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Tuft (Caveolated) Cells in Two Human Colon Carcinoma Cell Lines

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The presence of an unusual cell type in two human colon carcinoma cell lines is reported. The cells show the same morphology as "tuft" (caveolated) cells present in normal gastrointestinal epithelium. Tuft cells were seen in cell line LIM 1863 growing *in vitro* and in human colon carcinoma cell line LIM 2210 growing as subcutaneous solid tumour xenografts in nude mice. Characteristic morphologic features of tuft cells included a wide base, narrow apex and a tuft of long microvilli projecting from the apical surface. The microvilli are attached by a core of long microfilaments passing deep into the apical cytoplasm. Between the microvilli are parallel arrays of vesicles (caveoli) containing flocculent material. Two different but not mutually exclusive explanations for the presence of tuft cells are proposed. The first explanation is that tuft cells came from the resected tumour and have survived by mitotic division during subsequent passages. The second explanation suggests that tuft cells are the progeny of undifferentiated tumour cells. Descriptions of tuft cells in colon carcinomas are uncommon and possible reasons for this are presented. The morphology of tuft cells is consistent with that of a highly differentiated cell specialised for absorption, and these new models provide an opportunity to further investigate the structure and function of tuft cells. (Am J Pathol 1988, 132:521–525)

A MORPHOLOGIC STUDY of mouse stomach published in 1955 first reported an unusual epithelial cell characterised by an apical tuft of long microvilli.[1] Since that time, similar cells have been described in the gastrointestinal and respiratory tracts of many animal species including humans, dogs, rats, mice, bats, llamas, and toads.[1–6] They have had different names, including multivesicular, special, fibrovesicular, tuft, brush, and caveolated cells.

A detailed study in 1973[2] described unusual cells in rat stomach found by light microscopy, TEM, SEM, and histochemistry, and was the first to use the term "tuft" to describe the cell type. This study concluded that tuft cells were highly differentiated cells specialized for absorption. In the following year another study described the same cell type in mouse stomach, small, and large intestine, and gave it the name "caveolated cell" because of the caveolae (small caves) invaginating the apical surface between individual microvilli.[5] These workers agreed that the cells were highly differentiated but were unable to come to a conclusion about their function. They also noted the wide distribution of tuft cells in the gastrointestinal tract from the stomach to the rectum, and from the depths of the intestinal crypts to the apex of villi. A study published in 1979 charted the passage of caveolated cells from birth, deep in the crypts, to extrusion from the luminal surface of mouse colon using continuous infusion of ^{3}H thymidine and autoradiography.[7] These workers estimated that caveolated cells had an average life span of 8.2 days (*vs.* 4.6 days for vacuolated columnar and mucous cells.[7]

The present paper describes tuft cells in two human colon carcinoma cell lines, LIM 1863,[8] and LIM 2210.

Materials and Methods

Cultures of LIM 1863 Cells

Cell line LIM 1863 is unusual in that it grows as round floating organoids with a central lumen and

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cryptlike morphology. The organoids seldom attach to the flask floor. LIM 1863 cells were maintained at 37 C in RPMI1640 + 10% fetal calf serum (Commonwealth Serum Laboratories, Melbourne, Australia), 0.6 μg/ml insulin (Commonwealth Serum Laboratories), 1 μg/ml hydrocortisone (The Upjohn Co., Sydney, Australia), 10^{-5} a-thioglycerol (Sigma Chemical Co., St. Louis, Mo), 50 μg/ml penicillin, and 50 μg/ml streptomycin.

Xenografts of LIM 2210

Cell line LIM 2210 was propagated as subcutaneous xenografts in the flanks of nude mice directly from small pieces of tissue obtained from a resected specimen of a poorly differentiated human colon carcinoma. Cell line LIM 2210 has so far proved resistant to propagation in cell culture.

