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• LIVER CANCER •

Biological characteristics of HCC by ultrasound-guided aspiration biopsy and its clinical application

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Abstract

AIM: To probe the pathological biological characteristics of hepatocellular carcinoma (HCC) by the ultrasound-guided aspiration biopsy and assess the clinical application value of this method.

METHODS: The biopsy and DNA analysis by flow cytometry (FCM) were taken in 46 cases with HCC nodules, including 26 cases and 20 cases with nodules ≤3 cm and >3 cm in diameters respectively, and 12 cases with intrahepatic benign hyperplastic nodules. They were taken in 22 cases of 46 cases with HCC before and after the therapy. Fine-needles and automatic histological incised biopsy needles were used. The fresh biopsy tissue was produced into the single cell suspension, which was sent for DNA detection and ratio analysis of cell period. The ratio of each DNA period of cell proliferation of each group was calculated and compared with each other. The DNA aneuploid (AN) and apoptosis cell peak were observed and their percentages were calculated.

RESULTS: The ratios of S and G_2/M periods of DNA, which reflect cell hyperproliferation, in the group with HCC tumors >3 cm in diameter were markedly higher than those of the group with HCC nodules ≤3 cm in diameter and the group with the benign hyperplastic nodules (P<0.01 except A:B of S period, P<0.05). The ratios of the middle group were also apparently higher than those of the latter group (P<0.01). The ratio of DNA AN of 46 cases with HCC nodules was 34.8 % (16/46). None of the cases with the intrahepatic hyperplastic nodules appeared AN. The DNA AN appeared more apparently with the growth of the tumors. The AN ratio of the group with tumors >3 cm in diameter was 55 % (11/20), markedly higher than that of the group with tumors ≤3 cm in diameter which was 19.2 % (5/26) (P<0.01). The FCM DNA analysis of 22 specimens of hepatic carcinoma tissue before therapy showed that the aneuploid peaks appeared in 5 cases (22.7 %). The ratio of G_1 period rose after therapy while the S period and G_2/M ratios fell (P<0.01). The aneuploid peak disappeared in the 5 cases after the therapy, while the apoptosis peaks in 12 cases (54.5 %) appeared.

CONCLUSION: Addition to supply the information of the pathological morphology of the tumor, the ultrasound-guided fine-needle aspiration tissue could be sent for FCM DNA analysis to comprehend its pathological biological characteristics. This can not only provide the clinic the reliable information about the occurrence, development, diagnosis, curative effect and prognosis of tumors but also supply biological information for clinic to choose therapeutic schemes.

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INTRODUCTION

Recently, with the wide use of flow cytometry (FCM) in clinic, it becomes possible to make pathological diagnosis develop from qualitative analysis of the morphology to quantitative analysis[1-5]. The study of DNA analysis of the biological characteristics of hepatocellular carcinoma (HCC) model and hepatectomy specimens of man HCC has greatly developed[6-18]. But the study of DNA analysis of the pathological biological characteristics of HCC specimens acquired from the ultrasound-guided aspiration biopsy has been rarely reported[15,19]. In our study, the ultrasound-guided aspiration biopsy and the FCM DNA analysis were taken in 46 cases with HCC, including 22 cases with HCC before and after the therapy and 12 cases with intrahepatic benign hyperplastic nodules (IBHN) in order to probe the biological characteristics of HCC and assessed the clinical application value of this research.

MATERIALS AND METHODS

Subjects

Forty-six cases with HCC, including 26 cases and 20 cases with nodules 3 cm and >3 cm in diameters respectively, consisted of 39 males and 7 females. The mean age was 53.7 years (range, 29-72). Twenty-two of 46 cases taking ultrasound-guided interventional therapy included 16 males and 6 females. Twelve cases with IBHN comprised 7 males and 5 females and the average age was 47.5 years (range, 27-58). The diagnosis was established by cytopathological and / or histological examinations.

Instruments and methods

The apparatuses were Aloka-650 and 1700 with 3.5-MHz probe. All cases took aspiration biopsy with 22G PTC needles. Twenty-two cases with HCC took percutaneous ethanol or other anti-tumor drug injection therapy for 5-8 times after biopsy, then took aspiration biopsy once again. The 18G automatic histological incised biopsy needle (producted by C.R. Bard, Inc, American) was used if the specimen taken by the fine needle aspiration was unsatisfactory. The amount of platelet of the patient must be $>60\times10^9$/L and the mobility of thrombogen of the cases must be >50 %. The process of fine-needle aspiration biopsy was as follows. The point of puncture

in the skin was determined by ultrasonography. Then sterilization, drape and local paralysis were done. The fine needle punctured through the point into the focus. The needle was twitched up and down in the focus. At the same time, the negative pressure was kept in the syringe. The needle was taken out when the tissue and / or tissue with blood were seen in the syringe. The aspirated material was divided into three parts. One part was smeared on glass slides for pathological examination. The second part was placed in 10 % formalin or electron microscope (EM) liquid for electron microscopy. The last part was placed in 10 ml physiological saline for cytometric DNA analysis. The process of puncture of automatic histological incised biopsy was similar to that of fine-needle aspiration biopsy. The needle punctured to the periphery of the mass, ejected and excised the tumor tissue, then was extracted quickly. The sample was 19.0 mm in length and 1.0 mm in diameter.

