gadea G, Lagerqvist EL, Busson M, Cahuzac N, Brock C, Hollande F, Gire V, Pannequin J, Roux P. The p53 Isoform $\Delta 133p53\beta$ Promotes Cancer Stem Cell Potential. *Stem Cell Reports*. 2015;

4(4): 531-40.

21. Kazantseva M, Eiholzer RA, Mehta S, Taha A, Bowie S, Roth I, Zhou J, Joruz SM, Royds JA, Hung NA, et al. Elevation of the TP53 isoform $\Delta 133p53\beta$ in glioblastomas: an alternative to mutant p53 in promoting tumor development. *The Journal of pathology*. 2018; 246(1): 77-88.
22. Campbell H, Fleming N, Roth I, Mehta S, Wiles A, Williams G, Vennin C, Arsic N, Parkin A, Pajic M, et al. 133p53 isoform promotes tumour invasion and metastasis via interleukin-6 activation of JAK-STAT and RhoA-ROCK signalling. *Nature Communications*. 2018; 9(1): 254.
23. Kasthuber ER, Lowe SW. Putting p53 in Context. *Cell*. 2017; 170(6): 1062-78.
24. Aoubala M, Murray-Zmijewski F, Khoury MP, Fernandes K, Perrier S, Bernard H, Prats AC, Lane DP, Bourdon JC. p53 directly transactivates $\Delta 133p53\alpha$, regulating cell fate outcome in response to DNA damage. *Cell death and differentiation*. 2011; 18(2): 248-58.
25. Gong H, Zhang Y, Jiang K, Ye S, Chen S, Zhang Q, Peng J, Chen J. p73 coordinates with $\Delta 133p53$ to promote DNA double-strand break repair. *Cell Death Differ*. 2018; 25(6): 1063–79.
26. Lee JH, Jeong H, Choi JW, Oh HE, Kim YS. Liquid biopsy prediction of axillary lymph node metastasis, cancer recurrence, and patient survival in breast cancer: A meta-analysis. *Medicine (Baltimore)*. 2018; 97(42): e12862.
27. Luo CL, Xu ZG, Chen H, Ji J, Wang YH, Hu W, Wang K, Zhang WW, Yuan CH, Wang FB. LncRNAs and EGFRvIII sequestered in TEPs enable blood-based NSCLC diagnosis. *Cancer Manag Res*. 2018; 10: 1449-59.
28. Li Y, Elashoff D, Oh M, Sinha U, St John MA, Zhou X, Abemayor E, Wong DT. Serum circulating human mRNA profiling and its utility for oral cancer detection. *J Clin Oncol*. 2006; 24(11): 1754-60.
29. Camp RL, Dolled-Filhart M, Rimm DL. X-Tile. *Clinical Cancer Research*. 2004; 10(21): 7252–9.
30. Joruz SM, Bourdon JC. p53 isoforms: Key regulators of the cell fate decision. *Cold Spring Harbor perspectives in medicine*. 2016; 6(8): a026039.
31. Vandekerckhove G, Todenhofer T, Annala M, Struss WJ, Wong A, Beja K, Ritch E, Brahmbhatt S, Volik SV, Hennenlotter J, et al. Circulating tumor DNA reveals clinically actionable somatic genome of metastatic bladder cancer. *Clinical Cancer Research*. 2017; 23(21): 6487–97.
32. Arsic N, Ho-Pun-Cheung A, Evelyne C, Assenat E, Jarlier M, Anguille C, Colard M, Pezet M, Roux P, Gadea G. The p53 isoform $\Delta 133p53ss$ regulates cancer cell apoptosis in a RhoB-dependent manner. *PLoS One*. 2017; 12(2): e0172125.
33. Hofstetter G, Berger A, Schuster E, Wolf A, Hager G, Vergote I, Cadron I, Sehouli J, Braicu EI, Mahner S, et al. $\Delta 133p53$ is an independent prognostic marker in p53 mutant advanced serous ovarian cancer. *Br J Cancer*. 2011; 105(10): 1593–9.
34. Xie N, Chen M, Dai R, Zhang Y, Zhao H, Song Z, Zhang L, Li Z, Feng Y, Gao H, et al. SRSF1 promotes vascular smooth muscle cell proliferation through a $\Delta 133p53$ /EGR1/KLF5 pathway. *Nature Communications*. 2017; 8: 16016.