Morphology

Cultures of LIM 1863 organoids were centrifuged, washed in buffer, and fixed for 1 hour at room temperature in 5% glutaraldehyde. Nude mice bearing LIM 2210 xenografts were killed by cervical dislocation, tumours dissected from adjacent tissues, and pieces approximately 1 × 3mm fixed in a solution containing 3% formaldehyde, 4% glutaraldehyde, and 1% picric acid.

All tissues were then rinsed in 0.1 M cacodylate buffer and postfixed for 2 hours in 1% 0s04 in 0.1 M cacodylate buffer. Tissues were then placed in 1% uranyl acetate in 0.2 M maleate buffer for 1 hour, dehydrated through a series of graded ethanols, and embedded in Epon-Araldite. Thick sections (1 μ) were stained with toluidine blue and examined using light microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined in a Joel 100S electron microscope at 60 kv.

Results

Light Microscopy

Examination of 1-μ thick sections of LIM 1863 cells showed rounded organoids with a central lumen surrounded by polarised epithelial cells. The arrangement and morphology of the cells were similar to those seen in normal colonic crypts. Sections of LIM 2210 showed solid masses of moderately well-differentiated tumour cells with occasional acinar formation. Connective tissue and necrotic areas were scant.

Transmission Electron Microscopy

LIM 1863 Organoids in Culture

The arrangement of epithelial cells surrounding a cryptlike lumen seen with light microscopy was confirmed using electron microscopy (Figure 1). Tall columnar cells with stubby and irregular microvilli (similar to those seen on immature absorptive cells in normal crypts) were the most common cell type. Mucous cells were also present (Figure 2), although they were seen infrequently. A further subpopulation of cells similar in appearance to the vacuolated-columnar cells of normal colon were also evident (Figure 1), although they differed from normal vacuolated-columnar cells in that their vacuoles were in the basal third of the cell rather than the apical third.

Cells characterised by a tuft of long microvilli projecting from the apical surface were readily identifiable (Figure 3). Tuft cells were narrow at the apex and wide and rounded at the base with a core of microfilaments extending from the microvilli deep into the apical cytoplasm (Figure 3). Parallel arrays of small vesicles containing flocculent material were seen between the microfilament cores (Figure 4). Vesicles close to the surface were smaller than those deep in the cytoplasm. Nuclear morphology was variable and some showed features not seen in nonneoplastic colonic epithelial cells (Figure 3). Cells were close packed, with little intercellular space, although occasional intercellular spaces with interdigitations were seen in basal regions (Figure 1). The basal plasma membrane of some cells was flat, and on other cells it showed complex fine ruffles and filipodia (Figure 1). A basement lamina was absent.

Xenografts of LIM 2210

Although most sections showed solid collections of tumour cells with little intercellular substance or connective tissue, occasional acini were seen and some of these contained tuft cells (Figure 5). Tall columnar cells with regular microvilli similar in morphology to the absorptive cells on the luminal surface of normal colon were also present (Figure 6). The morphology of tuft cells in LIM 2210 xenografts was the same as in LIM 1863 organoids. The regular arrays of microfilaments in microvilli extended deep into the apical cytoplasm and then intermeshed with adjacent rootlets (Figure 7). Transverse microfilaments forming a terminal web were not prominent. Small vesicles containing flocculent material between microfilament bundles close to the apical surface were a prominent feature (Figure 7). Tuft cells were joined to adjacent cells with tight junctions (Figure 7).

