

Electron Microscopic Appearance of Cells from Carcinoma of the Prostate in Monolayer Tissue Culture*

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Summary. An electron microscopic study of human prostate cells grown in monolayer tissue cultures is presented. The cultures were established from tissue of 4 patients with adenocarcinomas of the prostate. The predominant cell type was identified as epithelial. These cells were character-

ized by close cell growth with many desmosomal junctions, formation of "lumina" between cells and atypical mitochondria. The ultrastructural appearance is compared to that of prostatic cancer in surgical specimens and culture cells derived from benign nodular hyperplasia of the prostate.

Since the well known HeLa strains from human cervical carcinoma were first established as a stable cell strain in monolayer culture (7), various other human epithelial tumors have been cultured successfully in vitro (10, 11). Considerable progress has also been made in the study of malignant human genitourinary tumors by means of tissue culture technique (2, 8, 15, 18).

One of the difficulties in the morphological study of cells in culture is the ascertainment of their origin. The light microscopic variations in the growth of prostatic explant cultures have been previously described (17) and attempts have been made to correlate various cell types with the appearance in histological sections.

We have recently been able to grow human prostatic cells obtained from patients with benign

nodular hyperplasia of the prostate in monolayer cultures. Ultrastructural studies of these cells revealed three main types: "epithelial" cells, smooth muscle cells and fibroblasts, usually growing in a mixed culture (12).

This manuscript summarizes our results of electron microscopic examinations of the different cell types identified in monolayer cell cultures of carcinoma of the prostate. These are compared to the appearance of cells derived from benign nodular hyperplasia of the prostate.

Materials and Method

Perineal biopsies were obtained from 3 patients with histologically proven adenocarcinomas of the prostate. In another patient tumor tissue was obtained from an operative specimen following radical prostatectomy. One of the patients had a clinically active carcinoma with elevated acid phosphatases (Stage IV) whereas the other 3 patients had clinically Stage I and II tumors with normal acid phosphatases.

Monolayer cell cultures were initiated by explanting or trypsinizing the tumor tissue and then grown as previously described (12, 13, 14).

Culture cells for examination by electron microscopy were obtained from confluent cultures

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and prepared in two different ways: 1. The cells were fixed in the culture dish, still adhering to the bottom, according to a method described by Ross (16). 2. Cells were scraped from the bottom of the culture dish with a "policemen rubber". Both cell preparations were fixed in 2.5% glutaraldehyde, postfixed in 1% OsO_4 , dehydrated in ethanol and embedded in Epon 812. The embedded cells were sectioned on a Porter-Blum ultramicrotome MT2, stained with uranyl acetate and examined in a RCA-EMU-3-G electron microscope.

For comparison, biopsy specimens from some of these same patients were also examined under the electron microscope.

Results

The ultrastructural examination of human prostatic cells in monolayer tissue culture, derived from patients with adenocarcinoma of the prostate, reveals predominantly one cell type, an epithelial cell, characterized by numerous junctional complexes (desmosomes) between adjacent cells. These desmosomes are exclusively located on sites where adjacent cells develop multiple interdigitations. The cells form intercellular spaces of var-

iable sizes. Some appear round in shape, appearing as "lumina". Abundant microvilli are present along all borders of these cells (Fig. 1).

The typical cell shows a moderately large nucleus, usually peripherally located. Some cells possess two nuclei. The majority of the nuclei is bizarre, elongated and curved and contains one or more prominent nucleoli (Fig. 2).

The cytoplasm contains a moderate number of rough surfaced tubular endoplasmic reticulum scattered throughout the cells. Unlike in cultured cells from nodular hyperplasia of the prostate the endoplasmic reticulum is not dilated. The mitochondria are mostly elongated, enlarged and the orientation of the cristae appear to be slightly different from the normal structure. Compared to prostatic cancer cells in surgical specimens, however, the cristae appear better preserved (Fig. 3). Various vesicles and vacuoles, some containing stacks of membranes some appearing as holes as well as osmiophilic dense bodies, probably lipid droplets, are seen. Free ribosomes mostly organized as polysomes are distributed throughout the cytoplasm. Most of the cells exhibit various numbers of fibrils arranged in small bundles, varying in shape and mainly located close to the cell surface (Fig. 3).