The examination method of FCM

The FACScan (B.D. company, America) was adopted. At first, the fresh biopsy tissue was produced into the single cell suspension[1]. Then it was stained by PI for DNA analysis. DNA content was measured and the ratio of each DNA period of cell proliferation was analyzed. The ratio of each DNA period, the percentage of the DNA aneuploid (AN) and the percentage of apoptosis cell peak were observed.

Statistical analysis

The average value and standard deviation of the percentage of the ratio of cell proliferation and DNA AN stem-line of each group were calculated respectively. The student-*t* test or student-*t*' test was used to test whether the mean of each group was different from each other. A difference was regarded as significance if $P<0.05$.

RESULTS

Table 1 showed the ratio of each DNA period of cell proliferation of each group. Statistical analysis showed that the ratio of S and G_2/M periods of DNA, which reflect cell hyperproliferation, in the group with HCC tumors >3 cm in diameter were markedly higher than those of the group with nodules ≤3 cm in diameter and the group with the benign hyperplastic nodules ($P<0.01$ except A:B of S period, $P<0.05$). The ratios of the middle group were also apparently higher than those of the latter group ($P<0.01$). The apparent characteristics were that the DNA of IBHN was steadily in G_1 period, which means the nonproliferation diploid (2c) state (Figure 1). While the DNA of HCC cell stayed in unstable division stage and appeared the cell hyperproliferation state (Figure 2) with the development of foci.

Table 1 The ratios of cell proliferation of HCC in different diameter and benign hyperplastic nodules (%, $\bar{x}\pm s$)

Characteristics of foci	Number of cases	DNA analysis of each period		
		G_1 period	S period	G_2 / M period
The group with HCC>3 cm (A)	20	40.3±6.8	43.5±7.4	16.2±1.7
The group with HCC≤3 cm(B)	26	57.7±5.8	30.4±5.2	11.9±1.1
The group with IBHN (C)	12	94.8±0.7	2.6±0.5	2.6±0.4

G_1 period A:B A:C B:C $P<0.01$; S period A:B $P<0.05$, S period B:C A:C $P<0.01$; G_2 / M period A:B B:C A:C $P<0.01$.

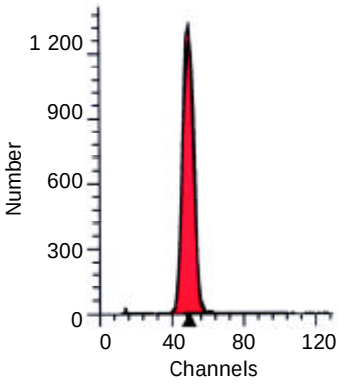

Figure 1 DNA analysis of aspiration biospy tissues acquired from intrahepatic benign hyperplastic nodules showed steady diploid (2C) peak that stayed in G_1 period (single red peak).

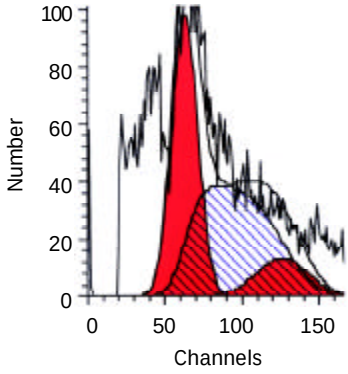

Figure 2 DNA analysis of aspiration biopsy tissues acquired from HCC nodules showed S period of hyperproliferation (the peak with oblique lines) and G_2 / M period (the second red peak).

The statistical materials also showed that the ratio of DNA AN stem-line of 46 cases with HCC nodules was 34.8 %(16/46) (Figure 3). While none of cases with IBHN appeared AN stem-line. The DNA appeared AN more apparently with the growth of the tumors. The AN ratio of the group with tumors >3 cm in diameter was 55 % (11/20), markedly higher than that of the group with tumors ≤3 cm in diameter which was 19.20 % (5/26) ($P<0.01$).

