Direct Cloning of Human Parathyroid Hyperplasia Cells in Soft-Agar Culture

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ABSTRACT

Parathyroid specimens removed from patients with clinical hyperparathyroidism were cultured in a two-layer soft-agar system. Four patients had parathyroid hyperplasia and one had a parathyroid adenoma. Colonies grew from single-cell suspensions of each specimen. Plating efficiency ranged from 0.001 to 0.05%. No colonies grew from two normal bovine parathyroid specimens. Parathormone was detected in 0.9% NaCl solution incubated with the culture plates of three of the four human specimens tested. Parathormone levels determined by radioimmunoassay ranged from 10.4 to >100 ng/ml. Plates tested serially showed a progressive rise in parathormone levels with time and an increase in colony size and number. Microscopic evaluation of the cellular layer showed clusters of cells morphologically consistent with parathyroid origin. Colonies remained viable for approximately 3 weeks. These data confirm that malignancy of tissue in vivo is not necessary for colony formation in agar and that human parathyroid hyperplasia or adenoma cells produce and secrete parathormone in this system.

INTRODUCTION

The ability of cells to form colonies in soft agar is one of the properties that characterizes transformed or malignant cell lines (2, 8). Several reports have described the ability to predictably grow colonies in agar from suspensions of cells taken from the fresh human tumor tissue of solid tumors, malignant ascites, and bone marrow sources (3, 4). With the exception of bone marrow colonies grown with specific trophic factors added, such as a colony-stimulating factor, there have been no reports of nonpassaged, nonmalignant human tissue which has been shown to form colonies in agar. We describe the growth in a 2-layer soft-agar system of colonies formed from single-cell suspensions of parathyroid specimens removed for surgical correction of hyperparathyroidism due to parathyroid hyperplasia or adenoma. This paper describes the biochemical and histological characteristics of these parathyroid colonies in agar and discusses the implications of the growth of this abnormal but nonmalignant tissue in this system.

MATERIALS AND METHODS

Patient Studies. Patients with suspected hyperparathyroidism were referred to the Surgery Branch, National Cancer Institute, for evaluation. All patients studied had hypercalcemia, hypophosphatemia, normal renal function, inappropriately elevated serum PTH² levels, and increased urinary calcium excretion. No other cause of hypercalcemia was found after extensive clinical evaluation. Patients underwent parathyroid exploration, and a portion of each parathyroid gland removed was submitted for histological examination. A portion of the largest gland removed from 5 consecutively explored patients was sent immediately to our laboratory for agar culture.

Collection of Cells. A small portion of an excised parathyroid gland was taken immediately from the operating suite to the laboratory for culture. A single-cell suspension was made by mechanically teasing the tissue apart under sterile conditions and then by passing the cellular fragments through progressively smaller needles. These cells were then washed twice in Hanks' balanced salt solution (Grand Island Biological Co., Grand Island, N. Y.). Cell viability was determined by the ability to exclude trypan blue, and the cells were counted in a hemocytometer. Because of the average viability of 1.0 to 10.0% and the small specimen size, the number of nucleated viable cells in the 1 ml of final plating medium ranged from 5.0 x 10⁴ to 1.0 x 10⁵ cells. Specimens of normal bovine parathyroid tissue were handled in the same fashion.

Culture Assay for Parathyroid Cells. The culture assay used was similar to that described by Hamburger and Salmon (3, 4) except that no conditioned medium was used. One ml of 0.5% agar in enriched McCoy's medium 5A was prepared as an underlayer in 35-mm Petri dishes. The parathyroid cells were suspended in modified CMRL Medium 1066 (Grand Island Biological Co.) and 15% horse serum (Flow Laboratories) with a final agar concentration of 0.3%. This cellular layer was allowed to gel and was then incubated at 37°C in an atmosphere of 7.5% CO₂ and air at 100% humidity.

Growth of Colonies. The plates were examined several times each week for evidence of colony growth. Colonies were defined as round aggregates of 50 or more cells arising from a single cell.

Histological Characterization. Each specimen underwent routine histological preparation and examination prior to culturing. Histological examination of colonies growing in agar was performed with a technique similar to that described by Salmon and Buick (6). Briefly, plates which demonstrated colony growth were washed once with 0.9% NaCl solution for 10 min and then fixed with a 3% solution of glutaraldehyde in Hanks' balanced salt solution. The portion of the cellular layer to be examined was then outlined with a 25-gauge needle by cutting through the cellular layer but not through the bottom agar layer. The cellular layer was then washed into a Petri dish filled with 0.9% NaCl solution and floated onto a glass slide. The agar film on the slide was kept from wrinkling during the drying period by placing on it an overlay of cellulose acetate.

