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Flow Cytometric Study on Tumor Antigen TA-4 in Uterine Cervical Squamous Cells

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Abstracts Flow cytometric analysis was made of a tumor-antigen, TA-4, in cells from squamous cell carcinoma of the utrine cervix. The cells were stained by the indirect immunofluorescent antibody technique using a rabbit antibody specific to TA-4 and fluorescein isothiocyanate conjugated anti-rabbit IgG antibody. FCM histograms of the carcinoma cells showed a broad but conspicuous peak with intense fluorescence pattern not seen in normal squamous epithelium. Would be possible to distinguish malignant from non-malignant cells of the uterine cervix it by the FCM analysis. Possible application of the technique to cytological screening of cervical cancer is discussed.

Key Words: Flow cytometry, Tumor antigen, Squamous cell carcinoma, Uterine cervical cancer

ntroduction

A tumor-antigen TA-4 which was purified from squamous cell carcinoma of the uterine cervix is specifically demonstrated in the circulation of patients with squamous cell carcinoma by a radioimmunoassay[1]. This marker has been used for estimating the prognosis or extent of disease in cervica squamous cell carcinoma[2]. However, it is also clear that the present radioimmunoassay method for TA-4 is not sensitive enough to detect early stages of the disease. The endeavors have been made to utilize the antigen for cytological diagnosis of uterine cervical cancers. A great problem arises in application of TA-4 to clinical cytology since normal squamous epithelium contains approcimately 20% of TA-4 activity demonstrated in the tumor tissue extracts[3]. Quantitative evaluation of the antigen on a single cell level would be necessary to discriminate malignant cells, which may contribute to not only reliable diagnosis of early cancer but also advances in cancer cell biology. Recently, flow cytometry (FCM) has developed and this technique is widely used for cancer diagnosis by measurement of content of nucleic acid and/or protein on a cell. Flow cytometry also permits the quantitation of some antigenic substances on a cell by the use of fluorochrome labeled antibody. In the present study, we attempted FCM evaluation of TA-4 activity in cells from squamous cell carcinoma and normal squamous epithelium of the uterine cervix, and indicated that this method would be useful to distinguish malignant cells from non-malignant cells.

Materials and Methods

Squamous cell carcinoma tissues were obtained from 2 patients with squamous cell carcinoma of the uterine cervix. Normal squamous epithelium of the uterine cervix was obtained from 38-year-old patient with leiomyoma of the uterus. Each tissue was treated with 0.1% collagenase (Sigma Chemical Co., U.S.A.) solution in PBS at 37° for 45 min. The dispersed cells were filtered through 50 μm nylon mesh to obtain a suspension of single cells and fixed in 70% cold ethanol. The cells were washed twice with PBS and were stained by the indirect fluorescent antibody technique using anti TA-4 serum (rabbit) and FITC conjugated anti-rabbit IgG (goat). For DNA analysis aliquats of cell suspensions were stained with propidium iodide (Calbiochem-Behring Co., U.S.A., 50 μg/ml in PBS) after RNAse (Sigma Chemical Co. U.S.A.) treatment at 37° for 30 min. FCM analysis was performed using FACS III (Becton Dickinson Co., U.S.A.). The relative fluorescence intensity of each cell was recorded as frequency histogram, based on a linear scale that was divided into 256 channels. Prior to each analysis the instrument was adjusted using biologic standard, i.e., propidium iodide stained hamster spleen cells, so that the peak of the fluorescence histogram was set to a particular position. G1/0 peak was set to 40th channel in this experiment. In both experiments, the wave length employed for excitation of the dyes was 480 nm and emission integrated above 530 nm was selected.

Results

Cells from normal squamous epithelium demonstrated a diploid pattern in a DNA histogram (Fig. 1), while a malignant case (case B) showed diploid as well as aneuploid cell population. In another malignant case (case A), however, neither aneuploidy nor polyploidy was apparent on the DNA histogram, which indicated that FCM profile in this case was indistinguishable from that of the non-malignat case. Figure 2 shows a FCM profile of TA-4 acitivity in non-malignant subject which was poor in the antigen per cell. The fluorescence histogram of malignant case (case A) was superimposed on the pattern of non-malignant case. When cells with fluorescence intensities outside the gain of the histogram were eliminated,

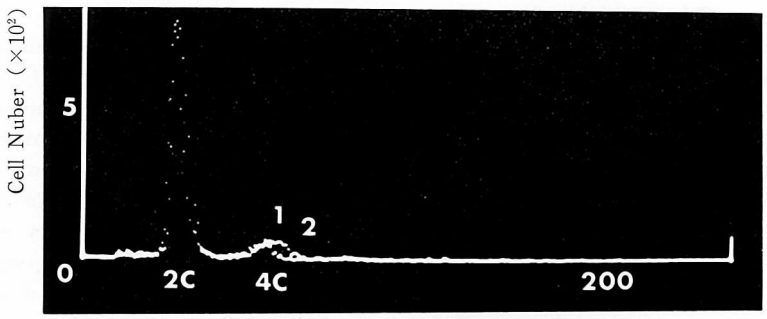

Fig. 1 Flow cytometric analysis of DNA content in (1) normal squamous epithelium from the uterine cervix and (2) cervical squamous cell carcinoma (case A). A profile of DNA distribution of the malignant cells is difficult to be distinguished from the normal diploid pattern. 2C and 4C denote diploid and tetraploid DNA contents, respectively.

