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VIRUS-LIKE NUCLEAR DEGENERATION IN MALIGNANT MELANOMA ☼

By

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Introduction

The viral etiology of several tumors was established at a very early date (2, 9, 21, 22). Recent reviews confirm the role of viruses in tumorigenesis for an evereextending list of tumors have established morphological, physiological, and physical characteristics of some viruses (16). Virus-host relationships have been examined and cytological observations of host correlated to phasic patterns of viral multiplication (12).

Until recently, attempts to depict a virus-like particle in an association with human tumors have been unsuccessful. Negroni and others (13,17, 23) demonstrated virus-like particles with human leukemia, and virus-like bodies have been involved in studies of Burkitts Lymphoma (10). No viruses have been reported from malignant melanoma.

This report characterizes nuclear inclusion bodies found in some cells of a malignant melanoma and the presence within them of structured particles showing morphological characteristics of a large virus.

Materials and Methods

Clinical examination of H. A., a 37 year old white female with a two month history of blurred vision in the right eye, revealed a best corrected visual acuity of O. D. 20/50 and O. S. 20/20. The pertinent physical findings included episcleral vessels inferiorly and temporally in the right eye; a large pigmented tumor was visualized in the anterior

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choroid extending onto the adjacent ciliary of the same eye from 5-10 o'clock. Visual field examination of the affected eye revealed an absolute scotoma which corresponded to the area of the tumor. The eye subsequently was enucleated.

The tumor was removed and fixed immediately for light and electron microscopy. The tissue for light microscopy as done routinely in pathology laboratories. For electron microscopy, pieces 2 mm^2 or smaller were taken. After fixation in 1% (w/v) osmium in phoshopsaline buffer (0.2 M), pH 7.5 at 0°C for 1 hour, the tissue was dehydrated and embedded in Epon (15). Sections showing silver interference colors (18) were taken using the Porter-Blum microtome. The sections were lifted on copper grids, stained with uranyl acetate (25) and lead citrate (20), and examined in an Elmiskop 1.

Description

Light microscopy – Light microscopy confirmed the diagnosis of malignant melanoma (19) of the choroid and ciliary body. The tumor was the mixed type consisting of spindle type and epithelioid cells (3) (see pl. 1 a, b, c).

Electron microscopy – Since extensive detail has been given on the cell types, organelles, inclusions and relationships in malignant melanomas (1, 7, 8), these items are not described again in this report. Attention is focused primarily on nuclear degeneration as observed in the relevant tumor.

Many nuclei were as described in previous reports (11, 14, 26, 27), here there were nuclei of many melanoma cells showing a particularly characteristic and repetitively appearing degeneration. Varying stages of this degeneration were evident. Within the nucleus of affected cells there appeared oval, round, or channeled masses with a specific content. Hereafter, these masses will be referred to as the "nuclear inclusion body". It is characterized as a specific entity having a membrane and a definitive internal content to be described herein.

Nuclear inclusion bodies (Plate 1) were found only in pigmented cells; however, in such cells the cytoplasmic granules were comparatively larger, coarser and more electron-dense than pigment granules in the typical cells of malignant melanoma.

The nuclear inclusion body, surrounded by a dual membrane (Pl. lf), was composed of many vesicular of a relatively uniform size, variations depending on the content of the vesicular particles (Pl. le). The particle size ranged from 260 to 300 millimicrons. The smaller particles were diffusely electron dense the vesicularity being obscured by a melanin or lipidlike overlay; in others, the vesicular membrane was more distinct but the internal structure of the particle structurally electron dense. Some particles showed a centrally located nucleoid, other less electron density and a tendency of the nucleoid to be located laterally rather than centrally. Such variasions might be interpreted as a maturation series. The vesicles described were embedded in an opaque matrix. No evidence was seen of the particles traversing the inclusion body membrane to enter the nucleus proper. No determination has been made as to whether the particles here described are DNA or RNA; at present, they have not been successfully grown in tissue culture.

With the exception of the nuclear inclusion body, nuclear morphology of affected cells did not differ markedly from that of other cellular nuclei of tumors without inclusion bodies or of normal cells from other tissues. The chromatin was diffuse and the nucleonema formed a banded reticulum existing as a single mass or as a dual mass at opposite poles of the nucleus. Of the five malignant melanomas examined in this laboratory, only the one tumor presented such nuclear inclusions.

