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Yoshiro Yabe*

Hiroko Koyama[†]

*Okayama University,

[†]Okayama University,

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Yoshiro Yabe and Hiroko Koyama

Abstract

A subcutaneous tumor of a patient with epidermodysplasia verruciformis was studied by the light microscopy, the electronmicroscopy and the immunofluorescent test. The tumor cells were histologically pleomorphic and electronmicroscopically contained varying amounts of cytoplasmic filaments without Z-band formation. The antimyosin serum stained the tumor cells, showing their myogenic origin. No virus or virus-like particles were observed in the tumor. Tumor antigens stainable by the patient's serum were not detected. Hamsters inoculated with the tumor extract at birth developed no noticeable diseases.

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ELECTRONMICROSCOPIC, IMMUNOFLUORESCENT AND VIROLOGICAL STUDIES ON A RHABDOMYOSARCOMA IN EPIDERMODYSPLASIA VERRUCIFORMIS*

Yoshiro YABE and Hiroko KOYAMA

*Department of Virology, Cancer Institute, Okayama University Medical School,
Okayama 700, Japan (Director: Prof. Y. Yabe)*

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Presence of C-type virus-like particles in a rhabdomyosarcoma of a patient with epidermodysplasia verruciformis was reported by OKAMOTO *et al.* (1). The authors, one of whom (Y. Y.) was a coauthor of that report, thoroughly re-examined the tumor of the same patient by the electronmicroscope and the immunofluorescent test, and found out that the tumor was convincingly a rhabdomyosarcoma but had no virus or virus-like particles in it. With this tumor, further virological studies were done by the immunofluorescent test and by inoculating its tissue extract into newborn hamsters. The present paper concerns the results of these studies.

MATERIALS AND METHODS

Case: The same patient as reported by OKAMOTO *et al.* (1); a 40-year-old man, K. K., with the typical skin lesions of epidermodysplasia verruciformis and a 4×5×3 cm subcutaneous tumor on the right lateral chest wall. The subcutaneous tumor was removed surgically. Three months later, a metastatic tumor was observed in the right inguinal region and removed surgically. The patient died of widely disseminated tumor metastases 9 months after the first operation. Autopsy could not be done. The primary tumor and a metastatic tumor were used for the present study.

Histological examination: The tissue was fixed in 10 % formalin and embedded in paraffin. The sections were stained with hematoxylin-eosin and Heidenhain's iron-hematoxylin.

Electronmicroscopy: A part of the tumor was fixed in 6 % glutaraldehyde, post-fixed in 1 % osmium tetroxide, dehydrated, embedded in Epon 812, sectioned and stained doubly with uranyl acetate and lead citrate.

Immunofluorescence for myosin: The human myosin and the guinea-pig antimyosin serum were prepared by the methods similar to those described by HIRAMOTO

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et al. (2). The fluorescein isothiocyanate-labeled rabbit antiserum against guinea-pig globulin was obtained from the Institute of Medical Science, Tokyo University. Using these two antisera, detection of myosin was done by the indirect method (2).

Immunofluorescence for tumor antigens: The patient's sera were obtained at the time of operation of the primary tumor and at the late stage with wide-spread metastases. The fluorescein isothiocyanate-labeled rabbit antiserum against human globulin was obtained from the Institute of Medical Science, Tokyo University. Using these sera, frozen sections fixed in acetone were stained by the indirect method.

Tissue extract: Tumor tissue was ground in a chilled mortar, suspended in phosphate-buffered saline to 20 %, centrifuged at 3,500 rpm for 15 minutes and the supernatant was preserved at -60°C for 1-3 days before inoculation. One-twentieth milliliter of thus prepared extract was inoculated into the muscular part of the thigh of hamsters within 24 hours after birth.

RESULTS

Light microscopy: The histology of the tumor was characterized by pleomorphism with round and spindle cells and occasional giant cells of which the cytoplasm was eosinophilic (Fig. 1). Strap-like cells were observed only occasionally, but convincing cross striations were not observed.

