

The Expression of hTER and hTERT mRNA in Human Tumors with Tissue Microarray*

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Abstract: To explore expression-patterns of hTER and hTERT mRNA in human tumors by tissue chip with in situ hybridization (ISH), simultaneously, evaluate the applicability and value of tissue chip in ISH. Tissue chips consisted of 230 specimens of human tissues (10 cases normal tissues, 13 cases benign tumors, 207 cases malignant tumors) were prepared through the patent tissue arrayer. The expression of hTER and hTERT mRNA in specimens were determined by ISH. The expression of hTER and hTERT mRNA in normal tissues, benign and malignant tumors were related to characterization of tumor ($p < 0.005$). The distribution of hTER and hTERT positive cells was regulated from limited in basal layer to diffused throughout most layers of squamous epithelium is correlated with increasing severity of histopathologic changes. That means the expression-patterns of tissue chip provides a rapid and powerful histological method that would be used in ISH and play an important role in tumor biology. And, the expression-patterns of up-regulation and dysregulation of hTER and hTERT of is an important histology sign could be used as a useful molecular marker for diagnosis and identification human tumor.

Key words: tissue chip (tissue microarray); tumor; human telomerase; in situ hybridization(ISH)

In 1998, Kononen^[1] reported a new type microarray technology named as tissue microarray (TMA), also called tissue chip (TC). In this paper we using tissue microarray and in situ hybridization (ISH) technology to detect the expression of hTER mRNA and hTERT mRNA in 230 cases of multi types of human tumor and normal tissue.

MATERIALS AND METHODS

1. Specimens

230 cases of Paraffin-embedded tissues from Department of pathology of Second hospital of Xi'an JiaoTong University were in this experiment, among which 207 cases were advanced carcinoma (gastric adenocarcinoma 22, oesophagus squamous carcinoma 21, duodenum adenocarcinoma 3, colon adenocarcinoma 12, recto-adenocarcinoma 5, hepatocarcinoma 7, Hepatocholangiocarcinoma 1, cholecyst adenocarcinoma 4, pancreas adenocarcinoma 2, larynx squamous carcinoma 8, maxillary sinus squamous carcinoma 1, amygdalo squamous carcinoma 1, lung squamous carcinoma 4, lung adenocarcinoma 2, transitional cell carcinoma of bladder 12, transitional cell carcinoma of ureter 2, transitional cell carcinoma of renal pelvis 1, clear cell carcinoma of kidney

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17, adenocarcinoma of mammary 26, thyro-papillary carcinoma 14, thyro-folliculi carcinoma 3, medullary thyroid carcinoma 3, endometrium carcinoma 2, cervix squamous carcinoma 5, Vulva squamous carcinoma 1, ovario-cystadenocarcinoma 3, leiomyosarcoma 2, B cell lymphoma 5, medulloblastoma 5, neuroblastoma 2, retinoblastoma 1, Wilms tumor 2, spermatocytoma 1, melanoma 2, neurofibrosarcoma 1, chondrosarcoma 1, 3 cases of malignancy fibrous histiocytoma), 13 cases of miscellaneous benign tumors and 10 cases of normal tissue (mainly from mucosa tissues) as control.

2. Tissue chip preparation

According to the method established in our institute, we prepared 4×6 type (row×column) tissue chip (23 samples for each chip). Totally, 20 slides were prepared with following method: Firstly the HE sections of the 230 cases of paraffin embedded specimens were read, and the corresponding fitting regions of the paraffin embedded blocks were excerpted. Then, the tissue arrayer prepared by ourselves was used specially for digging out tissue cylinder 1.5 mm in diameter and 3 mm in length from the donator blocks, Then every 23 cylinders were embedded in one paraffin block and labeled. Furthermore, paraffin section 5μm was prepared and mounted on the slides coated with poly-lysine.