35. Hsu FM, Cheng JCH, Chang YL, Lee JM, Koong AC, Chuang EY. Circulating mRNA profiling in esophageal squamous cell carcinoma identifies FAM84B as a biomarker in predicting pathological response to neoadjuvant chemoradiation. *Scientific reports*. 2015; 5: 10291–.
36. Jin Z, Olaru A, Yang J, Sato F, Cheng Y, Kan T, Mori Y, Mantzur C, Paun B, Hamilton JP, et al. Hypermethylation of tachykinin-1 is a potential biomarker in human esophageal cancer. *Clinical cancer research*. 2007; 13(21): 6293–300.
37. Fujita K, Mondal AM, Horikawa I, Nguyen GH, Kumamoto K, Sohn JJ, Bowman ED, Mathe EA, Schetter AJ, Pine SR, et al. p53 isoforms $\Delta 133p53$ and p53beta are endogenous regulators of replicative cellular senescence. *Nat Cell Biol*. 2009; 11(9): 1135–42.
38. Liu Z, Jiang M, Zhao J, Ju H. Circulating tumor cells in perioperative esophageal cancer patients: quantitative assay system and potential clinical utility. *Clinical cancer research*. 2007; 13(10): 2992–7.
39. Khan KH, Cunningham D, Werner B, Vlachogiannis G, Spiteri I, Heide T, Mateos JF, Vatsiou A, Lampis A, Damavandi MD, et al. Longitudinal Liquid Biopsy and Mathematical Modeling of Clonal Evolution Forecast Time to Treatment Failure in the PROSPECT-C Phase II Colorectal Cancer Clinical Trial. *Cancer Discov*. 2018; 8(10): 1270-85.

Tables

Table 1. Associations of tissue $\Delta 133p53$, p53 and $\Delta 133p53/p53$ ratio with clinicopathological features in ESCC.

	$\Delta 133p53$			p53			$\Delta 133p53/p53$ ratio		
	<	$\geq$	<i>P</i>	<	$\geq$	<i>P</i> value	<	$\geq$	<i>P</i> value
	0.0425	0.0425	value	0.0195	0.0195		2.6470	2.6470	
Age			0.3944			0.6353			0.1389
< 60	18	63		23	58		53	28	
$\geq$ 60	17	82		25	74		54	45	
Gender			1.0000			0.0166			0.1375
Male	30	124		46	108		88	66	
Female	5	21		2	24		19	7	
BMI			0.4820			0.0078			0.4763
< 24	17	80		18	79		60	37	
$\geq$ 24	18	65		30	53		47	36	
Smoking history			0.8146			1.0000			0.3624
No	6	30		9	27		19	17	
Yes	29	115		39	105		88	56	
Tumor location			0.9731			0.0295			0.0091
Upper/Middle	11	46		9	48		42	15	
Lower	24	99		39	84		65	58	
Pathologic M			0.1304			<			<
						0.0001			0.0001
M0	34	126		32	128		106	54	
M1	1	19		16	4		1	19	
Pathologic N			0.4211			<			<
						0.0001			0.0001
N0-N1	32	122		32	122		106	48	
N2-N3	3	23		16	10		1	25	
Pathologic T			0.0662			0.0009			<
									0.0001
T1-T2	20	58		11	67		67	11	

T3-T4	15	87	37	65	40	62
TNM stage	0.0011		<		<	
			0.0001		0.0001	
I-II	28	71	6	93	98	1
III-IV	7	74	42	39	9	72
Grade	0.0030		<		<	
			0.0001		0.0001	
G1-G2	24	59	9	74	71	12
G3-G4	11	86	39	58	36	61
Recurrence ^a	0.0371		<		<	
			0.0001		0.0001	
No	23	65	10	78	73	15
Yes	7	54	28	33	18	43

^a 149 of 180 patients with regular follow-up checks for recurrence after surgery.

Table 2. Associations of serum $\Delta 133p53$, p53 and $\Delta 133p53/p53$ ratio with clinicopathological features in ESCC.