The junctional complexes are composed mainly

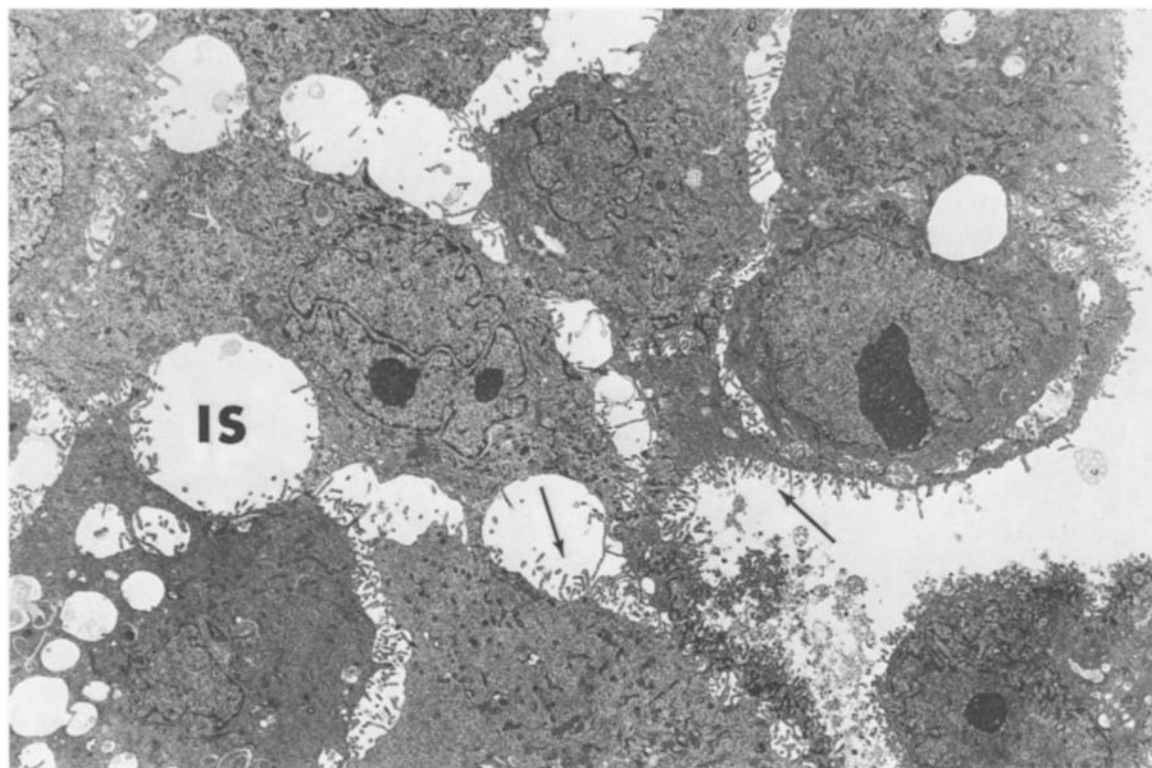


Fig. 1. Electron micrograph of human cells in monolayer tissue culture obtained from adenocarcinoma of the prostate. The adjacent cells exhibit lumina-like intercellular spaces (IS), variable in size. Along the luminal surface and at the free border of the cells there is an abundance of finger-like microvilli (arrows). Magnification: $\times 4100$

