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DIFFERENTIATION OF SPONTANEOUS AND VIRUS-TRANSFORMED OSTEOBLAST-LIKE CELLS BY COMPUTER-ASSISTED IMAGING CYTOMETRY

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INTRODUCTION

Non-transforming, bone tumor-inducing murine leukemia viruses (MuLV) (1) significantly affect growth and differentiation of osteoblast-like cells in vitro (2). In contrast, the FBR osteosarcoma virus (FBR MSV) transforms skeletal cells in vitro (3). Since retroviral infection and neoplastic transformation ensues via integration of viral DNA into the host genome, resulting in distinct changes in cellular morphology and functions, we studied morphological parameters of the nuclear chromatin of MuLV- and MSV-infected osteoblast-like cells at different stages of neoplastic transformation by imaging cytometry and discrimination on a multidimensional feature base.

MATERIAL AND METHODS

Osteoblast-like MC3T3-E1 cells were infected with OA MuLV (1) and two sublines were cultured further over a period of more than 100 passages. Non-infected MC3T3-E1 cells and cells transformed with the v-fos oncogene (MC3T3-E1-FBR) were used as controls. Micrographs of the different cell lines were obtained from Giemsa- and H33258-stained cells. Tumorigenicity of the cell lines was tested in syngeneic newborn mice. For imaging cytometrical analysis the cells were Feulgen-stained. Scanning conditions, feature extraction and statistical analysis were carried out as described (4).

RESULTS AND DISCUSSION

MC3T3-E1 cells exhibit a round, polygonal morphology and epithelium-like appearance in the confluent state (Fig. 1A). They were non-tumorigenic over a period of 104 cell culture passages. One cell line (MC 3T3-E1-OAnt), infected with OA MuLV showed increasing morphological heterogeneity (Fig. 1B) and similar appearance of the nuclei (Fig. 1F). The cells formed colonies in soft agar, however, they failed to develop tumors after infection into syngeneic mice. A different OA MuLV-infected cell line (MC3T3-E1-OAt, Fig. 1C,G) grew in soft agar and developed sarcomas in syngeneic mice. FBR MSV-infected cells (MC3T3-E1-FBR) were round-up, elongated and spindle-shaped (Fig. 1D). The size and morphology of the nuclei varied greatly. Numerous mitotic figures (Fig. 1H) were regularly found, indicative of the high proliferative activity of this cell line. DNA histograms of MC3T3-E1 control cells showed a bipartite G0/G1 peak between 2c and 4c, indicating the presence of two stem lines. MC3T3-E1-OAnt cells showed two distinct DNA peaks, one at 3c and a new peak at approximately 5c. Tumorigenic MC3T3-E1-OAt cells lacked a distinct stem line. They showed a diffuse aneuploid-hyperploid DNA distribution ranging from 6c to beyond 12c. A similar pattern with two stem lines was observed in v-fos-transformed MC3T3-E1-FBR cells. These data indicate an increase in DNA content and its diffuse multiploid distribution in the course of neoplastic transformation. A four-class-case discriminant analysis resulted in a correct assignation of 78.5% of all cells to one of the 4 cell lines. Individual distinction of the 4

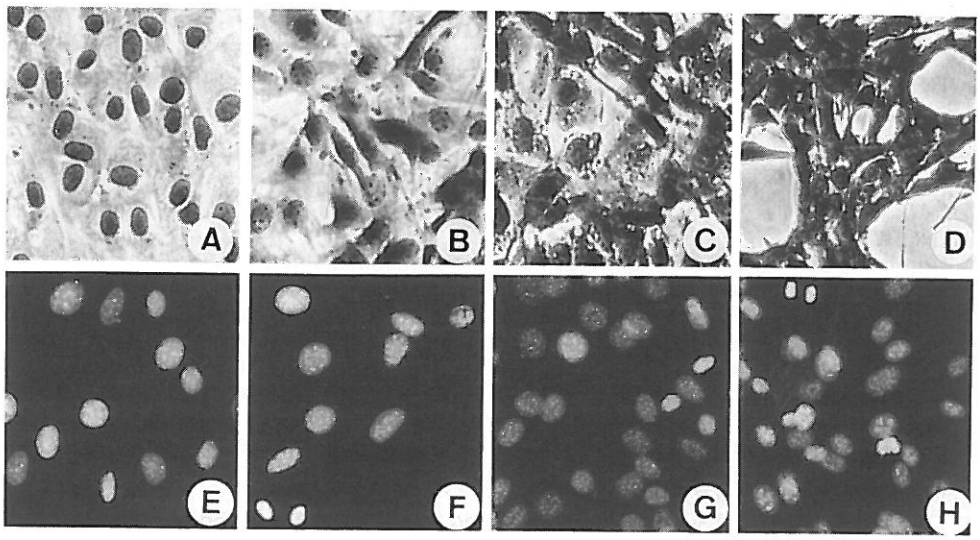

Fig 1 Microphotographs (A-D, Gimsa stain) and fluorescence micrographs (E-H, bisbenzimide-stain) of 3-day-old MC3T3-E1 cell cultures. A,E, control cells; B,F, non-tumorigenic, OA MuLV-infected cells; C,G, tumorigenic OA MuLV-infected cells; D,H, FBR MSV-transformed cells (x250).

cell lines carried out by discriminant analysis of the six possible two-class-cases was 79.6% to 96.6%. Since OA MuLV lacks transforming sequences (1) we consider MC3T3-E1-OAt cells to have undergone spontaneous transformation, a frequent event in permanent mouse cell lines. The role of the non-transforming virus in this process is not yet clear. In contrast, MC3T3-E1-FBR cells were transformed by the direct action of the v-fos oncogene. The discrimination of the two neoplastic cell lines on the basis of morphological parameters of the nuclear chromatin by 93.6% led to the assumption, that different pathways of cell transformation, can be identified and determined quantitatively by this type of analysis.

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