Cytological Differences between Normal and Malignant Human Cell Populations in Culture

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ABSTRACT

Cells from early-passage cultures of normal and malignant human tissues were analyzed for the presence of multiple nucleoli and tri- or multipolar mitoses; these properties are characteristic of malignant cell populations in tissue sections. For the cell types examined the presence of tri- or multipolar mitoses was characteristic of cells of malignant origin. Within epithelial cell populations, cells containing more than 4 nucleoli were found in populations of malignant but not of normal origin; this distinction did not apply to fibroblast populations. Large numbers of cells must be analyzed quantitatively to establish the distinctions described here, since they are properties characteristic of populations, not of individual cells. This approach may facilitate identification of normal and malignant cell populations in primary and early-passage culture.

INTRODUCTION

It is of considerable interest to study early-passage human cancer cells in culture. Such studies are of value, for example, in development of systems for predictive drug testing (1) and for basic mechanistic or diagnostic studies of biochemical properties, drug resistance, oncogene expression, responses to growth factors, chromosomal abnormalities, and genetic deletions (2–6). The heterogeneity of tumors both in terms of the spectrum of cell types which they contain and the ability of the tumor cells to grow in vitro makes such studies difficult to perform routinely. Contamination of carcinoma cultures by fibroblasts or macrophages can generally be recognized microscopically and confirmed by immunofluorescence, but normal and malignant epithelial cells are difficult to distinguish. Although many biochemical and biological properties have been associated with malignancy of cancer cells (7, 8), none of them are ideal for routine use; most of them apply to only a limited proportion of cancer cells or are exhibited by a range of normal cell types also, and often they require specialized techniques or involve a significant time lag before results are available. It would be a considerable advance for those of us involved in primary cell culture if a simple, rapid, quantitative method requiring a small number of cells could be used to determine the presence or absence of malignant cells in primary or early-passage cultures.

Histopathologists have developed a range of relatively simple criteria to detect malignancy in tissue sections. While some of these (such as invasiveness and metastasis) are not readily applicable to cells in culture, several of the characteristics used involve intrinsic properties of the cells and therefore might be relevant in vitro. The study described in this paper investigated whether or not simple histopathological criteria can be used in an objective and quantitative manner to distinguish normal from cancer cell populations in culture. It is sometimes assumed that the criteria used by the histopathologist to assess malignancy in tissue sections are applicable also to cells in culture. There is however (to our knowledge) no documented study of human cells using objectively assessable quantitative criteria which would allow laboratories engaged in primary culture to use these criteria with confidence. Previous studies using animal cells found correlations between malignancy and cytological properties, but this required expert assessment of multiple criteria and was not on a strictly quantitative and objective basis (9–11). Nevertheless, such multiple-parameter cytomorphological diagnoses of malignancy in vitro can be very accurate, although the level of accuracy varies with the evaluator involved (12).

The study presented here concentrates on criteria which can be quantified and analyzed in cell culture laboratories without special histopathological expertise and also concentrates on human cells. In choosing criteria for detailed analysis we rejected several, either because estimation would be somewhat subjective (e.g., altered cytoplasmic basophilia/acidophilia, nuclear hyperchromasia) or because we would expect such criteria to be relevant only in paired normal-malignant cell sets, and not relevant to comparison of different cell types (e.g., variation in cell or nuclear size and shape). We concentrated on criteria which we considered suitable for objective quantitative analysis: (a) number of nucleoli and (b) number of normal, tripolar, and multipolar mitoses. Some preliminary work was also done on nuclear:cytoplasmic ratio.

MATERIALS AND METHODS

Sources of Material for Primary Cultures

Tumor specimens (lymph nodes or primary lung tumor tissue) and normal lung and skin biopsy specimens were obtained from St. Vincent's Hospital, Elm Park, Dublin, Ireland. Amnion specimens were obtained from the National Maternity Hospital, Dublin, Ireland. All specimens were collected directly into sterile collection medium (1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum, penicillin (300 units/ml), streptomycin (300 µg/ml), tetracycline (150 µg/ml), and Fungizone (2.5 µg/ml) and were transported on ice to the laboratory.

Establishment and Maintenance of Cultures

Primary Cultures. Skin biopsies were set up as explant cultures; all other specimens were disaggregated enzymatically using a combination of 0.2% neutral protease (Dispase; Boehringer Mannheim 165–859) and 200 units/ml collagenase (type IV, Sigma C-5138 or type V, Sigma C-9263).

Growth Medium. All primary samples were cultured in a 1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, penicillin (100 units/ml), streptomycin (100 µg/ml), Fungizone (1.25 µg/ml), and hydrocortisone (0.4 µg/ml).

Fibroblast Removal. When predominantly epithelial cell populations were required fibroblasts were removed using 0.02% EDTA and 0.01%
trypsin at room temperature for 5–10 min (with careful microscopic monitoring).