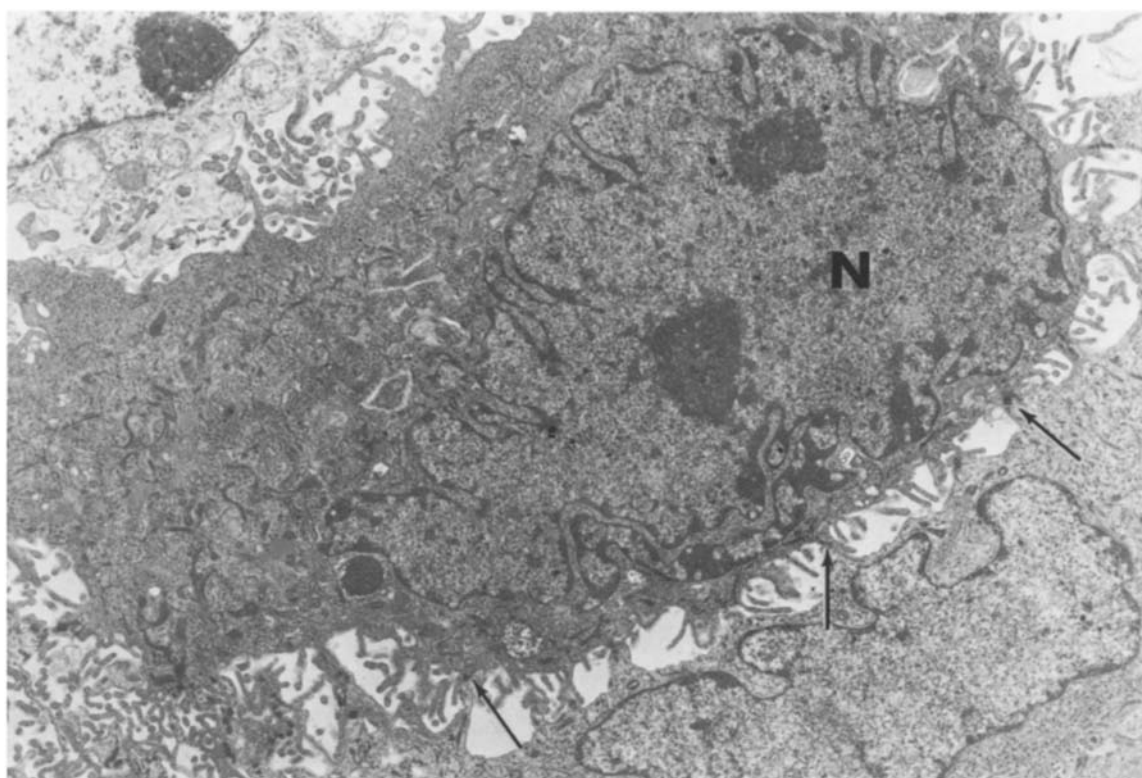


Fig. 2. Electron micrograph of a single cell in monolayer tissue culture originating from adenocarcinoma of the prostate. The peripherally located nucleus (N) is enlarged containing two prominent nucleoli. Note the abundance of intercellular junctions (arrows). Magnification: x 4400

