〈Brief Note〉

Cytodiagnosis of metastatic amelanotic melanomas to the lymph node by fine needle aspiration cytology: adjunctival value of immunohistochemical staining and electron microscopy

Chiyuki Kaneko[1] and Yuuko Kosuge[2]

Summary Amelanotic melanoma (AM) can mimic a wide variety of epithelial and nonepithelial malignant cells. The cytologic diagnosis of metastatic AM is challenging, given that the tumor cells may mimic those of a carcinoma or a sarcoma in cytologic materials obtained by fine-needle aspiration cytology (FNAC).

A single well-documented case of metastatic malignant AM to the lymph node with a cytologic evalutaion by FNAC was reported. The tumor cells were stained positively with S-100 and/or HMB-45 antibody. A correct cytologic diagnosis of AM may be made in a large number of cases by routine cytologic findings and immunohistochemical staining with S-100 protein and HMB-45 antibodies, as well as electron microscopy.

Key words: Amelanotic melanoma, Immunohistochemical stain, Lymph node, Fine needle aspiration cytology, Electron microscopy

1. Introduction

Malignant melanoma is known for its wide range of histologic patterns and its ability to mimic other malignant tumors[1)-3)]. Malignant melanoma is generated in the skin and sinus paranasalis by a malignant tumor of melanocytic origin[4)].

Malignant melanoma is usually quickly diagnosed by the presence of melanin granules. On the other hand, amelanotic melanoma is without melanin granules and is often difficult to differentiate from nonepithelial malignant tumors. Recently, fine-needle aspiration cytology (FNAC) was used to treat widespersed mammary glands, thyroid area, and lymph nodes. As for the reports of amelanotic melanomas that metastasize to the mammary gland[5)] and the ovary[6)]. However, no other detailed study has been reported on transmission electron microscopy.

This study stresses the importance of immunohistochemical staining and electron microscopy for a

[1)]Department of Cytopathogy, Fujita Health University School of Health Sciences, Toyoake, Aichi 470-1192, Japan
[2)]Department of Pathology, Kitasato Junior College of Health and Hygienic Sciences, Minamiuonuma, Niigata 949-7241, Japan
Address reprint requests to: Chiyuki Kaneko, Ph.D. Fujita Health University School of Health Sciences, 1-98 Dengakugakubo, Kutsukake-Cho, Toyoake-City, Aichi 470-1192, Japan
Received for Publication May 26, 2009
Accepted for Publication July 2, 2009

correct diagnos is of metastatic amelanotic melanoma with to the lymph node.

2. Materials and methods

1. Subjects

Eight malignant cases were studied (5 males and 3 females). The salient clinical features of these cases are shown in Table 1.

Written informed consent was obtained from all volunteers, and our study was in compliance with the rules for human experimentation at our institution.

Among the cases, there were two examples in the ophthalmology area, one example each of a subnasal cavity, of the skin, one of the rectum, three of metastasis in the lymph nodes and five among eight melanoma, cases, one of a metastasis in the lymph node of a stomach cancer patient, one of a malignant melanoma metastasis, and one each of a metastasis in the lymph nodes of one amelanotic melanoma patients.

The specimens were obtained using touch smears and FNAC. Eight cases were smear fixed immediately in 95% ethyl alcohol and air-dried. All 8 were stained by the Papanicolaou (Pap) method and May-Grunwald-Giemsa(MGG). The remaining specimens were fixed in formalin and analyzed by immunostaining and special staining. The monoclonal antibodies used were HMB-45 (1 : 50, DAKO) and S-100 (1 : 350, DAKO). For immunostaining, the routine avidin-biotin peroxidase complex (ABC) method was employed, and color was developed with 3,3'-diaminobenzidine tetrahydrochloride(DAB). As for special staining methods, Grimelius and Masson-Fontana's stains were used.