Electronmicroscopy: Electronmicroscopically, the nuclei of tumor cells varied from round to spindle and frequently showed deep indentations. The nucleoli were prominent (Fig. 4). The cytoplasm of tumor cells contained a moderate number of mitochondria, granular endoplasmic reticulums and varying amounts of filaments. In some cells, filament bundles and slightly dilated endoplasmic reticulums were arranged parallel to each other, suggesting the relation between myofibrils and sarcotubules. However, no convincing Z-band formation was observed.

The tumor was thoroughly studied electronmicroscopically for virus particles, but no virus or virus-like particles were observed.

Immunofluorescence for myosin: The antimyosin fluorescent antibody, which specifically stained human skeletal muscle (Fig. 2), stained the cytoplasm of many cells in the tumor (Fig. 3).

Immunofluorescence for tumor antigens: In the immunofluorescent test for tumor antigens, both of the sera obtained from the patient at the first

Fig. 1. Hematoxylin-eosin stain of tumor. Pleomorphism is marked. $\times 300$.

Fig. 2. Fluorescence photomicrograph of normal human skeletal muscle stained with anti-myosin serum. Staining is seen in the sarcolemma as well as in the muscle striations. $\times 600$.

Fig. 3. Fluorescence photomicrograph of tumor stained with anti-myosin serum. Positive immunofluorescence for myosin is evident. $\times 600$.

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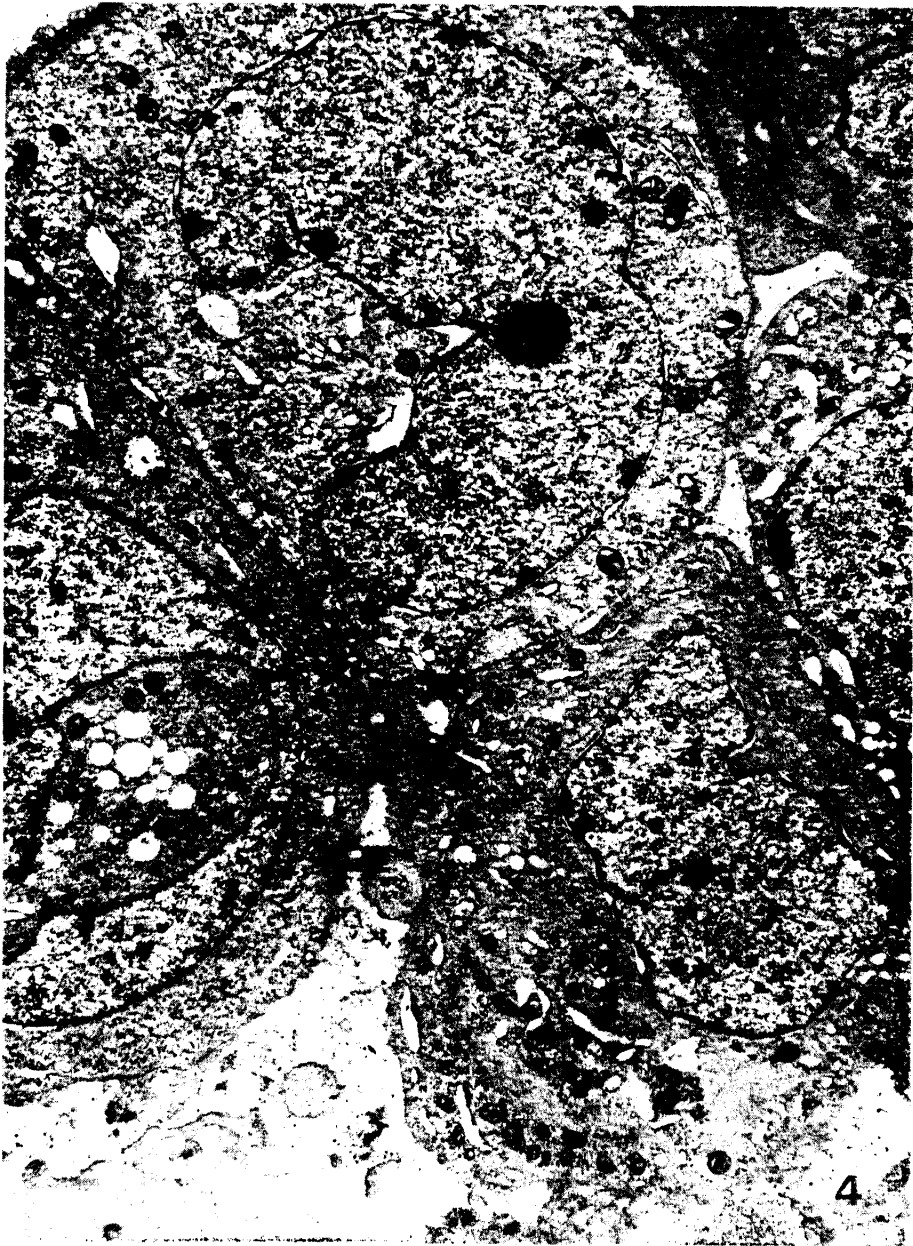


