CORRELATION OF PLOIDY AND CYCLIN B1, P34^{cdc2} AND PCNA EXPRESSION IN F9 TERATOCARCINOMA CELLS

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Among tumor populations, teratocarcinoma is a valuable model due both to its clinical incidence (it mainly affects young males and is potentially curable), and to its histologic peculiarities (its cells, which are originally undifferentiated, can undergo a process of differentiation giving rise to a number of tissues, including embryoid bodies similar to blastula stage embryos when the cells are grown in mouse peritoneum). Thus, it is useful for studying the processes of tumorigenesis, embryonic development, proliferation and cell differentiation (Atencia et al., 1994). Recently, with the identification of various molecules controlling the passage from one stage of the cell cycle to the next, considerable new light has been shed on the mechanisms that regulate cell proliferation. Certain "checkpoints" exist that are crucial to this regulation process and are located at the G1, G2 and metaphase levels. At each of these points, different molecules come into play, including cyclins, the proteins that regulate the activity of their corresponding cyclin-dependent kinases ("CDKs"). One of these kinases, p34^{cdc2}, when activated by cyclin B, enables the cell to pass the G2 checkpoint and enter mitosis. Within the cell cycle, a key process is DNA synthesis, in which are involved enzymes such as the DNA polymerase δ, one of whose auxiliary proteins is proliferating cell nuclear antigen ("PCNA"). This protein also plays a regulatory role at the G1 checkpoint. Analysis of these molecules makes it possible to study proliferation in various normal and tumor cell lineages. Another way of analyzing the proliferation of a cell population is to measure DNA content, which varies in each cell throughout the cycle and whose pattern changes depending on the degree of proliferation and anaplasia of the cells making up the population.

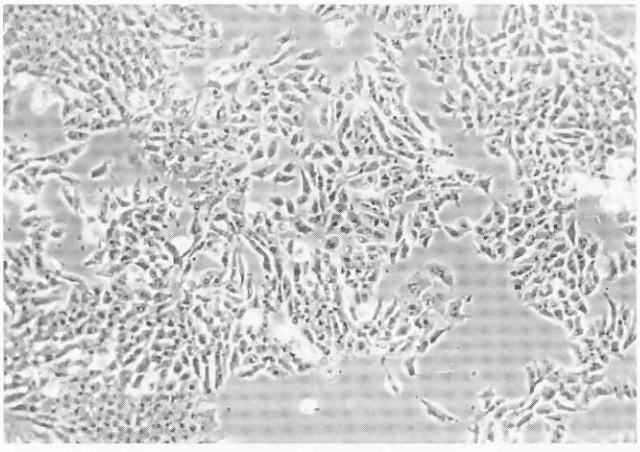

Figure 1. Microphotograph of F9 teratocarcinoma in culture, obtained using phase contrast microscopy.

fluorescent stain with propidium iodide. As 2c control, we used a lymphocyte population obtained from normal mouse thymus. DNA content patterns were then used to determine the percentage of cells in different cell cycle phases employing a gaussian adjustment of data. The proliferation markers PCNA, p34^{cdc2} and cyclin B1 were evaluated using indirect immunofluorescence techniques. The cells were fixed and permeabilized with paraformaldehyde, methanol and triton X-100. Then they were incubated in the primary antibody at 4°C for 16 h, followed by 30 min in the secondary antibody, also at 4°C. The results were analyzed using fluorescence microscopy and marker expression was quantified by flow cytometry.

To analyze the proliferation pattern of the F9 teratocarcinoma cell line, we quantified its DNA content and expression of the proteins PCNA, p34^{cdc2} and cyclin B1. The techniques used were cell culture, immunofluorescence and flow cytometry. The cells were cultured in DMEM medium with 10% fetal calf serum, incubated at 37°C in a humidified, 5% CO2 atmosphere. To prevent cell differentiation, the culture flasks were gelatinized. Every two days, the cultured cells were subcultured with a 0.5%-EDTA 0.2% solution, avoiding cell-damaging confluence. The cultures were not kept longer than 1 month to avoid possible mutations, and cells were replenished from a reservoir kept in liquid nitrogen. Quantification of DNA content was done using two different methods: microspectrophotometry and flow cytometry. For the former procedure, the cells, which were seeded on chamber-slides (Nunc) at a rate of 10,000 cells per well, were fixed in methanol and stained by the Feulgen method. To quantify DNA content by flow cytometry, we used

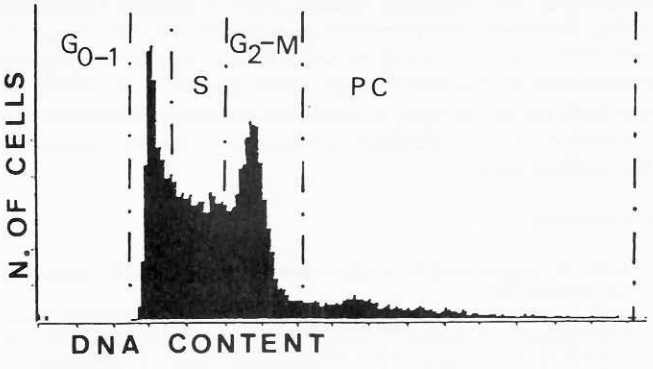

Figure 2. DNA content of an F9 teratocarcinoma cell culture stained with propidium iodide and analyzed by flow cytometry. PC: Polyploid cells.

