TP53 gene deletion in esophageal cancer tissues of patients and its clinical significance

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Abstract. The aim of this study was to examine TP53 gene deletion in esophageal cancer (EC) tissues obtained from patients and to evaluate its clinical significance. Forty surgical specimens from patients with esophageal squamous cell carcinoma were examined for TP53 gene deletion using the fluorescence *in situ* hybridization (FISH) technique. Thirty-two male and 8 female patients were enrolled, with an average age of 56 years. TP53 gene deletion was significantly higher in poorly-differentiated EC cases compared to well-differentiated cases (P=0.028). The TP53 gene deletion rate was also significantly higher in the group with lymph node metastasis compared to the group without lymph node metastasis (P=0.0313). The TP53 gene deletion rate was shown to be correlated with the level of differentiation and lymph node metastasis in EC; it may therefore be an important molecular marker for evaluating the condition of EC in patients.

Introduction

Esophageal cancer (EC) is one of the most common gastrointestinal cancers in the world and there is a clear regional and ethnic difference in terms of its incidence rate and etiology factors (1). The incidence of EC has been as high as 68.88/100,000 among the Kazakhs living in the Xinjiang Uyghur Autonomous Region (Northwest region of China). In China, the histopathological type of EC is different from that found in Europe and America and majority of cases are squamous cell carcinomas. EC is related to the activation of multiple genes and is a multi-step process. Oncogene activation and tumor suppressor gene inactivation are the basis for the development of molecular genetics. Currently, the p53 gene is the most important tumor suppressor gene and is located on human chromosome 17p13.1, which regulates the cell cycle and induces cell apoptosis (2,3).

Fluorescence *in situ* hybridization (FISH) is a cytogenetic technique that has gradually developed in recent years, involving the use of fluorescent labeled DNA probes to detect cell changes within the chromosome (4). Several studies have demonstrated that p53 gene deletion is important in the development and progression of EC (5-7). However, reports on the p53 gene deletion associated with Xinjiang Kazakh EC patients are absent.

In this study, we used FISH to detect TP53 gene deletion in 40 esophageal carcinoma cases in Kazakh patients, in order to analyze its clinical significance in evaluating patients' prognosis based on molecular pathology.

Patients and methods

From October 2010 to December 2011, 40 Kazakh EC patients were admitted to the Department of Thoracic Surgery, First Affiliated Hospital of Xinjiang Medical University, and underwent surgical resection. There were 32 male and 8 female patients, with an average age of 56 years (ranging from 31 to 82). None of the patients had received any preoperative radiotherapy, chemotherapy or other special treatment.

Pathological diagnosis confirmed esophageal squamous cell carcinoma in 40 cases post-operatively, including well-differentiated tumors in 14 cases, moderately differentiated tumors in 12 cases and poorly differentiated tumors in 14 cases (Table I). Ten samples of normal esophageal tissues (>5 cm from the tumor) were collected as normal controls. Informed consent was obtained from all patients.

Touch preparations of cells were made on glass slides from fresh specimens and air-dried overnight at room temperature and then stored at -80˚C ready for FISH. The same specimens were stained with hematoxylin and eosin (H&E) for pathological evaluation. FISH detection was followed by Nakamura's improved Vysis protocol (8). Direct fluorochrome-labeled centromeric probes were used for enumeration of different

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Abbreviations: FISH, fluorescence *in situ* hybridization; EC, esophageal cancer; CEP17, centromere of chromosome 17; SSC, standard saline citrate

Key words: TP53 gene, Kazakh, esophageal squamous cell carcinoma, FISH

Table I. Results of p53 and CEP17 by FISH.