Discussion

The question arises whether cells having nuclear inclusion bodies are phagocytic or transformed cells. Wellings and Siegel (27) have described phagocytes with large, non-uniform cytoplasmic granules, distinguishing between normal complement granules and phagocytosed granules. The dissimilarity of the cytoplasmic granules of these affected cells to other granules of non-affected cells is remarkable. Thus one would question the source of these cytoplasmic granules if they were phagocytized and if not, such cells would not be identical to the phagocytic cells described previously. In addition, these granules are not the amorphous masses depicted by the above authors but discrete particles overlaid by an electron¾dense material, the stroma being quite unlike that striated condition earlier described. No sequential patterns indicating the arising of these granules from the Golgi complex were observed (26). The

configuration of the small melanin granules in non-affected melanoma cells, however, does agree with the earlier descriptions (27).

While it is possible to say that the affected cell type differs cytologically in other respectes form the non-affected cells, it is not possible to determine whether it is a transformed cell or a migrant cell other than a phagocyte. If it is a migrant cell, questions arise as to its source. If it is a transformed cell, there are questions concerning the role of the nuclear inclusion body and its products in cellular transformation.

Whether other celles of such a developing tumor will undergo a transformation similar to that of those having inclusion bodies should be considered. Differentiations should be made as to whether the affected cells are phagocytes, migrant cells other than phagocytes, or **in situ** transformed cells.

The possibility has been examined that the nuclear inclusion bodies may have been sectional cuts through "nuclearhofs". Examination of serial sections did not reveal this to be so. In addition, extensive random sampling did not demonstrate a lateral "nuclearhof" pattern, thereby negating this possibility. Differences in the texture of the matrix of the nuclear inclusion body from the cytoplasm implied a marked difference in their composition.

Another point worthy of discussion is the demonstration of nuclear iclusion bodies and contained particles within one tumor but not within tumors from other individuals. Two possibilities are worth examining in this respect. First, it may be that the alterations noted were singular to the one individual examined and was representative of advanced cellular necrosis. The second possibility is that the etiology of this tumor was different from that of those described previously.

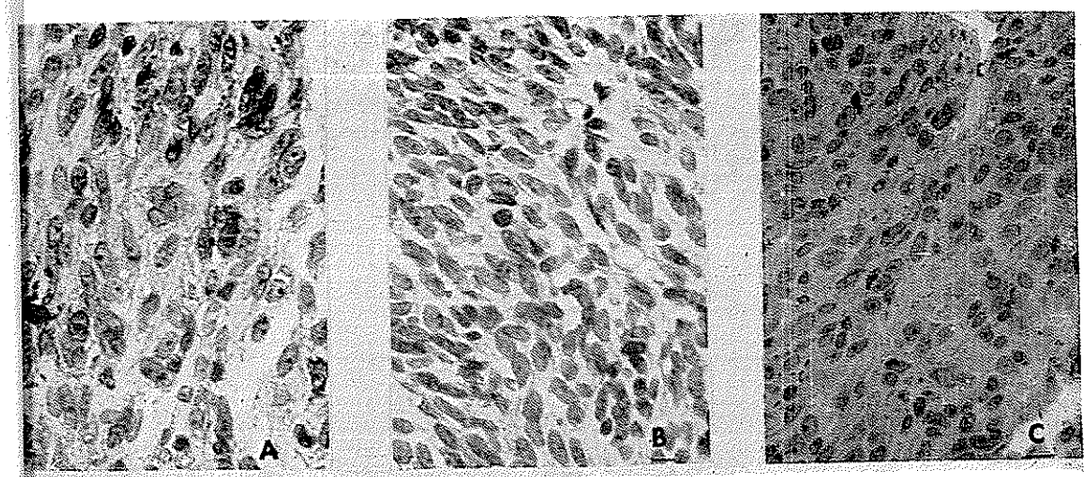
Undeniably the condition described was singular to the one tumor as it has not been demonstrated in other melanomas. However, such changes have not been described as typical of advanced necrosis from melanomas; neither have ultrastructural studies on typical necrotic alteration from other tnmors showed this specific modification. Cellular necrosis is first evident cytoplasmically, but the cytoplasmic characteristics of the melanoma cells appear typical of viable tumor cells. These consideration, that the etiology of this tumor is entirely different form other tumors

examined in this laboratory, would appear to be more reasonable. Cytological differences in this melanoma compared to similarly diagnosed tumors from other patients (4) would substantiate this proposal. The origins of neoplasia are diverse; the course of neoplasia may be determined by the causative agent (24).

Additional characterizations will have to be made to determine the chemical composition, infectivity and physical properties of such particles as they occur in malignant melanoma. Determinations should be made whether they are actually viruses, microplasma, or anatomical alterations due to physiological variation.