3. In situ hybridization

hTER and hTERT ISH Kits contain the cDNA probes labeled with digoxin were purchased from the Department of pathology, Beijing medical university, and the ISH was carried out just following the direction of manufacture. The sections were deparaffined, hydrated with 0.1mmol/L HCl, digested with proteinase K (100μg/ml), refixed with 4% formalin, washing the sections were dehydrated with graded ethanol solution. 20μl of hybridization solution was added on each section and hybridized overnight 4 h, after blocking with normal horse serum, the sections were reacted with anti-Dig-AP, developed with NBT/BCIP.

Sections hybridized with prehybridization sol without probes, and sections treated with RNAase and hybridized with the probes in parallel were used as negative control.

4. Evaluation of the quality of tissue microarray

Compare the tissue chip sections hybridized with probes were compared with the section prepared by conventional method in the following aspects: wrinkles, defection, shedding off and especially whether the results of hybridization were consistent with the real or no.

5. Determinate the results of ISH

When purple products appeared in the cytoplasm, it was judged as positive. The intensity was gauged into 4 grades: negative (-): no purple color could be detected; weakly positive (+): purple granules appeared in the cytoplasm of tumor cells and positive cells were less than 25% of total cells; mediated positive (++) : purple products appeared in the cytoplasm of tumor cells and positive cells were between 25% to 50% of total cells; strongly positive (+++): dark purple products existed in the cytoplasm of tumor cells and positive cells were more than 50% of total cells.

6. Statistical analysis

Differences between the groups were tested by χ^2 test.

RESULTS

1. The results of tissue chip

Tissue sample 1.5 mm in diameter arrayed on the slides in order, the tissue structure is integrity, 18 cylinder

tissue samples shed from slide, there were 221 cylinder tissue samples can be analysis, the shedding rate is $3.9\% \pm 2.4\%$, the structure of donator paraffin blocks is integrity and can be experimented continually. Visualized under microscope, the sections were very clear and had a very weak background and a well contrast (Figure 3).

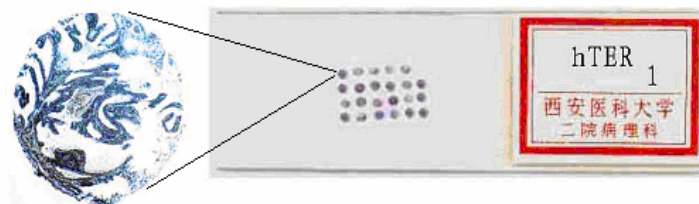


Fig. 1 The image of specimen in the 4×6 type's tissue chips

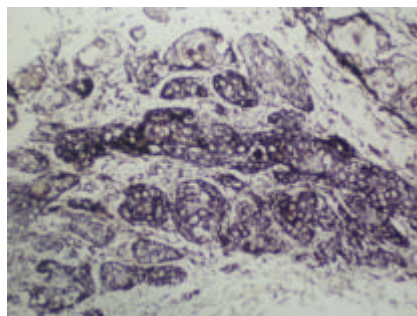


Fig. 2 hTER mRNA expressed in oesophagus squamous carcinoma, dark purple products existed in the cytoplasm of tumor cells. Developed with NBT/BCIP, ×400.

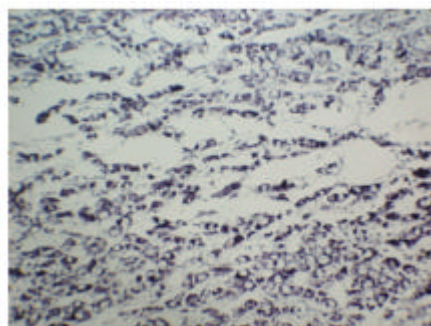


Fig. 3 hTERT mRNA expressed in gastric adenocarcinoma, dark purple products existed in the cytoplasm of tumor cells. Developed with NBT/BCIP, ×400.

2. The results of ISH

The expression of hTER and hTERT mRNA was showed in table 1.