	Δ133p53			p53			Δ133p53/p53 ratio		
	<	≥	<i>P</i>	<	≥	<i>P</i>	<	≥	<i>P</i> value
	0.0870	0.0870	value	0.0300	0.0300	value	2.9160	2.9160	
Age			0.5088			0.9394			0.5852
< 60	41	40		34	47		50	31	
≥ 60	55	44		41	58		65	34	
Gender			0.0105			0.1322			0.0759
Male	76	78		68	86		94	60	
Female	20	6		7	19		21	5	
BMI			0.9363			0.0516			0.0287
< 24	52	45		34	63		69	28	
≥ 24	44	39		41	42		46	37	
Smoking history			0.2320			0.7055			0.6981
No	16	20		14	22		24	12	
Yes	80	64		61	83		91	53	
Tumor location			0.2748			0.2229			0.5974
Upper/Middle	27	30		20	37		38	19	
Lower	69	54		55	68		77	46	
Pathologic M			0.0086			0.2331			< 0.0001
M0	91	69		64	96		112	48	
M1	5	15		11	9		3	17	
Pathologic N			0.018			0.0870			0.0133
N0-N1	88	66		60	94		104	50	
N2-N3	8	18		15	11		11	15	
Pathologic T			0.1035			0.2856			0.3214
T1-T2	47	31		29	49		53	25	
T3-T4	49	53		46	56		62	40	

TNM stage	0.0008		0.0276		< 0.0001	
I-II	64	35	34	65	78	21
III-IV	32	49	41	40	37	44
Grade	0.0857		0.4333		0.0131	
G1-G2	50	33	32	51	61	22
G3-G4	46	51	43	54	54	43
Recurrence ^a	0.0005		0.0007		< 0.0001	
No	56	32	26	62	83	5
Yes	21	40	35	26	18	43

^a 149 of 180 patients with regular follow-up checks for recurrence after surgery.

Table 3. Univariate and multivariate survival analyses of OS in patients with ESCC.

	Univariate		Multivariate	
	HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value
Age (≥ 60 vs < 60)	1.203 (0.758– 1.908)	0.4332		
Gender (Male vs Female)	2.012 (0.873– 4.641)	0.100		
BMI (≥ 24 vs < 24)	1.188 (0.750– 1.882)	0.4624		
Smoking history (Yes vs No)	1.618 (0.923– 2.836)	0.0931		
Tumor location (Lower vs Upper + Middle)	1.231 (0.694– 2.182)	0.4777		
Pathologic M (M1 vs M0)	3.695 (2.134– 6.397)	$<$ 0.0001	2.296 (1.247– 4.225)	0.0076
Pathologic N (N2–N3 vs N0–N1)	2.066 (1.182– 3.610)	0.0109		
Pathologic T (T3–T4 vs T1–T2)	1.591 (0.992– 2.551)	0.054		
Grade (G3–G4 vs G1–G2)	2.107 (1.293– 3.433)	0.0028		
Recurrence (Yes vs No) ^a	2.546 (1.549– 4.184)	$<$ 0.0001	2.184 (1.298– 3.672)	0.0032
Tissue $\Delta 133p53/p53$ ratio (≥ 2.6470 vs < 2.6470)	4.496 (2.742– 7.375)	$<$ 0.0001	3.864 (2.293– 6.511)	$<$ 0.0001
Serum $\Delta 133p53/p53$ ratio (≥ 2.9160 vs < 2.9160)	2.559 (1.617– 4.050)	$<$ 0.0001	2.503 (1.465– 4.276)	0.0011

^a 149 of 180 patients with regular follow-up checks for recurrence after surgery.

Table 4. Univariate and multivariate survival analyses of PFS in patients with ESCC.