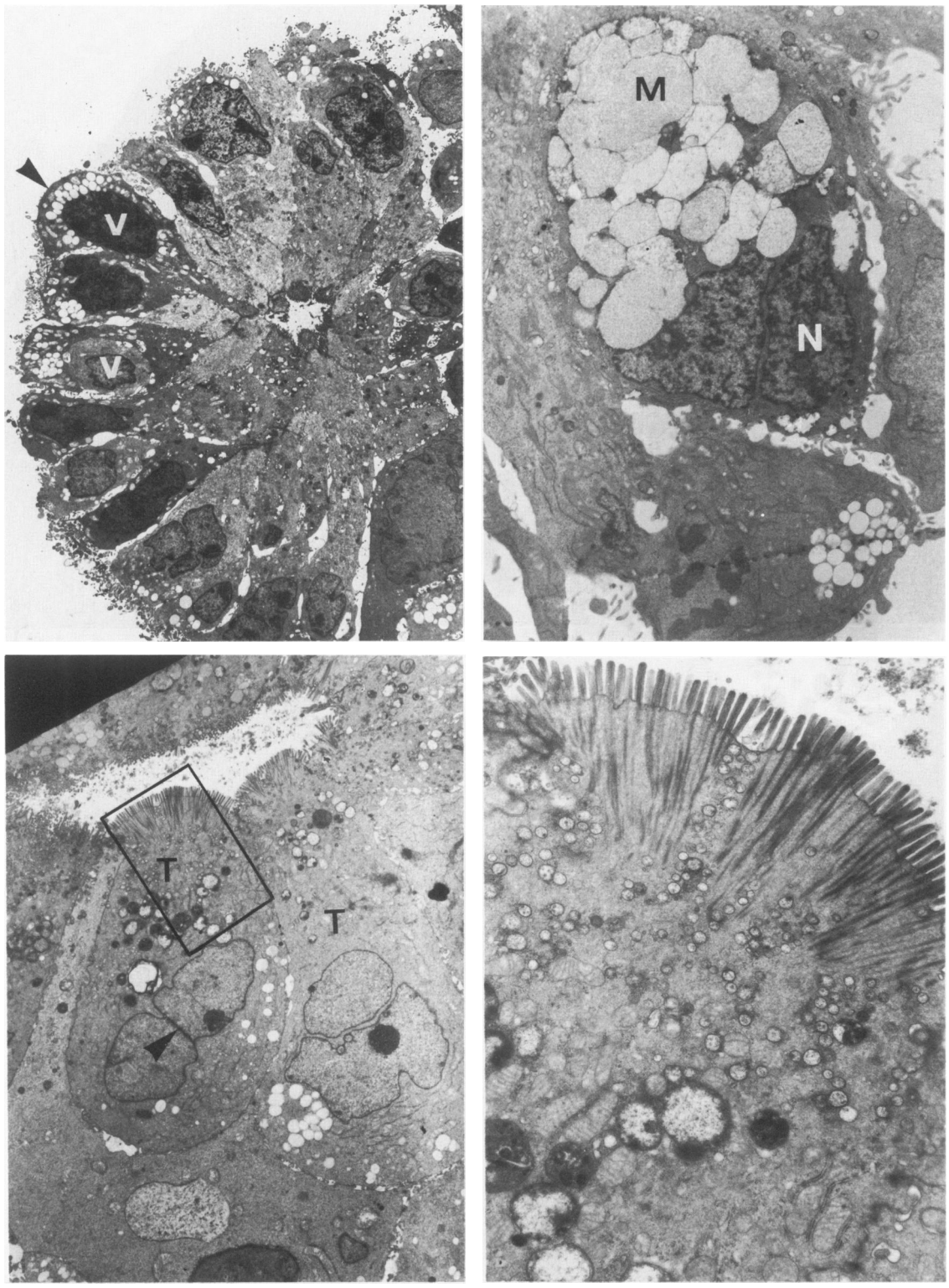

Figure 1—Low-power electron micrograph of a section through LIM 1863 cells that grow as floating organoids and have a cryptlike morphology. A small central lumen is surrounded by polarized epithelial cells. Many cells (V) contain vacuoles and are similar in appearance to those seen in the lower parts of normal crypts, except that here the vacuoles are located basally instead of apically. The outside (basal) surface of most cells show numerous fine ruffles and filipodia although some cells showed a smooth basal surface (black arrowhead). ×700. **Figure 2**—Electron micrograph of section through LIM 1863 cells showing a mucous cell with basal nucleus (N) and apical mucus droplets (M). ×3000 **Figure 3**—Electron micrograph of LIM 1863 cells showing several tuft cells (T) abutting the lumen of an acinus. Tuft cells show a wider base and narrow apex. The nucleus of one tuft cell shows an unusual "bar" across the surface (black arrowheads). ×1800 **Figure 4**—Higher power electron micrograph of the area inset in Figure 3, showing details of tuft microfilaments and apical vesicles (or caveolae) lying between adjacent roots. Vacuoles become progressively larger with passage away from the luminal surface. ×6000