Fig. 4. Electronmicrograph of tumor. Cells contain varying amounts of filaments. Parallel arrangement of filament bundles and slightly dilated granular endoplasmic reticulums and/or mitochondria is seen in the lower right cell. $\times 7,500$.

operation and at the late stage with wide-spread metastases did not stain the tumor cells.

Animal experiment with tissue extract : Twenty-six of 34 hamsters inoculated with the tumor extract at birth, survived over 3 weeks and died or sacrificed in 203-548 days. None of them had any noticeable diseases.

DISCUSSION

It has been reported that the histological diagnosis of rhabdomyosarcomas is often very difficult, and that some probable rhabdomyosarcomas are not stained even with the antimyosin serum (2). In the present tumor, the histological examination and the electronmicroscopy gave only some suggestive findings for the diagnosis of rhabdomyosarcoma. Its conclusive diagnosis of rhabdomyosarcoma was possible only by its positive immunofluorescence for myosin. It appears, therefore, that, for the diagnosis of rhabdomyosarcomas, the immunofluorescent test with the antimyosin serum would be superior to the electronmicroscopy.

Viral induction of rhabdomyosarcomas in animals has been reported (3), and a few negative or inconclusive results in the virological studies of human rhabdomyosarcomas have been also reported (4, 5). Epidermodysplasia verruciformis appears recently to be considered as a genetic condition with a decreased resistance against the wart or wart-like virus (6). Therefore, the development of rhabdomyosarcoma in the patient of epidermodysplasia verruciformis appears to be of interest. However, the negative results in all the attempts to detect virus particles and tumor antigens in this tumor and to induce tumors by its tissue extract are unfavorable to consider its viral etiology.

SUMMARY

A subcutaneous tumor of a patient with epidermodysplasia verruciformis was studied by the light microscopy, the electronmicroscopy and the immunofluorescent test. The tumor cells were histologically pleomorphic and electronmicroscopically contained varying amounts of cytoplasmic filaments without Z-band formation. The antimyosin serum stained the tumor cells, showing their myogenic origin. No virus or virus-like particles were observed in the tumor. Tumor antigens stainable by the patient's serum were not detected. Hamsters inoculated with the tumor extract at birth developed no noticeable diseases.

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REFERENCES

1. OKAMOTO, T., YABE, Y. and OHMORI, S.: Virus-like particles in rhabdomyosarcoma with epidermodysplasia verruciformis. *Dermatologica* **141**, 309, 1970
2. HIRAMOTO, R., JURANDOWSKI, J., BERNECKY, J. and PRESSMAN, D.: Immunochemical differentiation of rhabdomyosarcomas. *Cancer Res.* **21**, 383, 1961
3. MOLONY, J. B.: A virus-induced rhabdomyosarcoma of mice. *Natl. Cancer Inst. Monograph* **22**, 139, 1966
4. FREEMAN, A. I. and JOHNSON, W. W.: A comparative study of childhood rhabdomyosarcoma and virus-induced rhabdomyosarcoma in mice. *Cancer Res.* **28**, 1490, 1968
5. McALLISTER, R. M., NELSON-REES, W. A., JOHNSON, E. Y., RONGEY, R. W. and GARDNER, M. B.: Disseminated rhabdomyosarcomas formed in kittens by cultured human rhabdomyosarcoma cells. *J. Natl. Cancer Inst.* **47**, 603, 1971
6. LEVER, W. F.: Histopathology of the skin, 4th Ed., P. 380, J. B. Lippincott Co., Philadelphia, Toronto, 1967