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Cell Cycle Dependent Con-A Binding

—Flow Cytometric Analysis—

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Abstracts Changes in lectin binding activity occuring during the cell cycle were determined flow cytometrically in exponentially growing YU-101 cells by a technique of gated analysis based on cellular DNA content after double staining of the cells with fluorescein isothiocyanate (FITC) and propidium iodide (PI). Fluorescence intensity from FITC conjugated concanavalin A (Con A) increased during the cell cycle and reached maximum in G2+M phase. Binding of the lectins was reduced by half in G1 phase after cell division. Binding activity of Con A and in YU-101 cells was linked closely with cell cycle. It is suggested that the nuclear changes are associated with alterations of the cellular membrane and that molecular communications between the cell membrane and the cell nucleus control periodic cell cycle. The methodology developed in these studies gives reliable data since a procedure for cell synchronization which brings out some effects on the normal function of the cell cycle is unnecessary.

Key Words: Con A, Flow cytometry, Cell Cycle, DNA

Introduction

The cell surface undergoes changes during the cell cycle[1]. The significance of the membrane change for growth regulation has been suggested and differences in the binding of lectins between the mitotic and interphase cells have been shown[1,2,3,4,5]. However, there are a little reports dealing with relationship between cell cycle and lectin binding, since a cumbersome and time-consuming procedure for cell synchronization has been generally required and yet it induces some metabolic distortion to cells within the cycle.

The technology of flow cytometry permits fluorescence analysis of single cells at rates of 50,000 per minutes. Through combined use of dual-parameter analysis of fluorescein isothiocyanate (FITC)-Con A and propidium iodide (PI), it is possible to make a simultaneous measurement of lectin binding and DNA content at the single cell level. We excite the two fluorochromes at once by a single laser beam and quantitate cell surface lectin binding and cellular DNA content in YU-101 cells at the same time. A technique of gated analysis facilitates measurement of FITC-fluorescence intensity in individual cells fractionated according to cellular DNA

content. We demonstrate in this paper a technique for the simultaneous flow cytometric analysis of cellular DNA content and lectin receptors and changes of Con A binding in vitro during the cell cycle.

Materials and Methods

The established cell line YU-101 was derived from poorly differentiated squamous cell carcinoma of the human lung by one of us (Kawasaki) was used in all experiments. Tumor samples for cell culture were obtained from a 61-year-old male who had recieved neither radiation nor chemotherapy. The cells were grown in monolayer culture and Dulbecco's modified Eagle medium (Nissui Seiyaku Co., Tokyo) supplemented with 10% calf serum. The cell line was maintained by transferring 10^5 cells to 5 ml of fresh medium every 10 days. The cells have been subcultured 30 times during past one year. The doubling time is 48 hours. The cells in exponentially growing phase were removed from petri dish with 0.04% EDTA-Phosphate buffered saline (PBS) and were washed with cold PBS. The cells (5×10^6) were incubated with 10 μg/ml FITC-Con A (Vector Laboratories, Inc., U.S.A) for 45 min on ice. Fixation was performed in 0.5% paraformaldehyde-PBS for 30 min after rinsed with PBS. In addition, the cells were wahed again twice in PBS and refixed in 70% ethanol overnight. All procedures were carried out on ice.

The fixed cells were rinsed in PBS and stained with 5 μg/ml PI dissolved in PBS after RNase treatment as previously described[6]. Flow cytometric analysis was perfromed using FACS Ⅲ cell sorter (Becton Dickison Co., U.S.A.). The doubly stained cells were excited with the 488-nm line from an argon ion laser. Red fluorescence from PI was collected through a 657-nm bandpass filter and recorded as measure of total DNA content and green fluorescence from FITC was collected through a 532-nm band pass filter and recorded as measure of density of lectin receptors. Compensention system was employed since the spectral characteristics of PI results in a "false" increase in detected signal of FITC. Furthermore, a gated analysis (it is possible to selectively analyze one of the chosen parameters based on criteria from another parameters) was carried out to clarify the relationship between cell cycle phases and the density of cell surface receptors of lectins.

DNA histograms were analysed by the method of Takahashi[7].

Results

Incubation of YU-101 cells in exponentially growing phase with FITC-Con A shows binding of the lectins to all the cells in a ring form distribution (Fig. 1). Nuclei stained with PI showed red fluorescence.