Table 1 The express ratio of hTER and hTERT mRNA in 230 cases of human tumor and normal tissue

	Cases (N)	Cases of analysis (n) (%)	-		+		++		+++	
			hTER	hTERT	hTER	hTERT	hTER	hTERT	hTER	hTERT
Malignant tumor (%)	207	201 97.1%	23 11.4%	36 17.9%	33 16.4%	62 30.8%	66 32.8%	53 26.4%	79 39.3%	50 24.9%
Benign tumor (%)	13	11 84.6%	5 45.5%	7 63.6%	5 45.5%	4 36.4%	1 9%	0 0	0 0	0 0
Control tissue (%)	10	9 90%	4 44.4%	7 77.8%	5 55.6%	2 22.2%	0 0	0 0	0 0	0 0
Tatol (%)	230	221 96.1%								

Note: $H_{ChTER} = 110.048$, $H_{ChTERT} = 100.245$, $p < 0.005$

3. The historical distribution of positive cells

As shown in Figure 2-3, hTER mRNA expressed in cytoplasm of tumor cells, hTER mRNA expressed weakly in normal tissue and limited to the basal cells merely. There are few weak positive cells in basal cell layer could be found. In benign tumor, hTER mRNA expressed weakly, the positive cells were diffusedly in most layer of tumor tissue. In invasive carcinomas, the expression of hTER mRNA was mediated and strongly positive, the positive cells diffused throughout most layers of squamous epithelium as mass shape, nests,

hTERT mRNA expressed in cytoplasm of tumor cells, hTERT mRNA expression and distribution had a similar regulation of hTER mRNA expression. hTERT mRNA expressed weakly and limited to the basal cells in normal tissue merely, In benign tumor, hTERT mRNA weakly positive cells were diffused in most layer in tumor, In invasive carcinomas, the hTER mRNA expressed mediated and strongly, mediated and strongly positive cells were diffused throughout most layers of squamous epithelium as mass shape, nests.

So expression of hTR RNA and hTERT mRNA was co-localized not only in carcinoma regions but also in benign tumor and normal epidermal layers in histological. That is to say there was a special historical distribution of hTER and hTERT positive cells in normal or tumor tissues

DISCUSSION

1. Tissue microarray

TMA was designed for detecting same marker in different specimens in situ. Using TMA technology, we can study several dozens of specimens in DNA, RNA or protein level in situ. At present, this technique has been used in many aspects^[2, 3].

TMA offers an efficient way to examine a large number of tumor specimens on a single slide. However, TMA is biopsy of a biopsy, so the important thing is to assess the possibility and validity of TMA^[4], especially examine a group of heterogeneity tumor with TMA. Many authors thought that arranged numerous 0.6-mm in diameter tissue core biopsy specimens into single paraffin block and validated that TMA can be used to detect heterogeneous or homogeneous protein or gene expression^[5-7]. Furthermore, Kim^[8] constructed TMA by arranging the cylindrical biopsies of 2.0 mm in diameter into a tissue array block. In this paper, we utilized the private Patent tissue arrayer be manufactured by ourselves (Patent NO: L 00207528.8 NO:ZL 00262393.5), to prepare tissue microarrays by arranging the cylindrical biopsies of 1.5 mm in diameter into a tissue array block to ensure the specificity and the high-through character of TMA.

In this study, there were 460 samples mounted onto 20 slides of 4×6 type tissue chip prepared in total. 18 cylindrical tissue samples shed off; the shedding rate was 3.9% ± 2.4%. 1/23 times of materials reagent and time for the common method was used in the examination only. So using tissue chip we can economize the experiment material, increased the efficiencies of test markedly. This result suggested that TMA afforded an efficient and expeditious method for cancer research.

