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Identification of Prolactin Cells in a Mammosomatotropic Tumor by the Unlabeled-Antibody Peroxidase–Antiperoxidase Method (37844)

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Experimentally induced autonomous mammosomatotropic tumors of the rat pituitary, which secrete prolactin, growth hormone, and sometimes adrenocorticotropic hormone (ACTH), have been studied extensively since they can be readily propagated by serial transplantation in isologous hosts (1). In the normal rat pituitary, prolactin and growth hormone are each produced by a morphologically distinct type of acidophil (2, 3) while ACTH is produced by yet a third type of cell (4). Although mammosomatotropic tumors in rats are known to be derived from pituitary acidophils (5), it has yet to be conclusively determined if individual neoplastically transformed acidophils can secrete both prolactin and growth hormone and possibly ACTH as well.

The MtTW15 mammosomatotropic tumor variant is of particular interest because it grows rapidly in female hosts and secretes large amounts of prolactin and growth hormone (6, 7) and, although less well-documented, low levels of ACTH (8). The independent experimental modulation of prolactin and growth hormone levels in plasma of MtTW15 tumor-bearing hosts (7, 9) suggests that this mammosomatotropic tumor consists of separate populations of hormone-producing cells. In light of these findings, we have attempted to characterize the cell type(s) responsible for prolactin production by the MtTW15 tumor by utilizing the unlabeled-antibody enzyme method of Sternberger *et al.* (10) for the immunocytochemical localization of prolactin in tumor cells as well as in cultured cells derived from this tumor variant.

Materials and Methods. Tumor. Female Wistar–Furth rats bearing subcutaneously implanted MtTW15 tumors (11) were generously supplied by Dr. R. M. MacLeod (Cancer Research Laboratory, University of Virginia School of Medicine, Charlottesville, VA). Animals were housed in environmentally controlled animal quarters with 14 hr light–10 hr dark lighting cycle and received food and water *ad lib.* Nine weeks after implantation, tumor nodules were removed aseptically, rinsed in sterile Earle's saline, and portions were prepared for cell cultures or for immunocytochemical staining and morphological evaluation.

Immunocytochemistry. Samples of excised tumor were fixed for 1–2 hr in 2.5% glutaraldehyde in 0.1 *M* cacodylate–HCl buffer (pH 7.4) and processed histologically. After deparaffinization, adjacent sections were stained either with H and E for routine morphology or by a modification of the unlabeled-antibody enzyme method (10) as reported for the ultrastructural localization of prolactin (12). Rabbit anti-rat prolactin antiserum was obtained from the NIAMDD (NIAMDD-A-Rat Prolactin-S-1) while soluble rabbit peroxidase–antiperoxidase complex was generously supplied by Dr. Ludwig A. Sternberger (Edgewood Arsenal, MD). As a specificity control, normal rabbit serum was used in place of the anti-prolactin anti-serum. Peroxidase activity was revealed by a modification of the technique of Graham and Karnovsky (13) using H_2O_2 and 3,3′-diaminobenzidine. Prolactin-positive cells were counted at 440× with aid of an integrating eyepiece and

expressed as a percent of nucleated tumor cells counted.

Cell cultures in roller tubes were rinsed with Earl's saline and fixed for 2 hr in a 50% absolute ethanol–50% acetone mixture. The cultures were then rinsed with phosphate-buffered saline, immunocytochemically stained for prolactin as described above, and examined by inverted bright-field microscopy.

Tissue culture. A portion of a tumor nodule ($\sim cm^3$) was placed in 90-mm plastic petri dish containing 4 ml of complete Ham's F10 medium (14). The tissue was then minced with microsurgical scissors to give a mixed suspension of tissue bits and single cells. A small volume of this suspension was put into roller tubes so that the walls of the vessel were completely wet. The cells were maintained as monolayer roller cultures (15) in Ham's F10 medium supplemented with 15% horse and 2.5% fetal calf sera, 100 Us penicillin/ml, and 100 mg streptomycin/ml, and were buffered with 0.015 *M* *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 0.015 *M* $NaHCO_3$, and 5% CO_2 in balanced air. The cultures were maintained at 37° in a CO_2 incubator with daily medium renewal for periods of 6–13 weeks.

Results. Nine weeks after implantation, large, palpable tumor masses measuring 3–4 cm in diameter were evident. Tumors were well-encapsulated and nodular in appearance, and vascularization of the surface was marked. Routine histological examination revealed markedly anaplastic cells of diverse size with prominent nuclei within nodules which were separated by connective tissue and vascular elements. The cells within tumor nodules were frequently arranged in sheets or cords in association with vascular sinusoids (Fig. 1, A and D).

