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Short Report

Transient differentiation of teratocarcinoma cells towards nurse cells - an intrinsic mechanism of cooperation in cell cultures?

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Introduction

Embryoid bodies (EBs) are spontaneously formed organotypic structures which preserve the properties of 3-D cultures (Itskovitz-Eldor *et al.,* 2000). They are called EBs because of their resemblance to morulae and early blastocysts. EBs contain totipotent cells (Andrews,1998) and constitute a highly dynamic structure and an interesting model for studying cell differentiation (Damjanov, 1993; 1996). Recently, we have reported the existence of four stem cell populations in the CE44 teratocarcinoma (Hilario *et al.,* 2001). The aim of this work was to study the cell dynamics present in the cultures of this teratocarcinoma.

Materials and Methods

CE44 teratocarcinoma is a tumor cell line which is maintained intraperitoneally by serial transfers of ascitic fluid to 129 Sv mouse. Mice bearing 21-day ascitic tumor hosts were killed and the peritoneal fluid was aspirated. Cultures were performed from plating out of 10^4 EBs per culture flask as previously described (Alvarez *et al.,* 1999). Morphological and flow cytometry studies were carried out with 4, 6 and 14-day monolayers.

For the cytometric study (Hilario *et al.,* 2001) we used an EPICS ELITE Cytometer (Coulter Electronics, Florida, USA).

Staining for DNA content

The cells ($1x10^6$ cells/ml) were fixed in 70% methanol (30 minutes at 4°C), and resuspended in 500 µl of PBS-EDTA 0.013M and 100µg RNase A (Sigma, MO, USA) and incubated for 30 min at 4°C. Propidium iodide (Sigma, MO, USA) was added to a final concentration of 20 µg/ml for 30 min. Control was performed using thymocytes from 129 Sv mice.

Results

Embryoid bodies attached just slightly to the surface of the flask. Thus, when in 48 hours the culture medium was changed, most of the EBs became detached and in their place scattered giant cells and to a lesser extent small cells remained. When EBs were strongly attached they flattened out and these cells later produced a nest of cells around the attached embryoid body. After, a crown-like arrangement of the cells could be observed, while the embryoid body was undergoing a considerable reduction in size.

These giant cells (Fig. 1A) showed a fibroblast-like morphology and their size reached up to 3 mm. in diameter (measurement taken with a reticule in the eyepiece of the inverted microscope). The nucleus was large with dispersed chromatin and one or more nucleoli. Cytoplasm showed a low density, and contained few organelles and a prominent cytoskeleton. The smallest cells were normally star-like and displayed a very dense cytoplasm masking the nucleus. During the tracking of growth of the cultured cells, one could see how small cells made their way toward the giant cells and settled just above them (Fig. 1B). In this position the small cells gave rise to nests of cellular proliferation. Thus, each of the large cells formed a growth site. Throughout this time, the number of giant cells remained more or less constant, but the number of small cells increased covering the giant cells. Cells of the nests proliferated and gave rise to an area of greater cell

TABLE 1

DISTRIBUTION, IN THE DIFFERENT STAGES OF THE CELL CYCLE, OF THE GIANT CELLS PRESENT IN THE MONOLAYERS, ON THE 4TH, 6TH AND 14TH DAYS OF CULTURE. DATA ARE EXPRESSED AS PERCENTAGES

		Cell Cycle		
	Diploids	G0/G1	S	G2/M
4 days	94.13 ± 2.6	57.72 ± 3.6	14.68 ± 0.7	21.73 ± 4.0
6 days	97.42 ± 7.4	68.45 ± 3.2	9.05 ± 1.0	19.70 ± 2.5
14 days	99.65 ± 4.8	63.88 ± 6.3	11.00 ± 1.6	24.50 ± 4.0

Three monolayers, coming from different animals were used. From each of them 5 samples were taken. To calculate the ploidy index, thymocytes coming from 3 mice were used.