Case	Age (years)	Gender	Copy no. 0 p53/CEP17	1 p53/CEP17	2 p53/CEP17	3 p53/CEP17	4 p53/CEP17	FISH
1	53	M	3/0	35/0	60/26	2/34	0/40	Deletion
2	65	M	4/0	33/0	40/30	15/45	8/25	Deletion
3	45	M	5/0	23/0	58/54	13/28	1/18	Normal
4	54	F	1/0	21/0	51/62	21/30	6/8	Normal
5	50	M	3/0	35/0	56/79	4/11	2/10	Deletion
6	51	F	0/0	25/0	74/77	1/12	0/11	Normal
7	41	M	4/0	21/0	69/72	5/15	1/13	Normal
8	63	M	5/0	11/0	84/68	0/18	0/14	Normal
9	46	M	0/0	18/0	76/71	6/21	0/8	Normal
10	56	M	9/0	36/0	53/46	2/39	0/15	Deletion
11	68	M	10/0	40/0	49/67	1/19	0/14	Deletion
12	63	M	14/0	44/0	33/51	9/41	0/8	Deletion
13	66	M	4/0	10/0	63/48	16/21	7/8	Normal
14	71	M	5/0	42/1	50/48	3/36	0/15	Deletion
15	44	M	3/0	30/0	60/69	4/29	3/2	Deletion
16	63	M	5/0	40/0	49/51	6/28	0/21	Deletion
17	71	M	7/0	44/0	48/68	1/20	0/12	Deletion
18	52	M	10/0	31/0	41/52	13/15	5/33	Deletion
19	45	M	11/0	45/0	39/61	5/30	0/9	Deletion
20	70	M	0/0	19/0	58/66	14/31	9/3	Normal
21	52	F	3/0	25/0	45/59	20/28	7/13	Normal
22	37	F	2/0	24/0	69/60	4/24	1/16	Normal
23	56	M	5/0	36/0	41/50	16/34	2/16	Deletion
24	56	M	2/0	39/0	54/60	3/21	2/19	Deletion
25	60	F	2/0	38/0	35/46	25/39	0/15	Deletion
26	64	F	3/0	40/0	42/41	15/53	0/6	Deletion
27	54	F	4/0	41/0	33/52	22/20	0/28	Deletion
28	66	M	4/0	35/0	41/56	20/30	0/14	Deletion
29	51	M	3/0	30/0	50/67	10/23	7/10	Deletion
30	45	M	5/0	23/0	58/49	13/30	1/21	Normal
31	58	M	3/0	44/1	50/45	2/41	1/13	Deletion
32	70	M	0/0	40/0	55/42	5/36	0/22	Deletion
33	64	F	4/0	20/0	63/49	12/32	1/19	Normal
34	50	M	1/0	28/0	47/50	22/34	2/16	Normal
35	62	M	0/0	25/0	68/70	6/17	1/13	Normal
36	52	M	3/0	33/0	58/62	6/36	0/2	Deletion
37	50	M	3/0	21/0	70/70	6/22	0/8	Normal
38	48	M	4/0	19/0	58/51	15/39	4/10	Normal
39	60	M	5/0	21/0	74/60	0/26	0/14	Normal
40	60	M	3/0	20/0	57/50	20/37	0/13	Normal

CEP17, centromere of chromosome 17; FISH, fluorescence *in situ* hybridization.

chromosomes. Spectrum orange-labeled and spectrum green-labeled probes for the TP53 and centromere of chromosome 17 (CEP17) were purchased from the manufacturer (Vysis, Inc., Downers Grove, IL, USA).

Cells were denatured with 70% formamide and then washed twice in standard saline citrate (SSC) at 74˚C and at room temperature, respectively, for 2 min in a water bath. The slides were then dehydrated through a graded ethanol series (70, 85