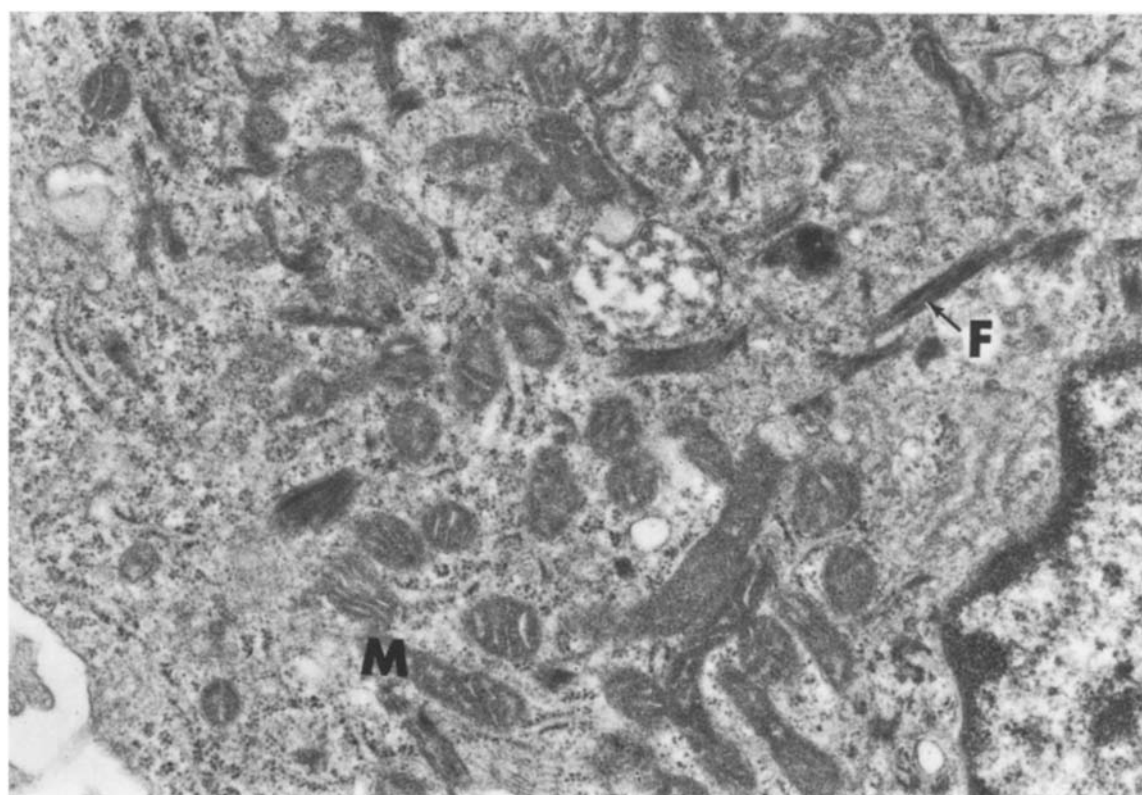


Fig. 3. Electron micrograph of a portion of an epithelial cell in monolayer tissue culture originating from an adenocarcinoma of the prostate. Elongated, distorted mitochondria (M) with unusually dense matrix are evident. The structure of the cristae is somewhat disorganized. Note the cytoplasmic fibrillogenesis with formation of irregular bundles (F). Magnification: x 30700



Fig. 4. Electron micrograph of desmosomal junctions (arrow) between cells in monolayer tissue culture originating from adenocarcinoma of the prostate. Magnification: $\times 64600$

of the desmosomal type as seen under high magnification (Fig. 4). The opposed cell membranes are separated by an intermediate zone, consisting of circumscribed electron-dense material. Bundles of tonofibrils sometimes converge on each side into the desmosome.

In addition to these epithelial cells some cultures show fibroblasts and smooth muscle cells. The connective tissue cells are identical to those described previously in monolayer cell cultures derived from benign nodular hyperplasia of the prostate (12).

Discussion

The knowledge of the ultrastructure of cells originating from human prostatic cancer in monolayer tissue culture is limited. Fraley and Ecker (5, 6) reported quite recently a spontaneous neoplastic transformation of normal adult human prostatic cells in monolayer tissue culture. The authors announced an ultrastructural study of these cells which has not been published as yet.

The ultrastructural appearance of prostatic carcinoma cells fixed immediately after biopsy has been studied in this laboratory as well as by other investigators (1, 4, 9). The malignant prostate cell as it appears "in vivo" exhibits one significant parameter of malignancy: a high number of pleomorphic abnormal mitochondria, enlarged, vacuolat-

ed and with distortion or lack of cristae, indicating a progressive degradation. In addition we observed an increased number of desmosomes, compared to the number observed in benign nodular hyperplasia.

In monolayer cell cultures derived from patients with adenocarcinoma of the prostate, an epithelial cell was identified, which was different from epithelial cells growing in cultures from benign nodular hyperplasia of the prostate (12). The malignant cell's most predominant ultrastructural feature was a close cell growth with abundant junctional complexes (desmosomes) between adjacent cells. Many interdigitations between cells were the predominant location of these desmosomes.

Another distinct feature, the formation by culture cells of intercellular lumina-like spaces, resembled the growth of cancer cells "in vivo", which may develop ectopic luminal spaces when invading connective tissue (9). The mitochondrial profiles of our cancer cells in culture, however, were different from those in surgical specimens. Although some mitochondria were enlarged and totally destroyed, the majority appeared intact but elongated, sometimes curved and the cristae somewhat distorted.

Circumscribed aggregates of intracytoplasmic fibrils of some culture cells appeared to be similar to the prostatic cytoplasmic fibrils observed by Fisher and Jeffrey in prostatic epithelial cells as they grow in nodular benign hyperplasia as well as in cancer of the prostate (4). The fibrillar material

in the culture cells, however, were organized in small bundles, mostly located close to the cell surface. In a more recent study, Tannenbaum, et al. (19) described cytoplasmic filamentous structures in benign prostatic cells adjacent to cancerous cells. Contrary to our findings these structures are located in a juxtanuclear position. Their significance is obscure.

Some of our cultures also contained connective tissue cells as previously described (12). This may indicate the high growth potency of these cells in vitro. In one culture, however, originating from a patient with a clinically very active carcinoma these cells were overgrown by cancer cells after several transfers.

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