Bull Yamaguchi Med Sch 33 : 9—12, 1986

Relationship between C-MYC Expression and the Cell Cycle in HL-60 Cells —Flow Cytometric Analysis—

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(Received June 20, 1986)

Abstract By flow cytometric measurement of cellular DNA, c-myc protein and cell volume, we attempted to assess the relationship between c-myc gene expression and the cell cycle in exponentially growing human promyelocytic leukemia cell line (HL-60). The protein product of c-myc gene was stained by an indirect immunofluorescence method using a specific antiserum to human c-myc protein. Bivariate distributions of cellular DNA, the c-myc protein contents and cell volume demonstrated the direct evidence that the c-myc protein increases with the progression of the cell cycle and then is halved by cell division.

Key Words : Oncogene, c-myc protein, Cell cycle, HL-60, Flow cytometry

Introduction

It is well known that oncogenes are implicated in oncogenesis and maintenance of transformed phenotypes and that the products of certain cellular oncogenes may regulate the process of self renewal of cells. The c-myc gene also plays an important role in cell proliferation[1,2]. The expression of c-myc gene is temporarily, highly elevated following the mitogenic stimulation of quiescent cells[3,4]. In exponentially growing cells, however, quantitative changes of c-myc gene product at single cell level are still unknown and no thorough analysis has been made to clarify whether or not c-myc expression is related to the cell cycle. The c-myc gene is amplified and expressed constitutively at elevated level in human promyelocytic leukemia line, HL-60[5,6]. The availability of a specific antibody to the c-myc protein makes it possible to evaluate the expression of c-myc gene in detail, in combination with a technique of flow cytometrical multi-parameter analysis. In this paper, to elucidate the change of the c-myc expression during cell cycle progression in exponentially growing cells, a simultaneous, quantitative measurement of cellular c-myc protein, DNA contents and cell volumes was performed in HL-60 cells.

Materials and Methods

HL-60 cells were cultured in RPMI 1640 medium (Flow Lab., U.S.A.) supplemented with 10% fetal calf serum (Flow Lab., U.S.A.) at 37℃ in a humidified atomosphere of 5% CO_2 in air. The cells were used in exponentially growing phase in which the doubling time of the

cells is about 24 hours (data not shwon). After rinse in phosphate buffered saline (PBS), the cells were fixed in 70% cold ethanol overnight. The fixed cells were washed twice with PBS and stained by an indirect immunofluorescence technique. Briefly, the cells were incubated with 1 : 40 diluted anti-human c-myc protein (Oncor Co., U.S.A.) for 1 hr and again washed twice in PBS. Subsequently, the cells were stained with FITC by treatment with 1 : 40 diluted FITC-anti sheep IgG antibody (Dako Co., Denmark) for 30 min at room temperature. The first antibody used has been authenticated to identify a protein of molecular weight 55,000 expressed by human c-myc gene and to react with human c-myc protein from HL-60 cells. Then, PI staining (5 ug/ml) for cellular DNA was performed after RNase treatment.

Flow cytometric measurement was carried out with FACS Analyzer (Becton Dickinson Co., U.S.A.) which was calibrated using lymphocytes so that coefficient of variation was about 2. 0. The cells were excited by 485 nm line from a mercury arc lamp. Red fluorescence was collected through a 580 nm long pass filter and reocrded as a measure of cellular DNA content. Green fluorescence was collected through 520 nm long pass and 535 nm band pass filters and recorded as a measure of amount of the c-myc protein. Three variables for each cell were stored on floppy disket in one experiment ; cell volume, PI and FITC fluorescence intensity The data were processed in list mode ; the values of each variable for each cell were stored in a list. Therefore, it is possible to generate univariate or bivariate distributions of any variable or combination of variables, respectively (Fig. 1). About 30,000 cells were counted.

Results

Background fluorescence of FITC was minimal in the staining procedure employed in this experiment. Although c-myc gene was transcribed throughout the cell cycle, green fluorescence varied greatly from cell to cell. A multivariate gating technique was employed to define clusters of cells. When the average fluorescence intensity of FITC or the average cell volume was plotted against each cellular DNA contents, the lines obtained were sigmoid rather than linear (Fig. 2-A & B). Furthermore, the dot line provided by plotting the average fluorescence intensity of FITC against each cellular volumes was almost linear (correlation coefficient r=0.995) (Fig. 2-C). The increase in c-myc protein during the progrssion through the cell cycle was paralleled by the increase in the cell volume. The average content of c-myc protein in G1 phase cells was about half of that in G2M phase cells. It is concluded that the amount of c-myc protein changed in parallel to the progression of the cell cycle.

