Ultrastructural Study of the Persistence of Colchicine-induced Cytological Changes in Harding-Passey Melanoma

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ABSTRACT

Electron microscopic observations were undertaken on Harding-Passey melanoma in control tumors, colchicine-treated tumors, and tumors that were passed through five transplantations in mice subsequent to colchicine treatment.

The untreated Harding-Passey melanoma tumor was composed of pleomorphic cells the cytoplasm of which contained tubulovesicular mitochondria and numerous free and membrane-attached ribosomes. Type A virus particles were observed in both mitotic and interphase cells. Colchicine-treated tumors exhibited several typical colchicine mitotic cells arrested in metaphase. A most significant feature of the colchicine administration was the appearance of microfilaments in both interphase and mitotic cells, a feature not observed in cells of the untreated tumor.

Electron microscopy of colchicine-treated tumors carried through five transplantations at biweekly intervals revealed the presence of microfilaments in tissues fixed at each of the transplantations.

It therefore appears that the cytological effects of colchicine resulting in the appearance of filaments in melanocytes of the Harding-Passey melanoma are more enduring than they were initially presumed to be.

INTRODUCTION

Whereas the beneficial effect of X-rays and radioactive isotopes in the treatment of human cancers has been definitely established, there remain certain types of tumors that are radioresistant, and melanomas belong to this group. Consequently, the effects of a number of chemical compounds and antibiotics on different animal melanomas are being investigated by several researchers (11, 45, 56). Suguria (52), following an extensive study on the inhibitory effects of chemical compounds and antibiotics on the Harding-Passey melanoma, observed that alkylation agents and antibiotics were equally effective. These chemical agents exert their beneficial effect not only by their tumoricidal action but also by promoting cellular maturation and differentiation.

Grobstein (19) defined cytodifferentiation thusly: "Since differentiation affects the cell in many ways and at many levels, there are a number of sets of criteria for identifying and characterizing the differentiated state. Since they are not always concordant, it becomes important to recognize the significance of each, and to specify criteria when one is characterizing differentiation. Cells may be said to be differentiated by morphological, behavioral, chemical or developmental criteria."

Evidence of submicroscopic differentiation is observed in both the nucleus and the cytoplasm. These changes are more conspicuous in the cytoplasm (13). Morphological changes in the nucleus during the course of development have been described by several investigators (3, 22). However, the site of functional realization of the cell, together with the respective structural arrangement, resides within the cytoplasm, the primary area of submicroscopic differentiation. During differentiation, changes in cellular organelles take place.

The appearance of microfilaments in the tumor cells of the Harding-Passey melanoma following colchicine treatment was first reported by Nathaniel et al. (37). The present study was designed to establish whether the microfilaments are transitory or persist through successive generations of tumor transfer without further treatment.

This report presents the ultrastructural features of the Harding-Passey melanoma following colchicine administration and compares these with those of the untreated control tumor. Subsequently, the cytology of the treated tumor is described as the tumor is passed through 5 transplantations in mice, with special attention paid to the presence of filaments.

MATERIALS AND METHODS

Female BALB/cJ mice bearing the Harding-Passey melanoma received i.p. injections of colchicine in doses of 0.5 mg/kg daily for 7 days starting 1 day after tumor transplantation. A further dose of 1.0 mg/kg was given daily for 7 more days. All animals were sacrificed 24 hr after the last injection, tumors were exposed by blunt dissection, and small pieces of tumor were then transplanted to new host animals. The remaining treated tumor tissue was fixed in buffered paraformaldehyde and glutaraldehyde according to the method of Karnovsky and further fixed in 2% osmium tetroxide in 0.2 M cacodylate with 0.4% sucrose for 1 hr. Following dehydration, tissues were embedded in Araldite. This fixed tumor is referred to as "treated tumor." After a 2-week growing period in the host animal, the tumor was removed and the above procedure of transplantation and fixation for electron microscopy was again performed. This tumor tissue is referred to as "treated tumor generation 1." The procedure of transferring the tumor was repeated 4 more times at 2-week intervals, and the resultant tissues are referred to as "treated tumor generations 2, 3, 4, and 5."
Sections 0.5 μm thick were cut from the Araldite blocks and stained with toluidine blue. Nonnecrotic areas of tumor were selected by light microscopy; thin sections then cut and stained with uranyl acetate and lead citrate. Sections were mounted on uncoated copper grids and visualized with the Philips EM 300 electron microscope.