Cell Lines. The following human lines were obtained from and cultured in media recommended by the American Type Culture Collection (Rockville, MD): SCC9, SCC 25, and RPMI 2650 (squamous cell carcinomas), Hep-2 and A431 (epidermoid carcinomas), HT1080 (fibrosarcoma), and SK-LU-1 (adenocarcinoma).

Preparation and Staining of Cultures. All cell lines and enzynemedissaggregated biopsy material were cultured in 25-cm² flasks. At 50–70% confluency, cultures were trypsinized (0.25% trypsin-EDTA) and seeded in sterile 8-well slides (Dynatech) at a final concentration of 2.5 x 10⁶ cells/well. Following incubation at 37°C in a 5% CO₂ atmosphere for 24–48 h the slides were thoroughly rinsed in phosphate-buffered saline and the cells were fixed and stained using absolute alcohol and sequentially stained with Carazzi's hematoxylin and 0.5% eosin. Phosphate-buffered saline/glycerol was found to be a satisfactory mounting medium for plastic, while glass slides were placed in xylene for a minimum of 1 h and mounted in DPX (BDH Chemicals). In some cases (e.g., skin explants) cells were fixed and stained directly in flasks.

RESULTS

Cultures (prepared as described above) were analyzed for the number of nucleoli/cell (Fig. 1) or for the presence of normal (Fig. 2) or tripolar or multipolar (Fig. 3) mitoses. Nucleoli in female cells could be distinguished from the Barr body on the basis of their greater size (Fig. 4).

Table 1 provides a summary of the analysis of numbers of nucleoli/nucleus for the various cell cultures studied. Results are presented as the percentage of nuclei which contained 1, 2, 3, 4, 5, or 6 nucleoli. For the populations analyzed it appears that nuclei of normal epithelial cells never contained more than 4 nucleoli, whereas nuclei of some of the cells in carcinoma populations contained 5 or 6 nucleoli. Some normal fibroblast populations also contained cells with more than 4 nucleoli/nucleus.

Table 2 concentrates on the mitotic cells in each population and expresses (as a percentage) the proportion of the mitotic cells displaying normal, tripolar, or multipolar figures. The results indicate that normal cell populations contain only normal mitotic figures, whereas within all of the malignant cell populations examined, some tripolar or multipolar mitoses were observed.

DISCUSSION

The results presented here indicate that human carcinoma cell populations in culture differ from normal epithelial cell populations derived from skin, lung, and placenta with reference to at least two cytological criteria; some cells in the population contain more than four nucleoli, and some of the mitotic figures are tripolar or multipolar. Recent studies of human hepatoblastoma and pancreatic carcinoma cell lines (13, 14) reported the presence of multiple nucleoli. A recent account
of a human renal cell carcinoma cell line (15) describes the cells as having only one or two nucleoli, but quantitative data are not presented, unfortunately.

In the case of normal human fibroblasts, once again tri- and multipolar mitoses are not observed; but in contrast to normal epithelial cells they may contain more than four nucleoli.

Since only a very small proportion of tri- and multipolar mitoses were seen, it was important to ensure that a sufficient number of mitoses was analyzed in both normal and malignant cell populations. It is clear from the data in Table 2 that a sufficient number of mitotic cells was analyzed in most of the normal cultures to ensure observation of abnormal mitoses if they occurred at frequencies similar to those prevailing in malignant cell populations. Since tripolar mitoses are observed not only in established cell lines but also in early-passage cultures of malignant origin and in malignant tissue in vivo, it is reasonable to attribute this property to the malignant population per se rather than to any effect of passage level or of the in vitro environment. Most of the normal cell populations described here were analyzed after 1 or 2 passages in vitro (a few were primary cultures; see Table 1) and we have not examined the effect of more extensive passaging on the number of nucleoli or the presence of tri- and multipolar mitoses. Several of the
cell populations of malignant origin were also analyzed after 1 or 2 passages (Table 1), however, and showed the properties described; it is clear, therefore, that, for the cell populations examined here, these properties are distinguishing characteristics for cells of malignant origin, at least at these early-passage levels.

Preliminary observations indicated that criteria such as more intense nuclear staining and irregularities of cell and nuclear size and shape did appear to correlate with malignant origin of the cells, but (as mentioned in the introduction) such criteria are difficult to score quantitatively and objectively. Detailed analysis of nuclear:cytoplasmic ratio was also performed on many of the malignant and normal cultures described here (data not shown). Both populations showed a very large spread in values. The average nuclear:cytoplasmic ratio for a particular culture showed no correlation with normal or malignant origin; in many instances, however, there was a tendency for malignant cell populations to contain cells with lower ratios. Further analysis of the possible relevance of this ratio in vitro is in progress. Other criteria including cell shape at metaphase (16) and results of specialized morphometric analyses (17) have been found relevant in distinguishing neoplastic and nonneoplastic rodent cells in vitro, and it would be of interest to investigate the applicability of such criteria to early-passage human cell cultures. The report by Gantt et al. (18) demonstrating increased G2 chromatid radiosensitivity as an early event in neo
cultures. The report by Gantt et al. (18) demonstrating in vitro (Rockville), 24: 1137-1146, 1988.

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