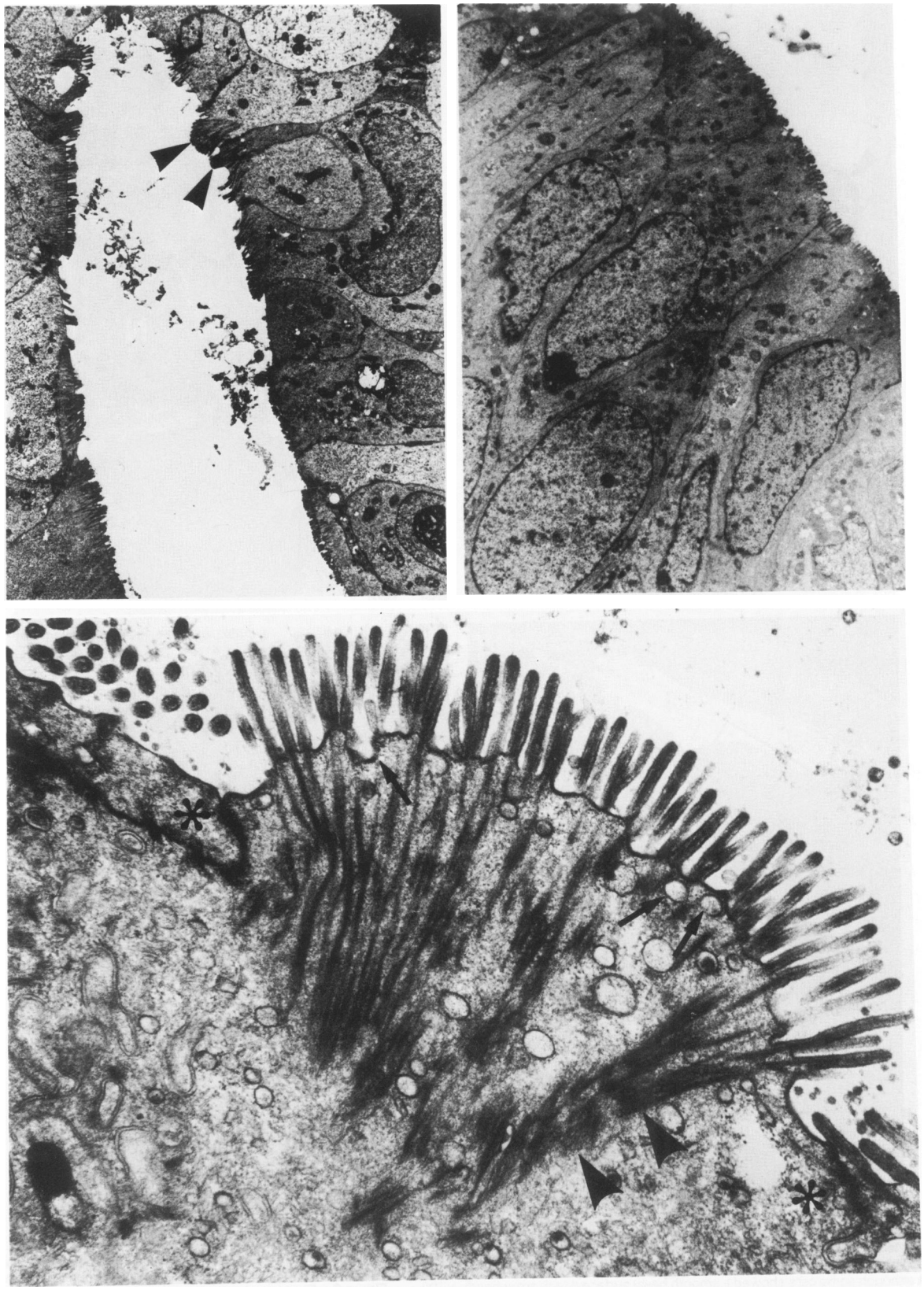

Figure 5—Low-power electron micrograph of human colon carcinoma cell line LIM 2210 growing as a subcutaneous xenograft in a nude mouse. Several tuft cells (black arrowheads) abutting an acinus can be seen. ×800 **Figure 6**—Electron micrograph of LIM 2210 xenograft showing polarized columnar epithelial cells with regular surface microvilli similar in morphology to those seen abutting the lumen in normal colon. ×2000 **Figure 7**—Electron micrograph of apical area of tuft cell in LIM 2210 xenograft. The regular arrays of microfilaments in the surface microvilli extend deep into the apical cytoplasm. Although adjacent bundles of filaments intermesh (black arrowheads) there is little evidence of transverse microfilaments forming a terminal web. Vacuoles close to and fusing with the apical plasma membrane can also be seen (black arrows). Tight junctions (asterisks) are also apparent. ×20,000

Discussion

Fairly convincing evidence now exists showing that, in normal colon, undifferentiated cells in the crypts (stem cells) give rise to all of the differentiated cells comprising the definitive epithelium, ie, absorptive, mucous, enteroendocrine, and tuft (caveolated) cells.[7,9-11] Tuft cells arise in the lower parts of the crypt and travel up the crypt wall to reach the luminal surface, and have an estimated turnover time of 8.2 days in mouse colon.[7] There are several possible explanations for the presence of tuft cells in the two cell lines examined. First, they may have come from the original resected human tumour and survived through mitotic division. Alternatively, tuft cells may be produced from undifferentiated tumour cells in the explant in the same way as tuft cells are produced from stem cells in normal colon.[7] The cell lines examined showed all of the differentiated cell types found in normal colon (except for enteroendocrine cells), giving support to the second idea that undifferentiated tumour cells give rise to differentiated tumour cells, including tuft cells.