Immunocytochemical staining for prolactin (Fig. 1, C and F) revealed positive staining in cells found singly or grouped in clusters in association with blood vessels. Prolactin cells were characterized by pleomorphic shape, ranging from stellate to columnar in appearance (Fig. 1, G–J), and the presence of diverse nuclear morphology. Approximately 10% of the nucleated tumor cells (1798 counted) stained positively for prolactin; however, preferential distribution within the peripheral or central portions of the tumor nodule was not obvious. Controls in which adjacent sections were exposed to normal rabbit serum in place of rabbit anti-rat prolactin antiserum were completely negative (Fig. 1, B and E).

Although growth of transplanted MtTW15 tumors *in vivo* was rapid, roller cultures of tumor cells derived from tumors 9 weeks after implantation were difficult to maintain under the conditions used, and growth, as evaluated by daily microscopic inspection of culture tubes, was slow. Migration of cells from tissue masses was evident within 24 hr; however, completely confluent monolayers of cells were not formed after up to 13 weeks of culture. Stellate and spindle-shaped cells with extensive processes were the most frequently observed cell types which remained adherent to the glass. Adherent ovoid cells were also seen, but these were not as numerous. Figure 2 shows representative MtTW15 tumor cells that have been cultured for 6 weeks and immunocytochemically stained for prolactin. Positive staining for prolactin was revealed in cells with extensive processes as well as in cells of ovoid shape.

Discussion. Our results obtained by immunocytochemical and cell culture techniques demonstrate that MtTW15 tumor nodules are composed of a variety of different cell types. Even within the population of tumor cells which produce prolactin, there appears to be marked variability in cellular morphology which may reflect the neoplastic origin of these cells. A previous histological evaluation of the MtTW15 mammosomatotropic tumor (16) also reported marked diversity of cell types; however, prolactin cells were not identified immunologically.

Nine weeks after transplantation, approximately 10% of the tumor cells in this particular MtTW15 strain appear to be capable of producing prolactin as shown by our immunocytochemical observations. These results are of interest in light of