2. Immunohistochemistry

Immunohistochemical staining was performed by the ABC method. After deparaffinization, the preparations were treated with 0.3% peroxidase in methanol for 30 minutes to block endogenous peroxidase and then washed for 10 minutes in PBS. Exposure to primary antibodies lasted for 2 hours in a moisture chamber, followed by washing 3 times in PBS between subsequent ABC steps for 30 minutes at room temperature. Color development of bound antibodies was achieved using DAB dissolved in tris-HCl buffer (pH 7.6) for three to 10-minutes and stopped in running water. Light counterstaining of nuclei was performed with Mayer's haematoxylin for 30 seconds, followed by dehydration, clearing and mounting.

3. Transmission electron microscopy (TEM)

The specimens were obtained from lymph nodes with samples fixed in 2-3% glutalaldehyde and 1-2% osmium tetroxide.

Next, after dehydration in a graded series of alcohols, the samples were Epon embedded by the inverted gelatin capsule method.

Ultrathin sections were cut with a diamond knife on an LKB Ultratome, stained with uranyl acetate and lead citrate and observed with a JEOL-1010 (Japan Electron Optical Laboratory, Tokyo, Japan) transmission electron microscope.

3. Results

Table 1 Summary of details for cases studied

Case	Age	Sex	Site	Pathological diagnosis
1	59	F	Ophthalmological area	Malignant melanoma
2	50	F	Ophthalmological area	Malignant melanoma
3	56	M	Paranasals sinus	Malignant melanoma
4	42	M	Skin	Malignant melanoma
5	80	F	Rectum	Malignant melanoma
6	70	M	Lymph node	Malignant melanoma
7	57	F	Lymph node	Carcinoma of the stomach
8	66	M	Lymph node	Amelanotic melanoma

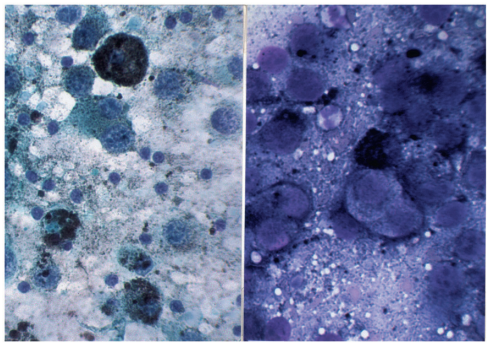
1. Cytological findings

One to 6 smears were observed to have an abundance of melanin pigment cytoplasm in the tumor cells. The melanin granules were plainly observed with Pap (Fig. 1, left) and MGG stain (Fig. 1, right).

The smears were cellular and showed discohesion. Tumor cells showed an elevated Nucleus/cytoplasm (N/C) ratio with nuclei round or oval in form and situated eccentrically.

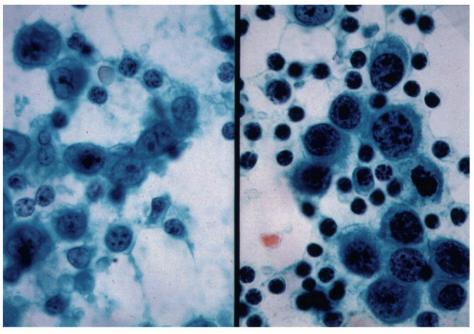
The chromatin was granular and unevenly distributed. MGG staining reveales morphological findings similar to those observed by Papanicolaou's stain. However, amelanotic melanoma smears were found to be without melanin granules (Fig. 2, left).

The tumor cells showed an elevated N/C ratio, with nuclei round in form, and prominent of nucleoli exhibiting a fine chromatin pattern. The cytoplasm was dark, and the pyknotic nuclei and their outlines not always well defined. In addition, metastatic carcinoma of the stomach to the lymph node was obserred with tumor cells showing large, round to oval nuclei exhibiting hyperchromasia.