To clarify the relationship of lectin receptors of YU-101 cells to cell cycle phases, a double staining method was employed and gated analysis was applyed with respect to DNA centent or Con A binding capacity. Cell cycle phases were determined on DNA content: G1 phase; 36%, S phase; 40%, G2+M phase; 24% (Fig. 2).

Figure 3 illustrates the changes in cellular binding of FITC-Con A during cell cycle. Fluorescence intensity from FITC varied greatly from cell to cell and so even in the fractionated cells based on cellular DNA content. The slanting feature of the dot plot might indicate a parallel increase in cellular DNA and Con A binding, although there is wide variation in Con A binding. Mean values of FITC fluorescent intensity were calculated for populations in G1, S and G2+M which obtained by gated analysis techniques. It increased distinctly with progression of cell cycle from G1 to S phases, and reached a maximum in G2+M phase. A linear relationship was seen between Con A binding and DNA content of the cells. Lectin binding in G2+M cells was elevated twice in G1 cells and namely, after mitosis, it was reduced by half (Fig. 4). Furthermore, a gated analysis based on fluorescence intensity from FITC revealed that the cells with higher fluorescence were in the G2+M phase, whereas cells with lower fluorescence were in the G1 phase (Fig. 5).

Discussion

It is well known that cells in mitosis are subjected to dramatic changes in their sur-

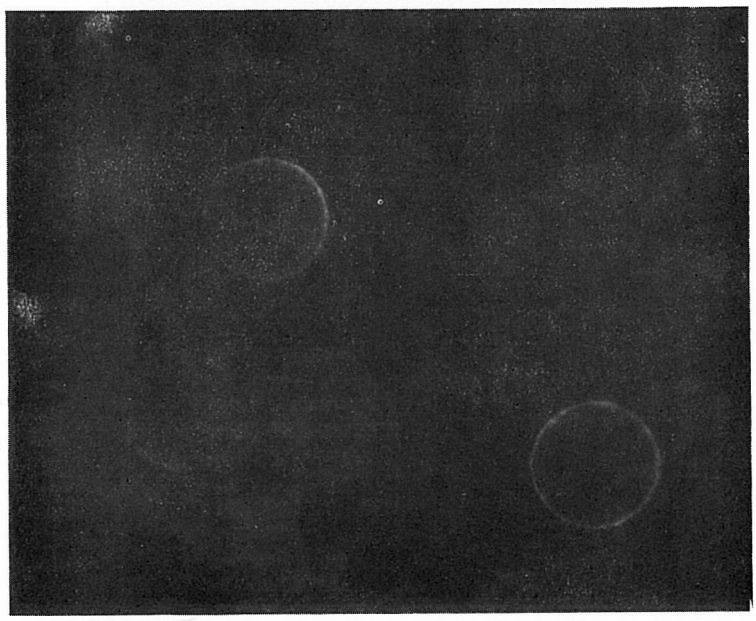

Fig. 1 Green fluorescence is demonstrtaed on the cell surface alone.

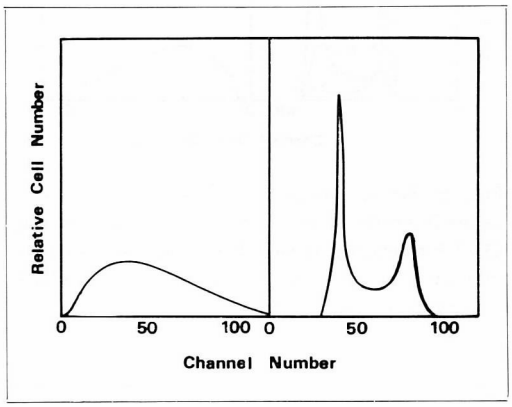

Fig. 2 DNA histograms of YU-101 cells in exponentially growing phase (right) and Con A-binding histogram (left).

face morphology. Moreover, the cell surface undergoes morphological alterations during the cell cycle and cells in mitosis demonstrate higher bind of Con A (3). Differential lectin binding to cells is an indication for differential surface glycosylation patterns among cells in each phases of the cell cycle. However, to what extent the lectin binding activity is depend on the phase of the cell cycle is not known.