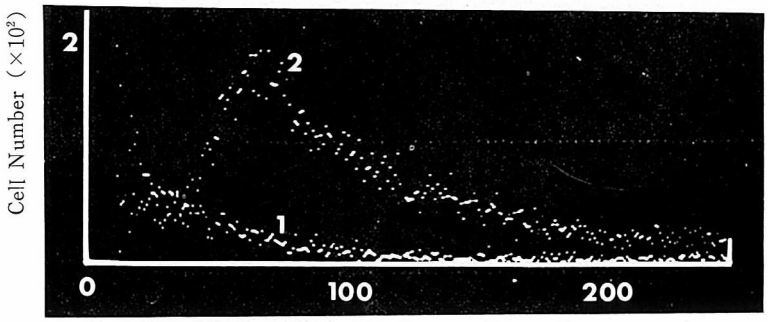

Fig. 2 Fluorescence intensity distribution of the binding of FITC-labeled antiTA-4 to non-malignant cells. Most of cells are poor in TA-4 activity.

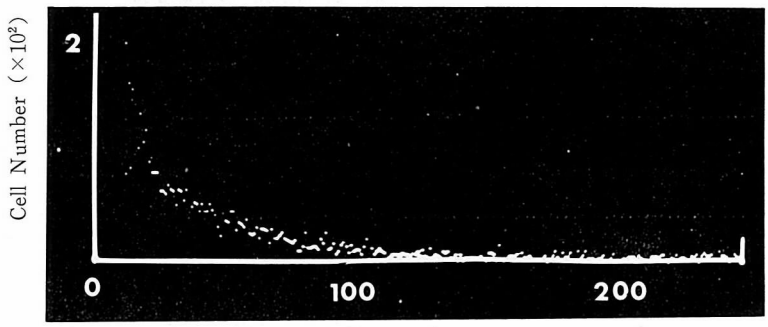

Fig. 3 Fluorescence intensity distribution of the binding of FITC-labeled antiTA-4 to squamous carcinoma cells of case A(2), which is superimposed on that of non-malignant cells (1). Increase in the number of cells with higher TA-4 activity is distinct in the malignant case.

approximately 70% of the normal epithelial cells appeared between channels 0 and 50. On the other hand, cancer cells formed a broad but conspicuous peak at the position of channel around 70. The percentages of cancer cells with fluorescent intensities greater than channel 50 were 82.9% (case A) and 81.7% (case B) (Fig. 4), respectively.

Discussion

In an attempt to use FCM for the detection of malignant cells, measurement of cellular DNA content has played a dominant role. Generally, normal cells show diploid DNA pattern while in malignant cells DNA profile often deviates significantly from the normal diploid value. However, as shown in

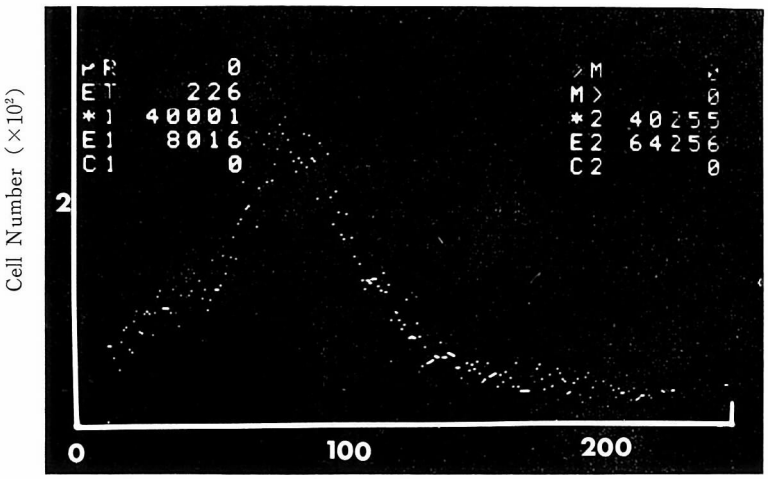

Fig. 4 Fluorescence intensity profile of the binding of FITC-labeled antiTA-4 to the malignant cells of case B, which is essentially the same pattern as that of caseA.

the present study (Fig. 1), case A showed neither aneuploidy nor polyploidy on the DNA histogram, which would be diffcult to be identified as malignant. FCM analysis on the basis of cellular DNA content is often inaccurate to distingush the malignant cell, and a considerable number of false negative and false positive results has been reported [4-6]. For precise diagnosis of carcinomas, the discovery of more useful parameters are awaited with great interest.

Recently, much attention has been forcused on tumor-markers which may be useful to distinguish malignant cells from non-malignant cells, unfortunately, no single marker yet discovered can qualitatively discriminate malignant cell. However, there are ,at least, quantitative differences in some tumor-markers between malignant non-malignant cells. TA-4 is also present in the normal squamous epithelium of the female genital tract, although the amount of TA-4 activity is apparently lower in the non-malignant cells than in the malignant cells[3]. Furthermore, previous report suggested that TA-4 would be present in all squamous cell carcinoma tissues[3]. Since FCM is a powerful strategy to measure differences in some characteristics on a cell, the combination use of FCM and TA-4 staining would be a promising aid to detect malignant cells. The present results clearly demonstrated that this is the case. Squamous carcinoma cells were characterized by distinctly different pattern of fluorescence from normal cells. However, for practical application of this technique to the screening of the cervical cancer, further investigation would be required to elucidate whether this method can detect a small population of malignant cells admixed with large number of the non-malignant cells.

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