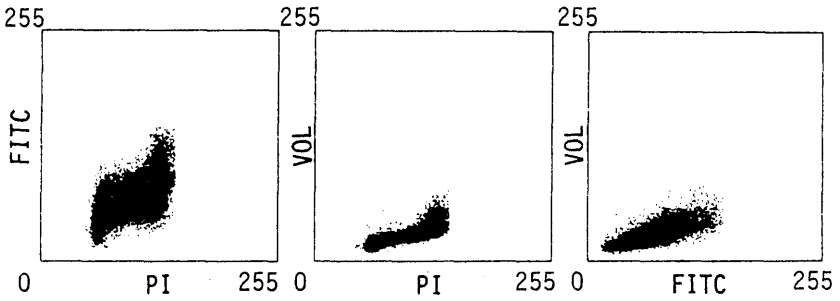

Fig. 1 Three plates of bivariate distribution obtained by reciprocal combination. Two of three parameters reveal the relationship among these parameters, although the amount of c-myc protein varies considerably from cell to cell. A peak of G1/0 based on cellular DNA is set at channel 55 on the axis of DNA content. Abscissa and ordinate : channel number (linear scale).

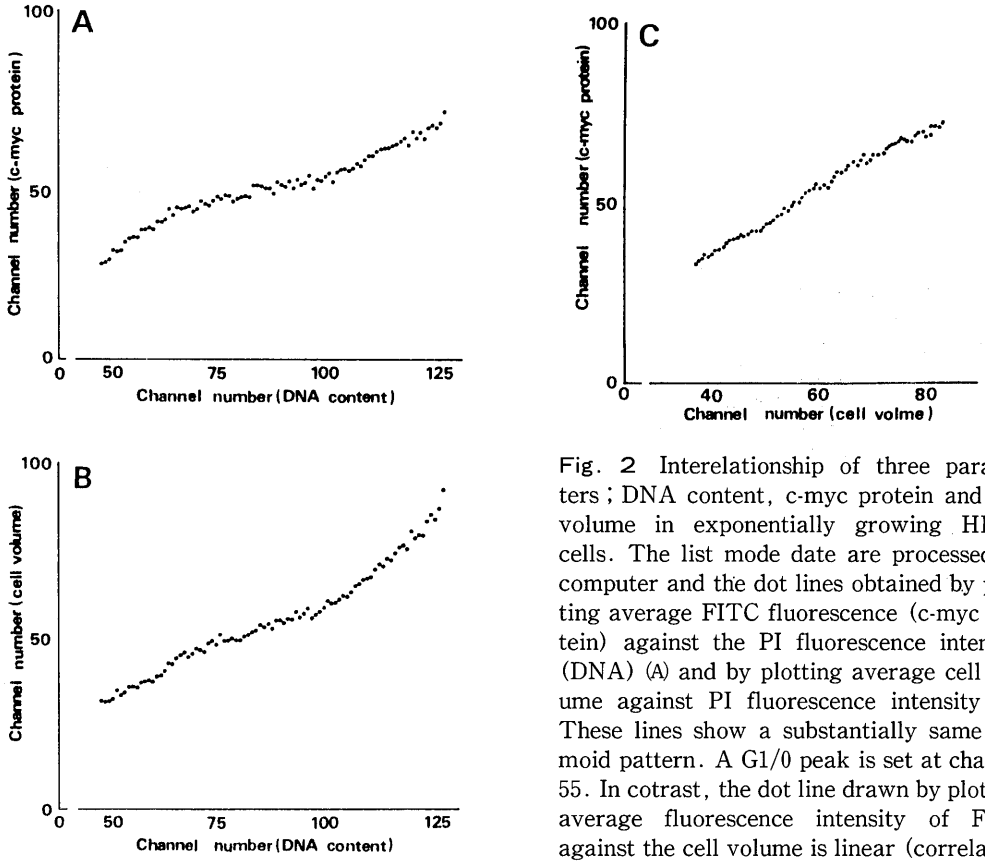

Fig. 2 Interelationship of three parameters ; DNA content, c-myc protein and cell volume in exponentially growing HL-60 cells. The list mode date are processed by computer and the dot lines obtained by plotting average FITC fluorescence (c-myc protein) against the PI fluorescence intensity (DNA) (A) and by plotting average cell volume against PI fluorescence intensity (B). These lines show a substantially same sigmoid pattern. A G1/0 peak is set at channel 55. In cotrast, the dot line drawn by plotting average fluorescence intensity of FITC against the cell volume is linear (correlation coefficient r=0.995) (C). These date indicate that the amount of the c-myc protein changes with the progression of the cell cycle.

Discussion

The amount of c-myc protein increases with progression of the cell cycle as well as cellular protein content and is divided into halves by mitosis. This suggests that the c-myc protein may be consistently required to maintain a constant level of cell proliferation. It is reasonable to speculate that, if half-life of human c-myc protein is extremely short as reported by Hann and his colleagues[7], the progression of the cell cycle from G1 to G2M phase should be accompanied by the increase in transcription rate of this gene. The c-myc protein is transcribed as a fixed percentage of total protein synthesis throughout the cell cycle of both avian and human cells[7]. Thompson et al. also reported that rRNA increases in proportion to cell volume and that the ratio of c-myc mRNA to total rRNA was constant throughout the cell cycle[8].

The analytical procedure used in this experiment provides a direct proof of the correlation between c-myc expression and the cell cycle in exponentially growing cells.

Acknowledgements

We are grateful to Dr. K. Tanaka for help in date processing. This work was supported in part by the Grant-in-Aid for Cancer Research (61-11) from the Ministry of Health and Wel-

fare.

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