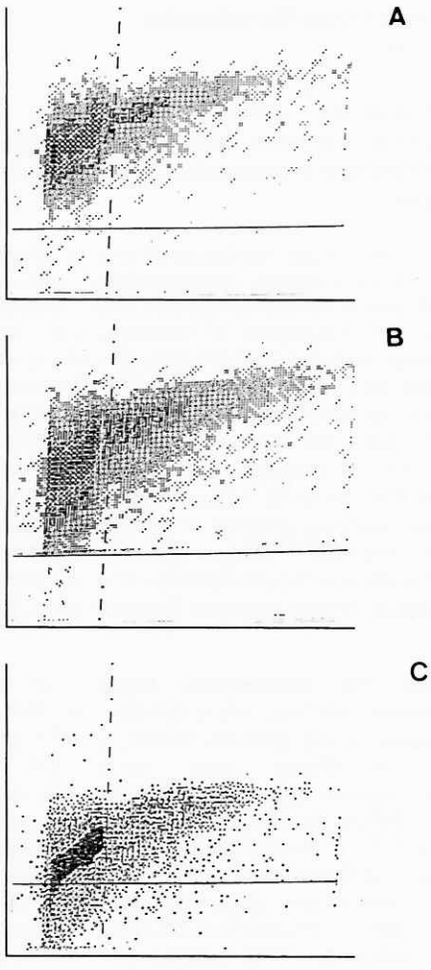

Figure 3. Biparametric graphic representation of PCNA (A), $p34^{cdc2}$ (B), and cyclin B1 (C) quantification (Y axis) related to DNA content (X axis). Negative cells are situated below the horizontal line, and polyploid cells to the right of the dashed line.

F9 cells adhere to the bottom of the culture flask and proliferate rapidly, with duplication times of 14-16 h. They are small cells, with a voluminous nucleus and one or more nucleoli, and form a tight monolayer (fig. 1). Following propidium iodide staining, cell nuclei are red under fluorescence microscopy when stimulated with green light. Quantification of the DNA content of F9 cells in suspension is shown in figure 2. There is a bimodal distribution, with 27.8% of the cells in G0/G1 and a large proportion of proliferative cells in the S and G2/M phases of the cell cycle (24.5% in S and 31.2% in G2/M). Also, 15.9% of the cells had a higher DNA content than the rest of the population and are located on the right of the bimodal curve. These are the high ploidy cells resulting from anomalous cell cycles, which are present in most tumors. Compared with the 2c pattern, the F9 cells are predominantly diploid. Polyploid cells reach as far as 8c and, very exceptionally, 12c. Observation using fluorescence microscopy of PCNA protein patterns in cells labeled by indirect immunofluorescence showed that the marker is located exclusively in the nucleus, adopting a spotted pattern that varies according to the cell, depending on the cycle phase and, more specifically, on the stage reached within the S phase. Quantification of the presence of PCNA by flow cytometry showed that all the cells express this protein with variable intensities following a gaussian distribution. The $p34^{cdc2}$ protein was located primarily in the cytoplasm. Quantification by flow cytometry revealed that all the cells expressed the protein in different degrees and with less intensity than in the case of PCNA. Cyclin B1, located in the cell cytoplasm, was more weakly labeled than the previous two proteins and was expressed only in 80% of the cells (fig. 3).

Taken together, these results show that F9 teratocarcinoma cells proliferate very quickly, since most of them were found in the S, G2 or M phases of the cell cycle, with only a relatively small percentage of cells in the G1 phase. This is typical of rapid cycle embryonic cells, with which teratocarcinoma bears such close similarity. Other authors have verified that in embryonic P19 carcinoma, the proportion of cells in G1 increases significantly following induction of differentiation 'in vitro' (Kranenburg et al., 1995). Moreover, the presence of a significant percentage of high ploidy cells indicates the existence of anomalous cell cycles, with DNA replication that is not followed by normal chromosomic segregation. We have studied these cells in other tumor models (de la Hoz and Baroja, 1993; Baroja et al., 1996). Also indicative of the proliferative potential of this cell population is the fact that all the cells express two proteins characteristic of cells in cycle, PCNA and $p34^{cdc2}$. Cyclin B1 is present in a large proportion of cells, although not in all. This fact is compatible with mitotic degradation, which is indispensable for completion of the division cycle.

References

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