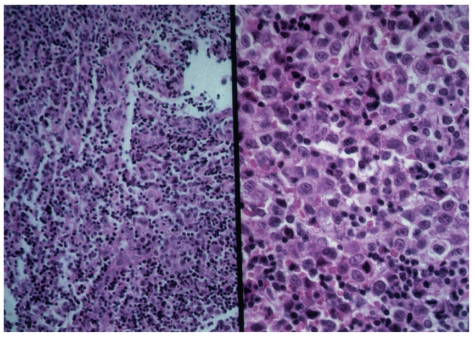
(Pap. stain x40) (MGG. stain x40)

Fig. 1 Malignant melanoma.
Melanin granule is plainly observed with Pap stain (left). Melanin granule is clearly observed with MGG stain (right).



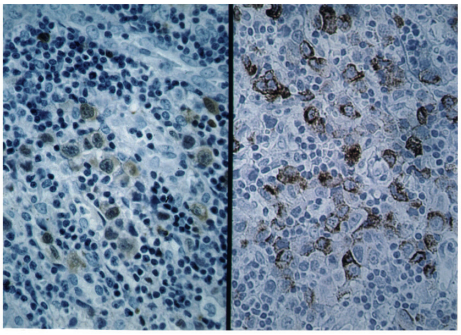
(Pap. stain x40) (Pap. stain x40)

Fig. 2 Amelanotic melanoma.
Varied nuclear and cytoplasmic features of amelanotic melanoma (left). Carcinoma of the stomach; Tumor cells showed large, round to oval nuclei exhibiting hyperchromasia (right).



(HE stain x20) (HE stain x40)

Fig. 3 Amelanotic melanoma.
Lymphocyte is mainly observed in the background. Tumor cells observed sporadically inside (left). Tumor cells are generally bright, and the nucleolus is clearly recognized. Melanin granule is not recog-nized (right).



(ABC method x40) (ABC method x40)

Fig. 4 Amelanotic melanoma.
Note positive cytoplasmic staining with S-100 antibody (left). Note positive cytoplasmic staining with HMB45 antibody (right).

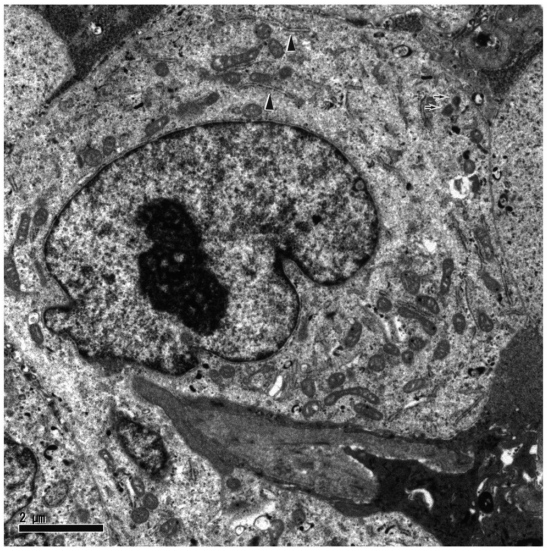


Fig. 5 Amelanotic melanoma.
Note melanosomes (arrow) and rough endoplasmic reticulum (arrow head) (TEM).

2. Histological, special and immunohistochmical findings

In case 8, HE stain revealed relatively light tumor cells and clear nucleoli, but without melanin granules (Fig. 3). Moreover, Grimelius and Masson-Fontana stains were positive in tumor cells. Immunohistological staining was markedly positive for both S-100 (Fig. 4, left) and HMB-45 (Fig. 4, right). The number of S-100 positivity cells were few while many positive cells were observed in HMB-45.

3. Transmission electron microscopy findings

Observations by electron microscopy detected melanosomes and a rough endoplasmic reticulum (Fig. 5), while no intracytoplasmic microtubules were observed.

4. Discussion

Malignant melanoma is derived from melanocytes and has a poor prognosis. This tumor often develops in the ophthalmological area, paranasal sinuses, skin, and several other tissues[1, 5, 6]. Its diagnosis is relatively straightforward when melanin granules are present.