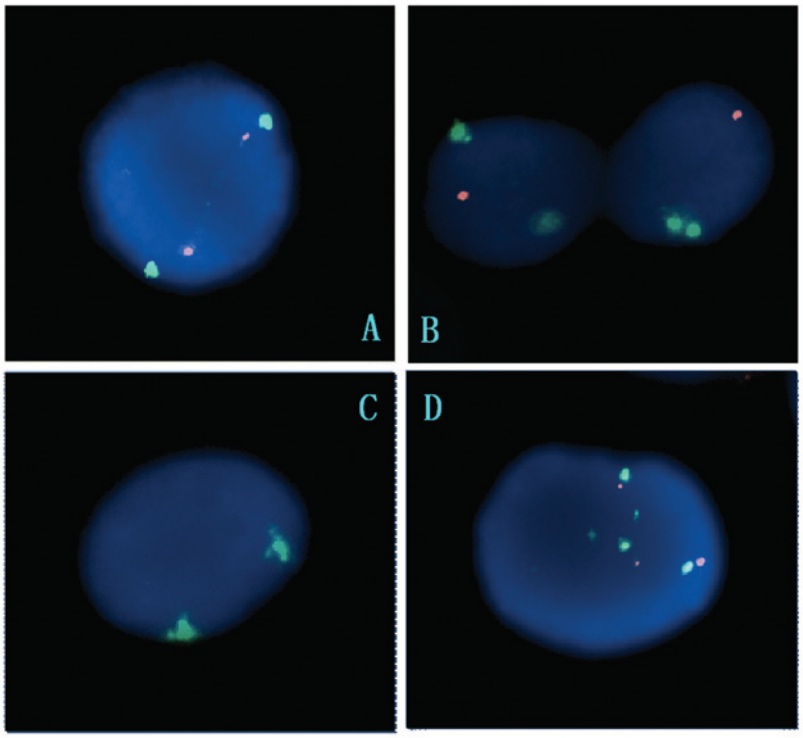

Figure 1. FISH analysis of TP53 (orange signals) and CEP17 (green signals). (A) Normal representative nuclei carrying 2 copies of TP53 and CEP17. (B) Partial deletion; nuclei carrying 1 copy of TP53 and 2 copies of CEP17. (C) Complete deletion; nuclei carrying 0 copy of TP53 and 2 copies of CEP17. (D) Amplification, nuclei carrying 3 orange signals and 5 green signals. FISH, fluorescence *in situ* hybridization. CEP17, centromere of chromosome 17.

and 100%, each for 2 min). We then applied 10 μl of hybridization solution containing 1 μl of each of the DNA probes, 7 μl of hybridization buffer and 1 μl of double distilled water. This was covered with a cover slip and sealed with rubber cement. Following incubation for 16 h at 42˚C in a humidity-controlled chamber, the slides were washed with an SSC solution for 5 min at 74˚C and at room temperature for 2 min. Then 5 μl diamidinophenylindole (DAPI, II) was applied to each spot and covered with a cover slip. The slides were observed under a fluorescence microscope that was connected to a cooled charge-coupled device camera. According to the kit instructions, under a fluorescence microscope, a special image acquisition and analysis system (Leica Microsystems, Ltd., Germany) was used for the signal count. In total, 100 nuclei were observed to obtain the number of p53 gene signals, such as 1 or 0; when nucleus fluorescence was >30%, we determined p53 gene deletion.

Microsoft Social Sciences 15.0 software (SPSS software, Chicago, IL, USA) was used for statistical analysis. The corrected χ^2 test or the Scheffe method were used for univariate analysis of data from each group. P<0.05 was considered to indicate a statistically significant difference.

Results

Orange (TP53) and green (CEP17) fluorescence signals were detected in all EC specimens. CEP17 hybridization signals were larger and brighter than TP53 gene signals. The representative FISH models included: normal, 2 intranuclear orange signals and 2 intranuclear green signals; partial deletion, ≥2 intranuclear green signals and 1 intranuclear orange signal; complete deletion, ≥2 intranuclear green signals and 0 orange signals (Fig. 1). Table I shows the TP53 gene deletion in the 40 esophageal carcinoma cases.

Table II shows the correlation between clinicopathological factors and TP53 deletion. Statistical analysis indicated that there were no significant correlations among TP53 gene deletion rate, age and gender (P>0.05). TP53 gene deletion rates were 28% (4/14) and 78% (11/14) in well-differentiated and poorly-differentiated EC, respectively (P=0.028). TP53 gene deletion rates were 38% (7/18) and 72% (16/22) in the lymph node metastasis and no lymph node metastasis groups, respectively (P=0.0313). A high frequency of CEP17 hyperdisomy was detected.