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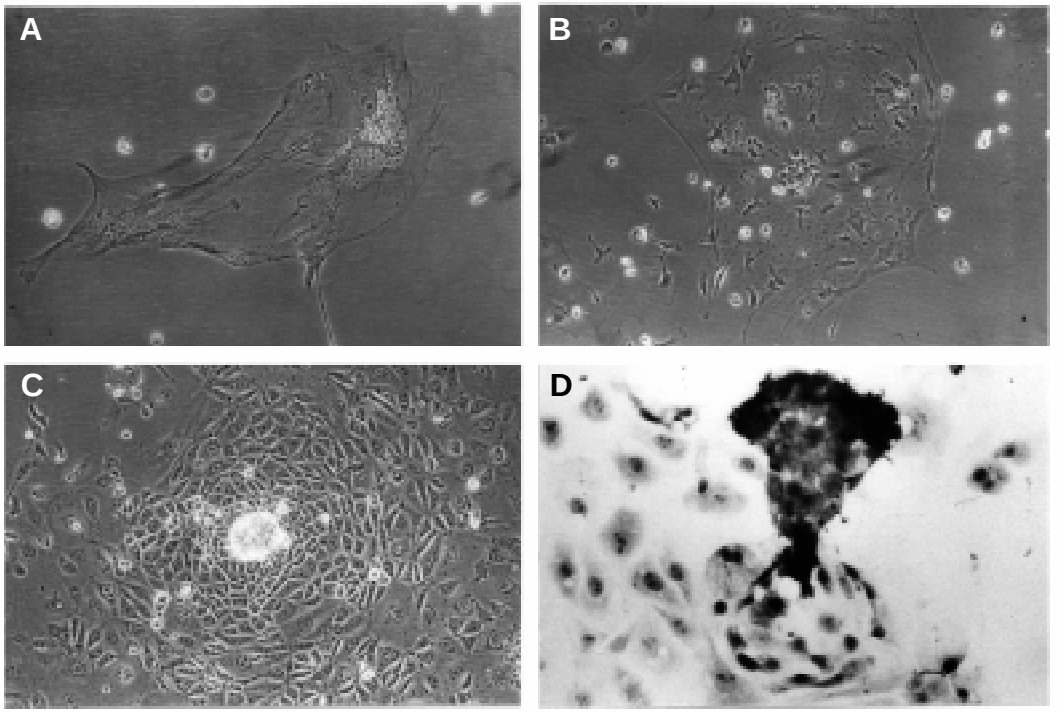

Fig. 1. Theoretical sequence in the formation of embryoid bodies in the monolayer. **(A)** *shows a giant cell with surrounding small cells.* **(B)** *Small cells lying on a giant cell. In* **(C)**, *a monolayer with a nest, and in* **(D)** *an embryoid body about to be shed from the monolayer. (A,B,C) were obtained by phase-contrast microscopy. (D) was obtained by conventional light microscopy from cultures on chamber slides stained with toluidine blue (Original magnifications: A,B,C, 6x; D, 200x).*

density, which formed a new embryoid body (Fig. 1C and 1D) that finally was shed from the monolayer. Monolayers were made up of a diploid cell population (Table 1). The distribution of the cells in the different stages of the cell cycle is shown in Table 1. Giant cells of cell cultures constituted the 23.44 ± 1.17% of total cells and showed a FALS of 50.11 ± 3.83 a.u. and a ISS of 60.24 ± 0.2 a.u.

Discussion

Our results show the presence of giant cells in low-density regions of monolayer cultures. These giant cells seemed to play an important function during cell proliferation in foci formation, which will develop new EBs.

The presence of giant cells in embryonal tumor cultures raise new questions about the cellular dynamics in these tumors. Moreover, giant cells are not a homogeneous cell population, at least with respect to cell differentiation. Recently, we have described the existence of four different stem cell populations in CE44 teratocarcinoma cultures (Hilario *et al.,* 2001). In this sense, the relation between giant cells and the proliferation and cell differentiation of the other cells of the culture remains to be elucidated.

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