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² The abbreviation used is: PTH, parathormone.
which was removed after the slide had dried. The dried sections were stained with hematoxylin and eosin and examined by light microscopy.

**PTH Assay.** Three representative plates from each specimen to be tested were chosen. Two ml of sterile 0.9% NaCl solution were added to the Petri dishes, and these dishes were allowed to stand overnight at 37°. The supernatant was aspirated the following day, frozen, and stored for PTH determinations. This procedure was repeated on the same 3 plates at approximately weekly intervals. The addition of 0.9% NaCl solution did not appear to alter the colony morphology or the growth characteristics compared to the plates of the same specimen which were not assayed for PTH. A standard radioimmunoassay for PTH was used, and all specimens were run with the same reference specimen as the control (1).

**RESULTS**

**Colony Growth.** Colonies (50 or more cells) grew from each of the 4 human parathyroid hyperplasia specimens and from the one parathyroid adenoma specimen. No colonies formed from the normal bovine parathyroid specimens. Plating efficiency ranged in those plates which developed colonies from 0.001 to 0.05%. Small clusters of 6 to 8 cells became visible within 4 to 6 days. By 10 days, most of the clusters had grown to dense round colonies. The average colony size by Day 14, the average time at which most colonies had reached maximum size and number, was 200 cells. After 3 weeks, individual cells in the agar began to appear pyknotic, and the colonies began to fragment. No attempt was made to refeed or subculture the plates.

**Morphological Characteristics of Cells.** Microscopic evaluation of cellular layers at 2 weeks revealed colonies composed of relatively homogeneous populations of polyhedral cells possessing cytological features consistent with parathyroid origin. Many of these cells possessed small central pyknotic nuclei and moderate amounts of eosinophilic cytoplasm characteristic of parathyroid chief cells. Specimens examined after 3 weeks in culture exhibited marked degenerative changes and loss of cellular cohesion.

**PTH Assays.** Of the 4 specimens assayed for PTH, 3 showed levels of hormone. Two of the 3 were assayed serially and showed a progressive increase of PTH level with an increase in colony size and number (Table 1). The third specimen was not assayed for PTH. A standard radioimmunoassay for PTH was used, and all specimens were run with the same reference specimen as the control (1).

**DISCUSSION**

The growth of colonies from all 5 human parathyroid specimens is of interest. The small size of the specimens, the low cell viability, and the resulting small number of viable cultured cells per plate did not seem to deter the parathyroid colonies from forming with a plating efficiency similar to other fresh tumor specimens which have been shown to grow readily in agar (4, 9). The presence of a marker (PTH) in addition to the characteristic histology confirm that these colonies were of parathyroid origin. These data demonstrate that nonmalignant, fresh human parathyroid hyperplasia cells can be readily cultured in soft agar. Growth of cells in soft agar is often cited as evidence for cell transformation and is generally limited to malignant tumor specimens (8), transformed cell lines which have been passaged in culture for long periods of time (2, 7, 8), or hematopoietic stem cells in the presence of specific growth factors, such as a colony-stimulating factor (5). While these parathyroid cells are not malignant by histological and clinical criteria, it is of interest that normal bovine parathyroid cells did not form colonies while all of the hyperplasia and adenoma specimens did. While we have not performed this assay on normal human parathyroid specimens, it is not unreasonable to propose that nonhyperplastic or adenomatous parathyroid tissues of humans may also, like bovine tissue, not form colonies in agar and that this property is associated with the altered state of growth regulation in these hyperplastic glands. The cellular changes which occur, allowing growth in agar, are as yet unknown, but our data demonstrate that cancer is not one of the necessary changes. It is possible that colony formation in agar might be a marker for, or in some way associated with, one or a variety of intracellular events. Repression of regulating loci or the addition of new information from an infecting virus could be one of these events which could both allow for growth in agar and be part of the gradual transformation process of a cell with normal proliferative control to one with frankly malignant characteristics in vivo. The ability to select those parathyroid cells which are able to form colonies in agar and to compare them with those which cannot may provide the answer to the question of what intracellular changes permit growth in agar.

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