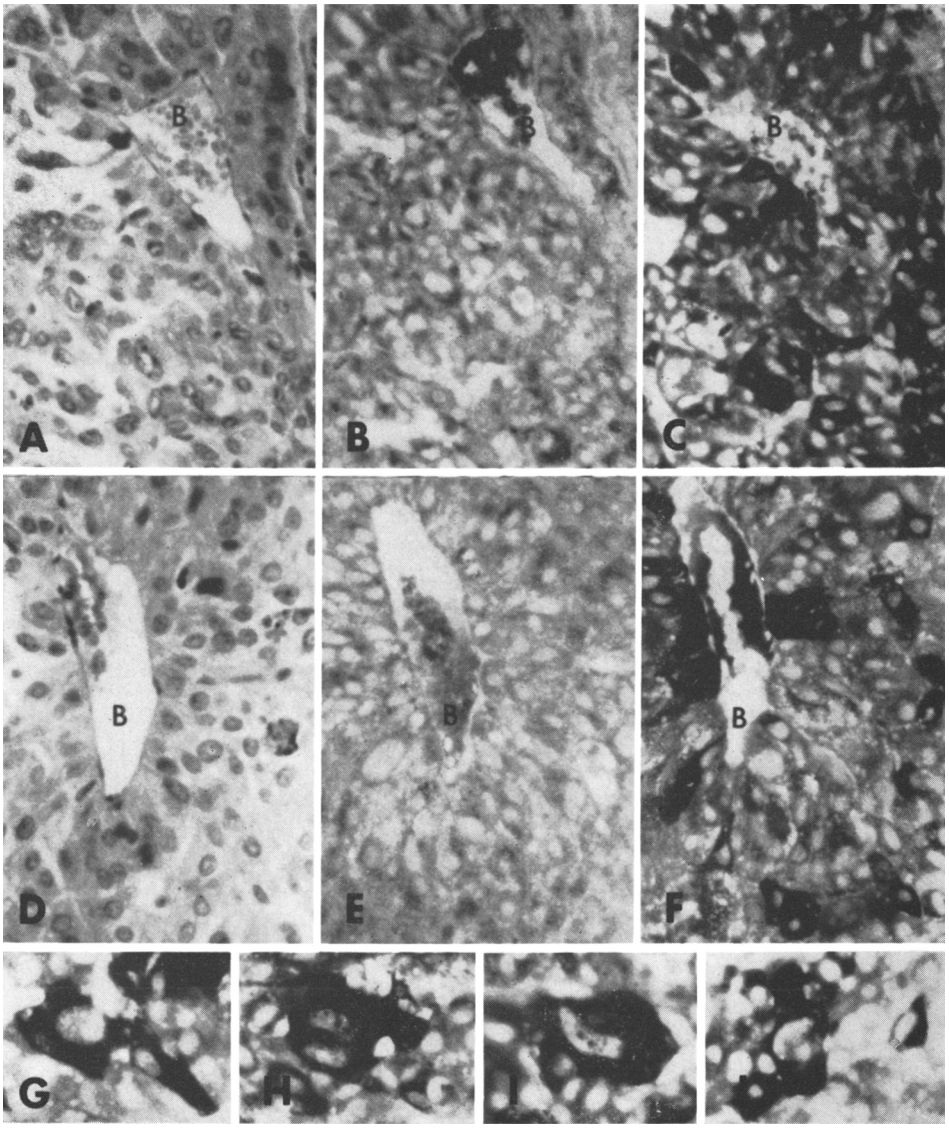

FIG. 1. Light microscopic immunocytochemical localization of prolactin in MtTW15 mammosomatotropic tumors 9 weeks after transplantation. Semiadjacent, 6-μm paraffin sections were stained with H and E (A and D) or were exposed for 48 hr to 1:1000 dilutions of either normal rabbit serum (B and E) or rabbit anti-rat prolactin antisera (C, F–J). Note polarization of prolactin-positive cells which appear black in micrograph around blood vessels (B) in C and F. Heterogenecity of prolactin-producing cells is indicated by bizarre nuclear morphology (G–J). Panels A–F, ×675; panels G–J, ×1700.

recent reports on plasma levels of prolactin and growth hormone in MtTW15 tumor-bearing animals (7, 9) which show hormone levels elevated by approximately 1000-fold over nontumor-bearing controls. Although a relatively small percentage of the tumor cells are producing prolactin, the absolute number of prolactin-producing cells may be very large because of the increased mass of these tumors in comparison to normal pituitaries. This also would explain the observation of Ito *et al.* (17) which showed that a small percentage of immunochemically identified prolactin cells

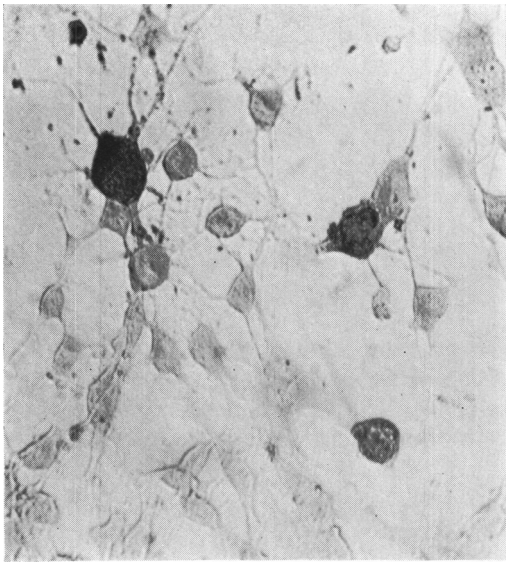

FIG. 2. Immunocytochemical localization of prolactin in a MtTW15 tumor cell culture maintained for 6 weeks. Prolactin production is suggested by positive staining in several cells of diverse morphology which appear black in contrast to adjacent unstained cells. ×1000.

(about 1% as judged by Fig. 10 in this reference) of the MtT.W5/St.H mammosomatotropic tumor variant produced markedly elevated plasma levels of prolactin although this variant is considered to be predominently a growth-hormone-secreting tumor.

Further studies will be required to determine if the porportion of tumor cells in the MtTW15 tumor which produce prolactin can be selectively altered by drug treatments of tumor-bearing animals and if the same cells which produce prolactin are also capable of producing growth hormone and ACTH as well.

Summary. To our knowledge, this is the first report on the immunocytochemical identification of prolactin-producing cells in the MtTW15 mammosomatotropic tumor variant. Cells staining positively for prolactin were characterized by extreme heterogenity of size, shape, and nuclear morphology. Only about 10% of the cells of tumor nodules were stained positively for prolactin. Roller cultures of MtTW15 tumor cells were not readily established; however, immunocytochemical staining revealed prolactin positive cells of diverse shapes in cultured cells as well.

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