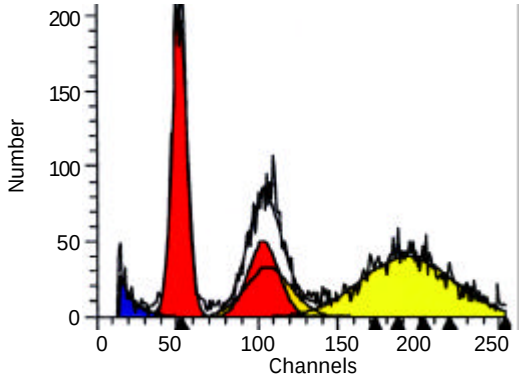

Figure 3 DNA analysis of HCC nodules showed aneuploid (AN) peak (the orange peak).

The pathological results of 22 cases among 46 cases with HCC after the ultrasound-guided percutaneous interventional therapy showed that the tumor cells appeared pyknosis and necrosis in various degrees. The Table 2 showed the results of FCM DNA analysis of the aspiration tissue. The ratio of G_1 period which reflects cell steady state rose apparently after therapy ($P<0.01$). While the ratios of S period and G_2/M period

which reflect cell hyperproliferation fell (P<0.01). After therapy the AN peaks disappeared in 5 cases which appeared before therapy and the apoptosis peaks appeared in 12 cases (54.5 %) (Figure 4) which was consistent with the cell pyknosis and dark nuclear staining showed by the histopathologic examination and the typical apoptosis cell or/and apoptosis body showed by the electron microscopy (Figure 5).

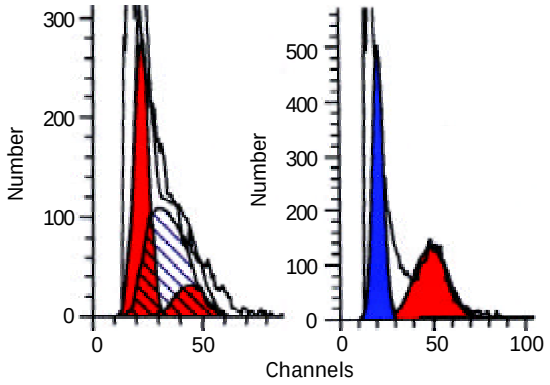

Figure 4 DNA analysis of HCC nodules showed hyperproliferation state before therapy (left) and apoptosis peak after therapy (the blue peak).

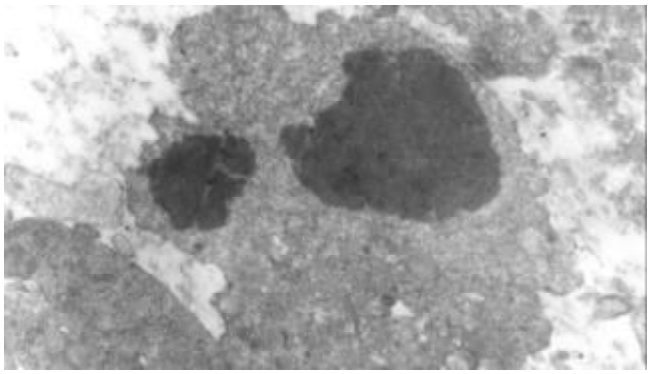

Figure 5 The same cases as those in figure.4 appeared typical apoptosis cells and apoptosis bodies after therapy.

Table 2 Cell proliferative ratios of 22 cases with HCC before and after ultrasound-guided interventional therapy (%, $\bar{x}$±s)

Time of detect	Each period of cell proliferation (%)		
	G_1 period	S period	G_2 / M period
Before therapy(a)	52.6±7.2	37.5±8.1	9.9±1.9
After therapy(b)	94.7±4.1	3.7±2.5	1.6±0.5

a:b of each period P<0.01.

DISCUSSION

The application value of cytopathological and histological examinations using ultrasound-guided aspiration biopsy has been affirmed in clinic[20-22]. There are also many scholars using many immunological methods to study the biological characteristics of hepatic tumors[23-45]. But the study of DNA detection of intrahepatic tumor tissue by ultrasound-guided fine-needle aspiration or incising biopsy[15,19] is rarely reported. On the basis of comparative study between ultrasound-guided fine-needle and heavy-needle biopsy, we made pathomophological examination of HCC nodules that were different in diameter, HCC nodules before and after therapy as well as benign hyperplastic nodules by ultrasound-guided puncturing biopsy. At the same time[46-48], the fresh biopsy tissue was produced into the single cell suspension which was sent for FCM DNA detection and ratio analysis of cell period. The changing process of cell proliferation was observed. Through the study of biological characteristics of hepatic carcinoma and pathological comparison of hepatic carcinoma tissue before and after the therapy, we probed the application value of DNA analysis in assessment of the therapeutic efficacy and prognosis.