Summary

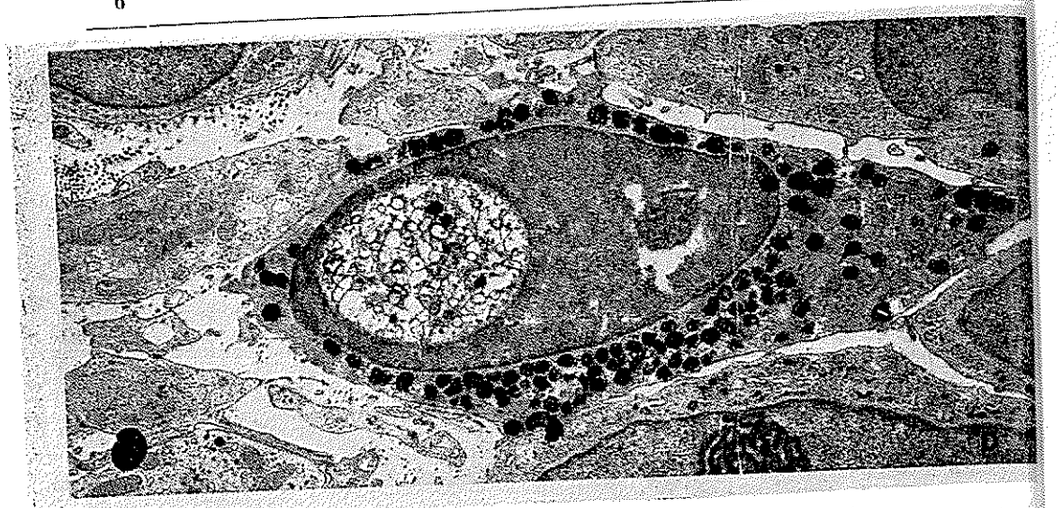
Nuclear inclusion bodies were observed in certain cell types of a malignat melanoma. These inclusion bodies contained virus-like particle 260 to 300 millimicrons in diameter. Variations in particle structure and content implied a maturation cycle.



a, b, c. Llght micrographs of malignant cells showing spindle and epithelioid cells. Micrograph c is a 2 micron section of tissue fixed in buffered osmium tetroxide which details cellular relationships and nuclear opacities with greater clarity.

Magnifications:

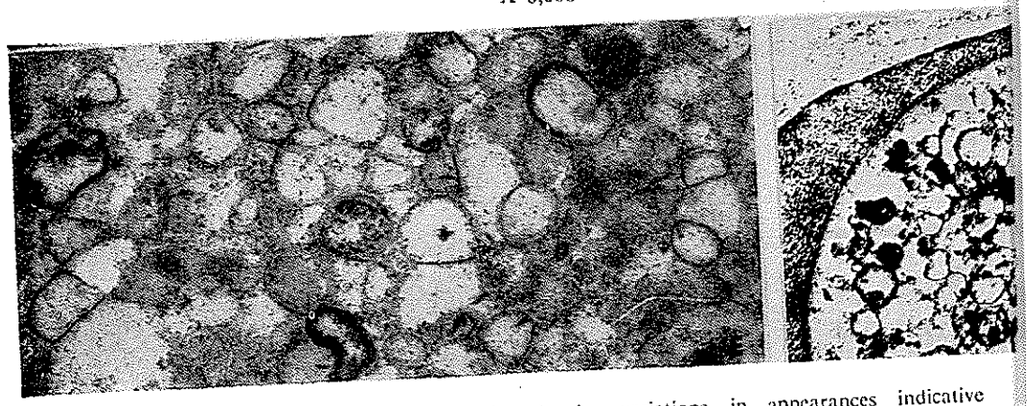
a. X 850 b. X 850 c. X 640



d. Electron micrograph of a pigmented cell with nuclear inclusion bodies and enclosed particles. Many pigment granules are distributed in the cytoplasm.

Magnification :

X 6,000



e. Detail of nuclear inclusion bodies showing variations in appearances indicative of a maturation cycle. Note that the bodies are contained in an amorphous matrix.

Magnification :

X 38,000

f. Detail of dual membrane surrounding nuclear inclusion body.