Some organoids of LIM 1863 cells were composed almost entirely of tuft cells, suggesting that tuft cells are either able to divide or that in these organoids, differentiation has been directed down this pathway. It is difficult to detect a tuft cell undergoing mitosis because of the absence of distinctive intracellular organelles enabling positive identification of mitotic figures.

The tuft cells observed in the study have essentially the same morphology as those described by others in the normal gastrointestinal epithelium.[2,5,7] Previous reports of tuft cells in colon carcinomas are uncommon.[12] This could be because tuft cells occur rarely in colon carcinomas, or because they have escaped detection previously. The latter explanation appears more likely for the following reasons. First, this study has presented evidence of tuft cells in two different human colon carcinoma cell lines. Second, detailed accounts of tuft cells have only been possible since the advent of electron microscopy, indicating they cannot be identified in routine, 5-μ paraffin sections. In addition, it seems possible that the characteristic appearance of tuft cells is attained only when they are in a cryptlike environment surrounded by other polarised epithelial cells, whereas in other situations, eg, within a solid mass of tumour cells, tuft cells may not be recognizable.

The observations of others that tuft cells originate in the lower parts of the crypts, have a consistent morphology, and migrate to the luminal surface[7] suggest that they are part of the differention lineage of the crypt stem cell. The function of tuft cells is still unclear, although the presence of small vesicles near the luminal surface and larger vesicles deep within the cells is consistent with that of an absorptive cell.

The presence of tuft cells in two human colon carcinoma cell lines provides an opportunity for more detailed investigations into their structure and function.

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