It is determined by the biological behavior of the cell itself that the normal tissue, reactive tissue and benign tumor all have normal diploid DNA. Like most other malignant tumors, HCC appears polyploid DNA, especially AN DNA. AN has become a reliable cytobiological signs of human malignant tumors[49]. On the other side, like other malignant tumors, the occurrence and development pattern of HCC also relate to a biological behavior that changes from a relative steady benign state in the early period to a high proliferative malignant state[3,4,7,50]. The study of HCC model of animal shows that, acted by the carcinogen the animal based on the hepatic cirrhosis appears the proliferation group of new born hepatocytes with diploid DNA stem-line which has highly proliferative activity and the potential ability of malignant tendency. That is the cytological basis of carcinogenesis later. The continuous changes of DNA stem-line in the process of occurrence and development of HCC form the characteristics of the various biological dynamic mechanism from early relative benign state to markedly malignant state of high proliferation. The course of DNA stem-line changing from 2C to AN is an important pathobiological sign. So the study of biological characteristics of HCC has the important clinical practical value for early diagnosis, selection of therapeutic schemes and judgment of prognosis of HCC. In our study, DNA analysis of HCC tissue by ultrasound-guided puncturing aspiration biopsy was made before and after therapy. The ratio of each period in cell period showed that the ratios of synthesis phase (S period) and mitosis phase (G_2/M period) which reflect high proliferation of HCC group were apparently higher than those of benign hyperplastic group (P<0.01) and that the ratios of S and G2/M period apparently rose with the growth of the tumors. As we all know, DNA, as the material basis of heredity of biotic bodies, is mainly located in chromosome of nucleus. The degree of maturation is closely relative to DNA content. The cell in non-proliferative state is characterized by 2C DNA stem-line. The DNA content increases multiply when cells enter division stage and DNA changes into AN stem-line. With the increasing of malignant degree of cell, the structure and amount of chromosome are more abnormal and the phenomena of changing into high proliferative state and AN is more remarkable. On the basis of the studies of animal HCC model and hepatectomic human HCC sample, Cong MW regards that the smaller HCC <3 cm in diameter is mainly characterized by diploid DNA stem-line which indicates relatively slow growth, lower malignant degree, tumors mostly having capsules, foci limiting, lower distant metastasis rate and lower occurring rates of thrombosis and satellite foci of tumor[3,4]. All these are the pathological basis of conducting radical operation. Our material also shows that 34.8 % of 46 cases with HCC appeared AN. The ratio of AN of the group with tumor >3 cm in diameter reached 55.0 % (11/20), markedly higher than that of the group with tumor ≤3 cm in diameter which was 19.2 % (5/26) (P<0.01). None of the cases with IBHN appeared AN. It is worth mentioning that, with the growth of tumor, the DNA gradually changes from low proliferation to AN stem-line at certain extent, which reflects that pathobiological characteristics is in relation to the size of tumor nodules. But it is not consistent thoroughly. In our study, the diameter of a small HCC nodule was only 1.1 cm, yet the DNA analysis characterized it by typical AN stem-

line (Figure 3).

Many scholars have made the researches in the relationship between the ploidy type and the prognosis of many tumors including HCC by FCM DNA analysis[51-55]. But the study of DNA analysis by ultrasound-guided fine-needle biopsy to assess the therapeutic efficacy has been rarely reported[56]. In our study, DNA analysis of tumor biopsy tissue shows that the AN stem-line which appeared before therapy disappeared after the ultrasound-guided interventional therapy with the cancer cell being killed. The ratios of S and G_2/M periods all fell obviously (P<0.01) and 54.5 % cases appeared physiological death process which was different from common necrosis, in another word, appeared apoptosis peak before G_0-G_1 period. All these changes were consistent with what had been seen in pathological examination. DNA content rose multiply and the cell appeared polyploid when the cell entered division stage. In pathological state, especially for cancer cells, the structure and amount of chromosome became abnormal and the phenomenon of polyploid and/or AN became more remarkable. Summarily, the content and periods of DNA decreased and increased with dying and recurring of the cancer cell[2,5,57]. The materials also show that DNA analysis could supply reliable biological information and objective quantification index.

This study shows that besides providing pathomorphological information of cytology and histology, the tissues acquired by ultrasound-guided puncturing biopsy could also be sent for FCM DNA analysis to comprehend the biological characteristics of tumors. So this method attains the objective of "one needle, three uses" and provides valuable clinical basis for diagnosis, therapeutic efficacy and prognosis assessment of tumors and is worth further studying.

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