Magnification

X 22,000

References

1. Bierring, F., Egeberg, J. and Jensen, O. A. (1963). Electron microscopy of a malignant melanoma of the choroid. Acta Ophthal. (Kbh.) 41:224;235.
2. Bittner, J. J. (1936). Some possible effects of nursing on mammary gland tumor incidence in mice. Science 84:162.
3. Callendar, C. R. (1931). Malignant melanoma tumors of the eye: A study of histological types in 111 cases. Trans. Am. Acad. Ophth. Otol. 36:131–142.
4. Cooper, W. C. and Hairstone, M. A. (1966). Ultrastructural studies of melanoma (unpublished data).
5 Dalton, A. J. and Hagenau, F. (1962). Tumors induced by viruses: Ultrastructural studies. Academic Press, New York.
6. Dmochowski, L. (1960). Viruses and tumors in the light of electron microscope studies: A review. Cancer Res. 20:977-1015.
7. Francois, J., Rabaey, M. and Lagasse, A. (1959). Etude au microscope electronique d'un sarcome de l'uvee. Ophthal. 137:65–73.
8. Francois, J., Rabaey, M. and Lagasse, A. (1960). Electron microscope date on pigment formation in malenoblastoma. A. M. A. Arch. Ophthal. 64:377-381.
9. Furth, J. (1933). Lymphomatosis, myelomatosis and endothelioma of chickens caused by a filterable agent. I. Transmission experiments. J. Exp. Med, 58:253–275.
10. Harris, R. J. (1964). A etiology of central African lymphomata. Brit. Med. Bull. 20:149–153.
11. Hogan, M. J. and Feeney, L. (1962). Ultrastructure of malignant melanomas of the choroid. Invest. Ophth. 1:544–555.
12. Hostkins, J. M. (1959). Host controlled variation in animal viruses. In Virus Growth and Variation. Ed. A. Isaacs and B. W. Lacey, Cambridge.
13. Inman, D. R., Woods, D.A. and Negroni, G. (1964). Electron microscopy of virus particles in cell cultures inoculated with passage fluid from human leukemic bone marrow. Brit. Med. J. :929–931.
14. Kroll, A. J. and Kuwabara, T. (1955). Electron microscopy of uveal melanomas. Arch. Opth. 73:378–386.
15. Luft, J. H. (1961). Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9:409–414.
16. Moore, D. H., Lasfargues, E. Y., Murray, M. R., Haagensen, D. C. and Pollard, E. O. (1959). Correlation of physical and biological properties of mouse mammary tumor agent. J. Biochem. Cytol. 5:85–92.
17. Negroni, G. (1964). Isolation of viruses from leukemic patients. Brit. Med. J. 927-929.
18. Peachey, L. D. (1698). A study of section thickness and physical distortion produced during micrototomy. J. Biophys. Cytol. 4:233–242.
19. Reese, A. B. (1963). Tumors of the eye. Hoeber Medical Div., Harper & Bros, N. Y.

20. Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17:208-212.

2 . Rous, P. (1911). Transmission of a malignant new growh by Means of a cell-free filtrate. J. A. M. A. 56:198.

22. Shope, R. E. (1932). A transmissible tumor like condition in rabbits. J. Exp. Med. 56:793-802.

23. Southam, C. M. (1963). The role of viruses in neoplasia with emphasis on human leukemia. J. Pediat. 63:138-157. 24. Squartini, F. and Se eri, L. (1962) Strain differences in the mammary tumor inducing virus as detected by the characters and behavior of neoplasms' In Ciba Foundation Symp. on Tumor Viruses of Murine Origin, 1953, pp. 82 106.

25. Watson, M. L. (1958). Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol. 4:475-478.

26. Wellings, S. R. and Siegel, B. V. (1959). Role of golgi apparatus in the formation of melanin granules in homan malignant melanoma. J. Ultr. Res. 3:147-154.

27. Wellings, S. R. and Siegel, B. V. (1960). Electron microscopy of human malignant melanoma. J. Nat. Cancer Inst. 24:437-441.

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Prevention by Cortisone of Histamine-induced Gastric Ulcer in Guinea Pig.

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Introduction

For many years histamine has been widely used in the experimental production of gastric ulcer. The drug has been given by various routes and in many species of laboratory animals (3). It is suggested that the ulceration is primarily due to increased acid secretion of the stomach. In addition, the vasospastic effect of histamine has been postulated as playing a contributory part in the histamine-induced ulcer by diminishing the vitality of the mucosal cells (5).

The study of the influence of corticoids on experimental gastric ulcer has been the subject of many interesting works. Andreani (1) has demonstrated the inhibitory effect of ACTH on histamine-induced ulcer by the method of Halpern and Martin. Selye et al. (6) have observed the inhibition of occurrence of 48/80 (a particularly potent histamine liberator) induced ulcer by pretreatment with cortisol.

In the present work the influence of cortisone on histamine-induced gastric ulcer is studied.

Materials and Methods

Studies were made in 75 guinea pigs of both sexes, weighing 220-300 g. The method of experiment was similar to that described by Eagleton and Watt (2). The animals were housed in individual cages and received no food for 24 hours prior to injection. Three groups of 25 guinea pigs were used. The first and second groups were injected intraperitoneally with 200 mg/Kg of cortisone acetate. Two hours later

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