However, when they are absent, its differentiation from malignant lymphoma, undifferentiated cancer, and other types of adenocarcinoma is sometimes difficult[7].

Watanabe *et al.*[6], reported immunohistochemical staining for Melan-A, S-100 and HMB-45 that showed immunoreactivity in the tumor cells, leading to a diagnosis of amelanotic malignant melanoma.

Recently, KBA. 62 antibody could function as a helpful diagnostic marker in such difficult cases[7].

Pagés *et al.*[7] have also evaluated the diagnostic accuracy of KBA. 62 in sentinel lymph nodes, by a comparison between anti-S-100 protein and HMB-45 antibodies.

The most effective panel of antibodies to be used in detecting occult metastasis in sentinel lymph nodes remains to be determined. Metastasis to regional lymph nodes is indeed the most important prognostic factor in early-stage melanoma.

Moreover, transmission electron microscopy is necessary to demonstrate melanosomes, intracytopalsmic microtubules[8], premelanosomes[9], or rough endoplasmic reticulum in cases with unusual cytologic and equivocal immunohistochmical manifestations[8].

In recent years, the use of FANC has become widespread in the cytology field[1, 3, 8, 10].

When lymph node metastasis of tumor cells is present, immunostaining should be actively applied. Since it has provel useful for early diagnosis and treatment. Diagnosis is straight forward in the presence of melanin granules but difficult in their absence.

In the future, FNAC is expected to be applied not only to lymph nodes but also to mammary and thyroid glands. In particular, for the diagnosis of malignant tumors showing lymph node metastasis, the results of immunostaining and electron microscopy are important and may prove useful for early diagnosis and treatment as well as the prediction of outcomes.

Moreover, observing intracytopalsmic microtubules was not possible due to melanosomes and a rough endoplasmic reticulum (eER).

References

1) Kobayashi G, Cobb C: A case of amelanotic melanoma spindle-cell melanoma presenting as metastases to breast and axillary lymph node: diagnosis by FNA cytology. Diagn. Cytopathol., 22: 246-249, 2000

2) Siddaraju N, Yaranal PJ, Mishra MM, Soundararaghavan J: Fine needle aspiratioin cytology in recurrent amelanotic melanoma: a case report. Acta Cytol., 51: 829-832, 2007

3) Rongioletti F, Smoller BR: Unusual histologic variants of cutaneous malignant melanomas with some clinical and possible prognostioc correlations. J. Cutan. Pathol. 132: 589-603, 2005

4) Edited by Rubin E, Farber JL: Pathology: Wallace H, Clark Jr: The skin, 1249-1252, J. B. Lippincott Company, Philadelphia, 1988

5) Mayayo Artal E, Gomez-Aracil V, Mayayo Alvira R, Azua-Romeo J, Arraiza A: Spindle cell malignant melanoma metastatic to the breast from a pigmented lesion on the back: a case report. Acta Cytol., 48: 387-390, 2004

6) Watanabe Y, Ueda H, Nakajima H, Minoura R, Hoshiai H, Noda K: Amelanotic malignant melanoma arising in an ovarian cystic teratoma: a case report. Acta Cytol., 45: 756-760, 2001

7) Pagés C, Rochaix P, Saati TA, Valmary-Degano S, Boulinguez S, Launay F, Carle P, Lauwers F, Payoux P, Guellec SL, Brousset P, Lamant L: KBA. 62: a useful marker for primary and metastatic melanomas. Human Pathology., 39: 1136-1142, 2008

8) Lai R, Redburn J, Nguyen GK: Cytodiagnosis of metastatic amelanotic melanoma by fine-needle aspiration biopsy: adjunctival value of immunocytochemistry and electron microscopy. Cancer (Cancer Cytopathol.), 84: 92-97, 1998

9) Tanaka N, Mimura M, Kimijima Y, Amagasa T: Clinical investigation of amelanotic malignant melanoma in the oral region. J. Oral. Maxillofac. Surg., 62: 933-937, 2004