RESULTS

Morphology of Untreated Tumors. Electron microscopic observations confirmed the optical microscopic findings that the tumor was highly cellular and that the cells were extremely pleomorphic (Fig. 1). Melanin granules were observed in all the cells of the tumor and formed the basis for identification of these cells as melanocytes. The amount of free and attached ribosomes varied in the melanocytes, accounting for light to heavy basophilia in toluidine blue-stained 0.5-μm-thick sections. The cytoplasm revealed tubulovesicular mitochondria and multiple Golgi complexes. Virus-like particles were observed in relation to the granular endoplasmic reticulum of many tumor cells. The tumor nuclei were prominent and exhibited considerable accumulation of chromatin along their margin. Mitotic cells in all phases of mitosis were observed in the tumor.

Morphology of Colchicine-treated Tumor. The most striking feature of colchicine treatment was the presence of numerous colchicine mitotic cells. The colchicine mitotic cell was enlarged and comprised of a central portion of chromosomes in a dense cytoplasmic matrix. The peripheral portion of the cell generally exhibited dilated cisternae of endoplasmic reticulum.

A significant feature observed in tumor cells treated with colchicine was the presence of microfilaments in both the mitotic and interphase cells. The microfilaments were found in bundles, with ribosomal rosettes often located outside the bundles rather than among the filaments (Fig. 2). In the interphase cells the microfilaments were more often perinuclear. In addition an increase in melanin granules with a tendency for marginal localization was evident in interphase cells.

Morphology of Treated Tumor Generations 1 through 5. The cytological features observed in the cells of the colchicine-treated tumor were also observed in the melanoma cells throughout the successive tumor generations, 1 through 5.

The most striking feature was the continued presence of filaments in tumor cells from generation 1 through 5 (Figs. 3 to 8). The microfilaments were observed in both mitotic and interphase cells in all tumor generations studied. In general the distribution of the microfilaments within the cytoplasm and the size of filament aggregates appeared to be similar in tumor cells of all generations.

There were 2 patterns of distribution of microfilaments. In several cells well-defined, conspicuous bundles of filaments were encountered (Figs. 4 and 8). Conspicuous collections of polysomes and granular endoplasmic reticulum often bordered such aggregates of filaments (Figs. 4 and 8). Cell organelles such as mitochondria and cell products such as melanin granules were found generally bordering the filament aggregates and rarely among them. These circumscribed bundles of filaments were more often encountered in interphase cells. Such collections of microfilaments may be located anywhere within the cytoplasm of the tumor cell (Fig. 4). However, they tend to be perinuclear (Fig. 8). In fact, we often located filament bundles by searching the perinuclear region.

A second pattern of microfilament distribution varied from a less defined bundle of filaments (Fig. 3) to a rather diffuse, random permeation of the cytoplasm by these filaments (Figs. 5 to 7). In Fig. 8 one observes both patterns of filament distribution; whereas a broad bundle of filaments borders 1 side of the nucleus, the rest of the cytoplasm on the other side of the nucleus displays a less defined and more diffuse pattern of distribution of microfilaments.

Both the localized and diffuse patterns of microfilament distribution were observed in both interphase and mitotic cells. However, the localized pattern was more commonly observed in interphase cells, whereas the diffuse pattern was prevalent in mitotic cells.

Similar to the colchicine-treated tumor, the cells of treated tumor generations 1 through 5 displayed considerable pigment within their cytoplasm. The pigment granules tend to locate principally along the peripheral aspect of the cell (Figs. 4 and 6).

As indicated earlier the microfilament aggregates were invariably limited by ribosomal particles, suggesting their possible role in filament production. Cytoplasmic organelles and viral inclusions in cells of treated tumor and treated tumor generations 1 through 4 resembled the untreated tumor.

DISCUSSION

The effects of chemical analog in experimental animal tumors are being investigated extensively with the hope of obtaining information that may be applicable to the treatment of human neoplasms. This is especially true of tumors that are not sensitive to radiation and are not amenable to surgery. Many of the chemotherapeutic agents are thought to exert a beneficial effect by altering the cellular cycle of growth, division, and differentiation.

The effects of colchicine on the microtubules of the mitotic cell have been recognized since the work of Eigsti and Dustin (14). The morphology of spindle tubules following colchicine therapy has been described by Harris and Mazia (21) in sea urchin eggs, Brinkley et al. (4) in the Chinese hamster cell, and Dougherty and Lee (12) in dividing rat hepatic cells.

Electron microscopic studies on Cloudman S-91 mouse melanoma (8) and Harding-Passey melanoma (37, 48) have not revealed the presence of microfilaments in tumor cells. Nathaniel et al. (37) reported the appearance of filaments in Harding-Passey melanoma tumor cells after colchicine treatment.