	Univariate		Multivariate	
	HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value
Age (≥ 60 vs < 60)	1.352 (0.877– 2.084)	0.1717		
Gender (Male vs Female)	1.580 (0.836– 2.986)	0.1589		
BMI (≥ 24 vs < 24)	1.320 (0.863– 2.020)	0.2009		
Smoking history (Yes vs No)	2.030 (1.164– 3.542)	0.0127		
Tumor location (Lower vs Upper + Middle)	1.363 (0.833– 2.230)	0.2173		
Pathologic M (M1 vs M0)	2.638 (1.522– 4.574)	$<$ 0.0001	2.171 (1.219– 3.866)	0.0085
Pathologic N (N2–N3 vs N0–N1)	2.066 (1.226– 3.482)	0.0065		
Pathologic T (T3–T4 vs T1–T2)	1.438 (0.932– 2.218)	0.1007		
Grade (G3–G4 vs G1–G2)	1.835 (1.180– 2.853)	0.0070		
Recurrence (Yes vs No) ^a	6.760 (4.138– 11.041)	$<$ 0.0001	5.212 (3.062– 8.873)	$<$ 0.0001
Tissue $\Delta 133p53/p53$ ratio (≥ 2.6470 vs < 2.6470)	3.677 (2.378– 5.684)	$<$ 0.0001	2.283 (1.387– 3.760)	$<$ 0.0001
Serum $\Delta 133p53/p53$ ratio (≥ 2.9160 vs < 2.9160)	4.735 (3.038– 7.379)	$<$ 0.0001	3.230 (1.947– 5.359)	$<$ 0.0001

^a 149 of 180 patients with regular follow-up checks for recurrence after surgery.

Figures

Figure 1

Kaplan-Meier curves of tissue $\Delta 133p53$, FLp53 and $\Delta 133p53/FLp53$ ratio in ESCC. mRNA expression of $\Delta 133p53$ (A), FLp53 (B) and $\Delta 133p53/FLp53$ ratio (C) in 42 paired adjacent normal and cancerous tissue of ESCC patients. ESCC, esophageal squamous cell carcinoma; FLp53, full-length p53. OS of 180 ESCC

patients divided by tissue $\Delta 133p53$ (D), FLp53 (E) and $\Delta 133p53$ /FLp53 ratio (F). PFS of 149 ESCC patients divided by tissue $\Delta 133p53$ (G), FLp53 (H) and $\Delta 133p53$ /FLp53 ratio (I). OS, overall survival; PFS, progression-free survival.

Figure 2

Kaplan-Meier curves of serum $\Delta 133p53$, FLp53 and $\Delta 133p53$ /FLp53 ratio in ESCC. Pearson's correlation analysis between tissue $\Delta 133p53$ (A), FLp53 (B), $\Delta 133p53$ /FLp53 ratio (C) and their respective level in serum of ESCC patients. OS of 180 ESCC patients divided by preoperative serum $\Delta 133p53$ (D), FLp53 (F) and $\Delta 133p53$ /FLp53 ratio (H). PFS of 149 ESCC patients divided by preoperative serum $\Delta 133p53$ (E), FLp53 (G) and $\Delta 133p53$ /FLp53 ratio (I).

Figure 3

The ROC analysis of tissue and serum $\Delta 133p53/FLp53$ ratio in Cox regression model. (A) Receiver operating curve in the OS model. (B) Receiver operating curve in the PFS model.

Figure 4

Dynamic changes of serum $\Delta 133p53/FLp53$ ratio reflect treatment response and recurrence risk of ESCC. (A) Changes of serum $\Delta 133p53/FLp53$ ratio before versus after (within 72 h following) resection. (B) OS of 180 ESCC patients divided by pre- and post-operative serum $\Delta 133p53/FLp53$ ratio, cutoff value was 2.9160. (C) Prognostic significance for time to recurrence in patients with persistent high serum $\Delta 133p53/FLp53$ ratio, conversion of serum $\Delta 133p53/FLp53$ ratio from high to low, conversion of serum $\Delta 133p53/FLp53$ ratio from low to high, and persistent low (cutoff value was 2.9160). (D) PFS of 149 ESCC patients divided by pre- and post-operative serum $\Delta 133p53/FLp53$ ratio, cutoff value was 2.9160. (E) An example of a patient (patient #151) with no history of radio- or chemotherapy, which was confirmed with brain metastasis on a subsequent CT scan after 20 months of tumor resection. (F) An example of a patient (patient #12) with no history of radio- or chemotherapy, which was confirmed with

no metastasis after 24 months of tumor resection. (G) 12 patients were monitored by CT scans every 4 months in a time range of 24 months, serum $\Delta 133p53/FLp53$ ratio were measured at each time until recurrence occurred. dashed line means $Y = 2.9160$.