The corresponding fitting regions selected from the donator blokes were according to their HE sections, so that, TMA technique can avoid the hemorrhage and necrosis area in cylindrical biopsy tissues, reduce their influence on the results of experiment. In addition, using this technologic, the experiment can be done under similar condition by arraying many samples on one slide, so the error of experiment could be decreased to the utmost extent. Packeisen^[9] thought that TMA can be used in situ research as a tool for internal quality control.

In this paper, TMA was used in the field of cancer research with ISH, and improved the efficiency of

histopathology research markedly. The results suggest that ISH-TMA is an efficient and expeditious technique can be reliably used to evaluate the prognostic value of expressed biomarkers, through examining up to several hundreds different clinical specimens in a single slide. At the present, as an efficient and expeditious biochip, TMA has recently become a widely accepted standard technology, and can be used for all different types of in situ tissue analysis.

2. Human tumor and telomerase

Telomerase, a ribonucleoprotein, is composed of a RNA component (hTER) and two protein subunits. One of these subunits, the catalytic subunit (human telomerase reverse transcriptase, hTERT), represents a reverse transcriptase. The expression of hTERT is closely correlated with telomerase activity. Kamamori^[10] suggest that ISH-based analysis of hTERT gene expression is superior to both TRAP telomerase activity and hTERT mRNA RT-PCR analysis as a means of determining telomerase status during carcinogenesis. Thus evaluating hTERT gene expression levels could be used as a marker of malignant progression^[11]. In this paper, we detected the expression of hTER and hTERT mRNA in 207 cases of human malignant tumor, 13 cases of benign tumor and 10 cases of normal tissue with TMA and ISH. The results showed that the expression of hTER mRNA and hTERT mRNA up-regulation ($p < 0.005$) following the progression of tumor. This suggest that the expression of hTER mRNA and hTERT mRNA has a significant correlation with occurrence and progression of tumor.

In this paper, we found that the expression of hTER mRNA was negative in 11.4% (23/207) malignant tumors, the expression of hTERT mRNA was negative in 17.9% (36/207) malignant tumors, but the expression of hTER mRNA was weak positive in 55.6% (5/9) normal tissues, the expression of hTERT mRNA was weak positive in 22.2% (2/9) normal tissues, That was to say hTR and hTERT mRNA were present not only in carcinoma tissues but also in normal epidermal layers. Many studies have also shown that hTER and hTERT mRNA can be detected in non-neoplastic tissues and normal tissues^[12,13]. Belair^[14] and Nakano^[15] thought that the expression of hTER and hTERT mRNA had a relationship with the high proliferation activity of cell. So we suspected that there possible were some atypical hyperplasia cells or high hyperplasia cells, for the atypical hyperplasia cells posses high proliferation activity as tumor cell, therefore the expression of hTER and hTERT mRNA were up-regulation in normal tissue. On the other aspect, the results suggested that the up-regulation expression of hTER mRNA and hTERT mRNA merely play an basic role in the process of tumor, and the levels of expression of the subunits of telomerase and their equilibration, transcription and the modification post transcription and other reasons we had not known at present may play an important role in the regulation of telomerase activity and the occurrence and progression of cancer.

This result also shown that the expression of hTER and hTERT mRNA was weak and the positive cells were limited to the basal cells in normal tissue and benign tumors, but that the expression of hTER and hTERT mRNA were mediated and strongly and positive cells were diffused throughout most layers of squamous epithelium as mass shape, nests in malignant tumors. So the expression of hTER and hTERT mRNA was co-localized not only in carcinoma regions but also in benign tumor and normal epidermal layers in histological, and there was a special historical distribution of hTER and hTERT positive cells in normal or tumor tissues. The expression-patterns are correlated with increasing severity of histopathologic changes. Other authors suggested it so^[16].

So we suggested that the up-regulation and dysregulation of hTER mRNA and hTERT mRNA had an important significance to diagnosis benign or malignant tumor, and perhaps the expression-patterns of hTER and hTERT could be determined as a useful molecular marker for diagnosing malignant tumor.

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