THE CLINICAL SIGNIFICANCE OF FLOW CYTOMETRIC DEOXYRIBONUCLEIC ACID MEASUREMENT OF DEPARAFFINIZED SPECIMEN IN BLADDER TUMOR

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A retrospective study of flow cytometric measurements on paraffin-embedded tumor specimens from 188 patients with bladder tumor was conducted. The results were analyzed in combination with the morphological variation of bladder tumors. It was found that the DNA ploid pattern, degree of infiltration and the multiplicity of bladder tumor were closely related with tumor recurrence, among which the DNA ploid pattern was most significant. In aneuploid bladder tumors the recurrence rate and mean annual recurrence frequency were 76.7% and 1.46, and those in the diploid bladder tumors were 18.7% and 0.33 respectively. Aneuploid was the most indicative parameter of the recurrence in bladder tumors. In addition, according to the DNA ploid pattern and DNA index (DI), the aneuploid tumors in our group were divided into 4 types, namely, tetraploid tumors, non-euploid with DI<1.5, non-euploid tumors with DI>1.5 and two-aneuploid tumors. The results showed that the recurrent rate of tetraploid tumors was relatively lower and it became higher and higher in the following order: non-euploid tumors with DI<1.5, non-euploid tumors with DI>1.5, and two-aneuploid tumors. This indicates that there are different biological behaviors in tumors with different ploid pattern. Finally, the correlation between DNA ploid pattern and tumor metastasis was also discussed.

The postoperative recurrent rate of bladder tumor is very high. It is no doubt that the rate can be lowered if the information relevant to recurrence can be obtained and therefore appropriate preventive methods are taken. Some variations were present in evaluation of the recurrence based only upon the morphological data previously. It is expected that flow cytometry (FCM) may improve the prognostic precision by evaluating the tumors at molecular level. Using FCM, we have measured the DNA content in deparaffinized specimens of bladder tumors, aiming to investigate the clinical significance of DNA ploid pattern, DI and the morphological parameters in predicting the recurrence and to establish the correlation between the DNA ploid pattern and the metastasis of bladder tumor.

MATERIALS AND METHODS

Sources of Specimens

Follow-up work has been done since 1988 on 330 patients with primary transitional cell carcinoma of bladder on whom operation was performed from 1976—1982. From 188 patients among them data were obtained with a follow-
up rate of 57%. Formalin-fixed, paraffin-embedded tumor specimens of the 188 patients who had undergone no antitumor treatment preoperatively were selected for study. The longest follow-up time was 12 years with an average time of 7 years. Among the 188 cases, 155 were male while 35 were female respectively. 91 patients died of neoplasm postoperatively with the survival time less than 4 years, one of the 91 cases only survived 3 months. This also took amount for that the mean follow-up time of this group was low.

One 4 μm section and two 40 μm sections were obtained continuously from each paraffin-embedded sample block. The former was hematoxylin-eosin stained again for pathological review to confirm the malignant diagnosis. The latter were used to prepare deparaffinized single nucleus suspension for FCM measurement. In addition, each fresh specimen from 27 bladder tumor was also separated into two parts. One was used for FCM while the other part was formalin-fixed, paraffin-embedded routinely, from which sections were obtained as described above for FCM measurement.

**Preparation of Deparaffinized Nucleus Suspension and Staining**

Using the improved Hedley's procedure, single cell nucleus suspensions were prepared from paraffin-embedded specimens. Initially, two 40 μm sections were placed in a 10 ml vial, deparaffinized in xylene, rehydrated in graded alcohols routinely, then minced. 2 ml pH 7.2 trypsin at the concentration of 0.25% was added into the vial, digested at 4°C for 12 hours, followed by a PBS rinse, sieved through a 60 μm diameter pore size nylon mesh. After being treated with 0.1% RNase, the nucleus suspension was stained with 0.05% propidium iodine (PI) at 4°C for 30 minutes in darkness without vortexing.

**FCM Analysis**

During the course of this study, EPICS V cytometer (coulter Co., USA) was used with which a 5 W argon ion laser was attached. 488 nm wavelength light and 630 nm light filter were employed. Before and after the measurement of the specimens, the normal human lymphocytes and cell suspension of deparaffinized uretal epithelial specimen were examined to establish the standard of normal value. From each sample, 5,000—20,000 (x=1,000) cells were measured. DI is the ratio of the DNA content of tumor cell to that of the normal cell in GO/G1 phase. Aneuploid tumor includes tetraploid, non-euploid, and two-aneuploid tumors. Tetraploid tumors show a sharp and high tetraploid modal in the DNA content histogram and the percentage of tetraploid cells is higher than 10%. Two-aneuploid tumor comprises 2 cell clones with different abnormal DNA contents and in histogram it is demonstrated as 2 aneuploid modals with different DI. In this study, x2-test and t-test were employed for statistic analysis of data.

**RESULTS**

Fresh samples and paraffin-embedded samples from 27 bladder tumors were examined by FCM. As shown in the Figure 1, results of two methods were similar in 25 patients (92.6%) comprising 11 diploid, 5 tetraploid, and 9 aneuploid tumors. Different results were present in only 2 cases: 1 fresh sample showed non-euploid histogram but paraffin-embedded sample diploid, while in the other case fresh sample showed tetraploid whereas the paraffin-embedded sample non-euploid.

The correlations between the pathological stage and grade of bladder tumor and the DNA ploid pattern can be seen from Table 1. Along with the progress of the pathological stage and grade, the percentage of patients with aneuploid cell stemlines increased obviously. Among the