The administration of colchicine to mice bearing the Harding-Passey melanoma tumor in this study resulted in several cytological changes in the tumor cells (29). The most striking feature was the appearance in the tumor cells of microfilaments, which were not observed in untreated cells. The second important feature was the continued presence of the filaments in these tumor cells even after 5 transplantations over a 10-week period. The third feature...
was the tendency for melanin granules to marginate or be located along the peripheral aspect of the cell. The significance of these cytological observations will be discussed.

Colchicine breaks down microtubules in dividing cells (43, 46) and in interphase cells (30, 32, 55). This action is brought about by colchicine binding to sites of interaction between protein subunits tubulin, the 65 diametric subunits of microtubules, thereby preventing their assembly into microtubules (49, 57).

Numerous investigations have shown that both colchicine and vinblastine induce the appearance in cells of filaments measuring 10 nm in diameter (2). Vinblastine, which is also a mitotic inhibitor, inactivates tubules by precipitating tubulin as large crystals (2, 33). Nathaniel et al. (37) observed the appearance of filaments in tumor cells of Harding-Passey melanoma after colchicine administration. In this communication the persistence of colchicine-induced filaments in tumor cells of Harding-Passey melanoma over a 10-week period is presented. Schubert et al. (47) reported the presence of microfilaments following colchicine and vinblastine treatment during in vitro differentiation of a mouse neuroblastoma.

The functional roles of microfilaments and microtubules have been investigated with respect to cell shape alterations, axonal growth, muscle contraction, and many other cell processes. Wessells et al. (58) and Spooner and Wessells (51) ascribed to the microfilaments and microtubules a contractile function responsible for a broad spectrum of cellular movements and developmental processes.

The breakdown or dissolution of microtubules by colchicine is connected with cessation of intracellular particle movement and has been observed in helizoan axopods (54), HeLa cells (15), neurons (28), and other tissues. Investigations by Yamada et al. (61) and Daniels (9) on the effects of colchicine on neurons in vitro suggest that microtubules are essential for growth and maintenance of neurites.

The alteration of skin color in fish, amphibians, and reptiles as a protective adaptation is well known. This change in skin color is associated with the intracellular movement of melanin granules in the dermal and epidermal melanocytes. When pigment granules are dispersed the skin is dark, and skin is light when pigment granules are aggregated (23). Fujii and Novales (17) observed filaments in the melanocytes of fish and suggested that they play a role in the movement of melanin granules. McGuire et al. (35) are of the opinion that microfilaments, which are abundant in frog epidermal melanocytes, are responsible for the dispersion of the melanin granules. McGuire and Moellmann (34) showed that dispersion of melanin granules by melanocyte-stimulating hormone was associated with the appearance of large numbers of filaments within the melanocyte dendritic processes. On addition of cytochalasin B, the filaments disappeared and the granules reaggregated. Cytochalasin B also reversed the dispersion of melanin granules induced by cyclic 3’:5’-adenosine monophosphate and theophylline (38). On the basis of these studies, Malawista (31), McGuire and Moellmann (34), and Novales and Novales (38) suggested that the intracytoplasmic filaments were the mediators of melanin granule dispersion.

Investigations of the effects of colchicine on the melanophores of Fundulus heteroclitus showed that there is a decrease in the microtubules of these cells associated with an increase in microfilaments and dispersion of melanosomes (59). Studies by Jande (26) and McGuire (36) also indicated that microtubules were primarily concerned with aggregation of melanin granules, whereas their disassembly into microfilaments aided dispersion.

The presence of microfilaments in human epidermal melanocytes has been reported (6, 39). Jimbow et al. (27) conducted a detailed study directed to characterization of ultrastructural events in melanocytes before and after exposure to sun in caucasoid, mongoloid, and negroid subjets. Both microtubules and microfilaments were observed in both exposed and unexposed skin. In the unexposed skin the microfilaments formed a distinct bundle, which was invariably perinuclear. After exposure to sunlight and UV of long wavelength, the microfilaments were located primarily in the dendritic processes, closely associated with melanosomes. In addition the microtubules were more frequently located along the periphery of the cytoplasm or in the dendritic processes.

The role of microfilaments and microtubules in the dispersion and aggregation of melanin granules has been very convincingly demonstrated by investigators mentioned previously. However, the actual mechanism by which the pigment granules are translocated is still obscure. Untreated tumor cells of the Harding-Passey melanoma contained few microtubules and no microfilaments. Following colchicine administration both mitotic and interphase cells displayed microfilaments. Well-defined bundles of filaments were more commonly encountered in interphase cells. The treated tumor cells also showed a tendency for the melanin granules to be located along the peripheral aspect of the melanoma cells. Light microscopic studies by Freidman and Drutz (16) reported a similar localization of melanin granules in tumor cells of the Harding-Passey melanoma after irradiation and nitrogen mustard treatment. In the light of the observations of Malawista (31), McGuire and Moellmann (34), and Jimbow et al. (27), one may speculate that the colchicine-induced microfilaments observed in this study probably are responsible for the peripheral displacement of the melanin granules.