Discussion

The causes of EC are complicated. At present, EC is considered as a multi-factor, polygenic variant, multi-staged disease. The variation of correlated proto-oncogenes and tumor suppressor genes is the key to tumorigenesis and development. Deletion of the tumor suppressor gene is the major mode of variation (9).

The p53 gene is currently thought to be the most important tumor suppressor gene and more than 50% of tumors are associated with its abnormality. p53 genetic mutation is important in the development of EC. Studies have indicated that patients with p53 genetic mutation had poorer prognosis and greater malignancy (10,11). Multiple studies have proven that p53 genetic mutation is an independent and reliable biological parameter for evaluating the disease prognosis (12,13). Due to p53 gene deletion or abnormality, cells with injured DNA enter into S stage, resulting in changes to heredi-

Table II. Correlation between p53 gene deletion and clinicopathological factors.

Clinicopathological parameters	Case	p53 deletion	P-value
Age (years)			
<50	11	3	0.0695
≥50	29	19	
Gender			
Male	32	19	0.4745
Female	8	3	
Differentiation			
Well-differentiated	14	4	0.028
Moderately differentiated	12	7	
Poorly differentiated	14	11	
Lymph node metastasis			
No metastasis	18	7	0.0313
Metastasis	22	16	

tary characteristics, chromosomal aberration and, ultimately, carcinomatous change.

FISH is a technique using fluorescently labeled single-chain nucleotide DNA probes, based on the base complementarity principle, to form double-stranded nucleic acid with complementary single chain nucleotides by specific binding, and to detect cell chromosome changes and definite gene copy number changes in order to diagnose malignant cells. FISH is capable of detecting all types of cytogenetic changes, including aneusomy, amplification and deletion. Recently, FISH has been used in the diagnosis of hematologic malignancy, lung, breast and renal cancer, and it is a technique with high sensitivity and specificity (4,14-17).

In the past, there were limitations in the detection of mutant p53 protein by immunohistochemical methods since viral infection and stress may also produce p53 protein aggregation, and therefore samples may have contained both mutant and wild-type variants. Several studies found that the p53 protein positive rate was lower than that observed using PCR-SSCP detection, which indicates that p53 mutation or abnormality in the malignant tumor is much higher than that shown by immunohistochemisty detection in real situations (18). The sensitivity of FISH is similar to isotope hybridization *in situ*, but the space resolution and gene mapping accuracy is higher; therefore, FISH plays a significant role in tumor studies (8,18,19).

In this study, FISH was applied using double-color DNA probes (fluorescent orange-labeled LSI TP53 probe and fluorescent green-labeled CEP17 probe) to detect TP53 gene deletion in Kazakh esophageal squamous cell cancer patients (7,20).

In the normal controls, in over 90% of nuclei, CEP17 and TP53 genes displayed 2 signals, indicating that FISH was successful in our study (data not shown). Among 40 EC cases, the mean TP53 gene deletion rate was 55% (22/40), and 28% (4/14), 58% (7/12) and 78% (11/14) in well-differentiated, moderately differentiated and poorly differentiated cases, respectively. This indicates that the TP53 gene deletion rate is correlated with the level of differentiation; the higher the differentiation, the higher the TP53 deletion rate. Moreover, the TP53 gene deletion rate also correlated with lymph node metastasis (P=0.0313). Similar studies support our results (21,22).

Our study used FISH to detect the TP53 gene in Kazakh patients with EC, and the results show for the first time that the TP53 gene deletion rate is correlated with the level of differentiation and lymph node metastasis in ECs, and may be an important molecular biological marker for evaluating the condition of ECs in Kazakh patients. Further study on the post-operative life span of these patients is required to confirm the correlation of TP53 gene deletion and EC prognosis.

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