To obtaine more information concerning the relationship between cell surface structures and the cell cycle, dual-parameter analysis was carried out by flow cytometry. Individual YU-101 cells showed ring form fluorescence of which intensity varied from cell to cell when exposed to FITC-Con A on ice. The shape of a X-Y dot plot contains information about how the rate of increase of the lectin receptor varies throughout the cell cycle. Bindings of Con A in YU-101 cells increased from G1 to G2+M phases, and after mitosis they were reduced by half.

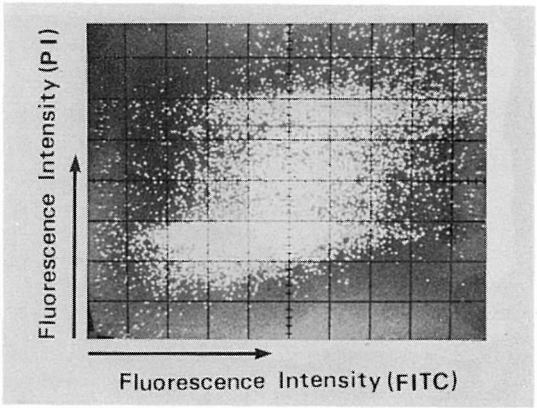

Fig. 3 Con A (FITC) versus DNA (PI) in X-Y plot. It indicates proportinanality between Con A binding and DNA content; higher Con A binding, higher DNA content.

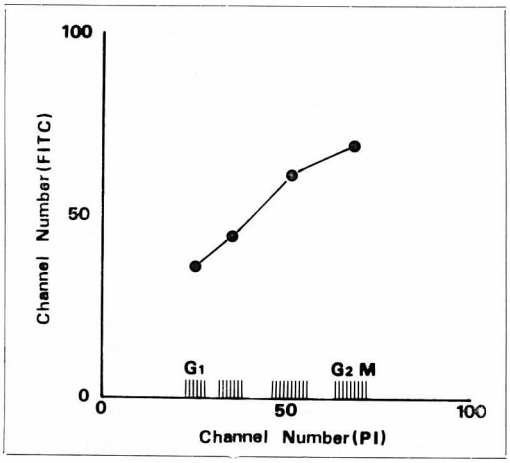

Fig. 4 Mean values of FITC fluorescence intensity (open circle) are obtained by gated analysis carried out in each windows (striped portion). Biding activity of Con A in YU-101 cells is shown as a function of the DNA content measured by FITC fluorescence. It increases with prog ression of the cell cycle, or fluorescence intensity of FITC is cut in half after cell division.

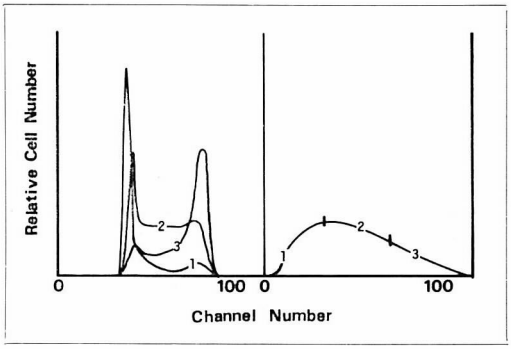

Fig. 5 Gated analysis based on FITC fluores cence intensity (Con A binding) gives interesting DNA histogram patterns. The fluorescence intensity of 1, 2 and 3 regions corresponds to DNA histograms1, 2 and 3, respectively.

The nuclear events relate with cyclic functions of the cellular surface membrane. Binding of Con A to mastocytoma cells increased in the G2 phase and reached a maximum in mitotic phase (3). Transformed mammalian cells aggregate in the presence of lectins, whereas normal cells do not (4). However, in mitosis, both of normal cells and transformed cells show high agglutinability (4). It has been suggested in synchro-

nized cells that although the factors involved in agglutination are still a matter of dispute, agglutination is modulated in the course of the cell cycle in both normal and transformed cells[4]. Without cumbersome and time-consuming procedure for cell synchronization, changes of lectin binding during cell cycle can be quantitatively determined in asynchronous cultured cells by a technique of gated analysis of flow cytometry coupled with double staining. Using the methodolgy developed in these studies. therefore, reliable data can be obtained since a possibility of cell cycle perturbation induced by synchronization would be eliminated. These studies extend our investigation into the significance of the cell surface for growth regulation and the resolution of this type of analysis would be considerably increased.

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