An extensive increase in neurofilaments in nerve cells has been observed following colchicine administration (60). This increase in neurofilaments is reported to be associated with inhibition of axoplasmic transport of monoamines (7) and protein (18, 28). Hansson and Norstrom (20) observed an increase in tangles of filaments in the glial cells of the rat hypothalamohypophyseal system after colchicine administration.

Microfilaments have been reported in a variety of cells performing diverse functions. The basal cells of the epidermis of adult Rana pipiens (42), as well as the basal cells of human esophagus and trachea (44), contain an abundance of filaments. Parakkal and Alexander (41) have offered an excellent review of vertebrate epithelia at the ultrastructural level. Jande (26) considered the filaments observed in darkened epidermal melanocytes of R. pipiens to be keratin. McGuire and Moellmann (34) reported that microfilaments that caused dispersion of melanosomes by melano-
cyte-stimulating hormone could be distinguished from similar filaments in keratinocytes by using cytochalasin B. Addition of cytochalasin B resulted in disappearance of microfilaments and aggregation of melanin granules, whereas the filaments in keratinocytes were not affected by cytochalasin B. The filaments observed in the tumor cells of the colchicine-treated Harding-Passey melanoma in the present study resemble the microfilaments observed in melanocytes by several investigators cited earlier. Ozzello (40) described filaments permeating the cytoplasm as perinuclear bundles in mammary carcinoma cells and suggested that they might be contractile proteins.

Glucocorticoids affect cellular differentiation in developing lung by slowing cell division and thereby promoting cell maturation and differentiation (5). Smith et al. (50) reported that cortisol applied to cultures of fetal lung when epithelial cells were in growth phase promoted maturation. On the other hand, they observed that, when the steroid was applied to more mature fetal lung, cortisone decreased cellular growth but promoted maturation. Adkinson and Klass (1) observed prednisolone to enhance cell maturation and differentiation in culture of pulmonary adenoma cells into cells morphologically indistinguishable from type II alveolar epithelial cells of the normal lung.

Investigations of Inoue (24), Swann and Mitchison (53), Eigi and Dustin (14), Davidson et al. (10), Brinkley et al. (4), and Inoue and Saito (25) showed that colchicine causes metaphase arrest. This effect is thought to persist for about 15 hr, after which the cell either degenerates or passes through the rest of the cell cycle. In the present study administration of colchicine to tumor cells of Harding-Passey melanoma induced the formation of microfilaments that persist for at least as long as 10 weeks, the duration of the study. These studies therefore add a longitudinal parameter and considerably extend the original investigations of Nathaniel et al. (37). Whether these microfilaments are similar to filaments observed in keratinocytes is beyond the scope of this report.

REFERENCES


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**Fig. 1.** Electron micrograph of a melanoma cell in a nontreated tumor showing a highly irregular nucleus, a prominent nucleolus, and a peripheral aggregation of chromatin. The cytoplasm exhibits multiple Golgi complexes, polysomes, mitochondria, and melanin granules. × 8,500.

**Fig. 2.** A conspicuous bundle of filaments (fil) bordered by ribosomes in a cell of colchicine-treated tumor. The presence of nuclear masses, some of which show perinuclear space while others do not, suggests that this cell is in early prophase or late telophase. Melanin granules serve as a marker and identify the cell as a melanoma. × 15,000.

**Fig. 3.** Electron micrograph demonstrating the presence of microfilaments (fil) in cells of treated tumor generation 1 (2 weeks after cessation of colchicine treatment). Note the large numbers of virus particles. × 34,400.

**Fig. 4.** Filaments in an interphase melanoma cell of treated tumor generation 2 (4 weeks after cessation of colchicine treatment). A conspicuous bundle of filaments (fil) bordered by ribosomes and mitochondria is clearly visualized. × 25,000.

**Figs. 5 and 6.** Electron micrographs demonstrating the presence of microfilaments (fil) in cells of treated tumor generation 3 (6 weeks after cessation of colchicine treatment). × 38,000; Fig. 6, × 37,500.

**Fig. 7.** Microfilaments permeating the cytoplasm of a cell in treated tumor generation 4 (8 weeks after cessation of colchicine treatment). × 28,725.

**Fig. 8.** Electron micrograph illustrating a well-defined bundle of filaments (fil), perinuclear in position and bordered by polysomes. Note the well-developed Golgi complex (G) and melanin granules adjacent to it. Treated tumor generation 5 (10 weeks after cessation of colchicine treatment). × 22,575.
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