Recent Progress in Diagnosis and Treatment of Melanoma

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Abstract: New methods for the diagnosis and treatment of melanoma are outlined. Dermoscopy provides a simple and effective means of diagnosing melanoma, and it is expected to be used widely in primary care settings. The CGH method that detects abnormalities in the number of DNA copies in the genome is very useful where differential diagnosis between melanoma and benign pigmented lesions is difficult in histopathological study. The effectiveness of sentinel lymph node biopsy has been confirmed with respect to surgery. This method eliminates the need for lymph node dissection when the sentinel lymph node is negative for metastasis, and can improve the patient’s QOL. The clinical use of leading-edge therapies for advanced cases, such as peptide vaccine and gene therapies based on achievements in immunological and molecular biological studies, is becoming a reality. A clinical study on gene therapy using liposomes containing the IFN-β gene has been approved by the Ministry of Health, Labor and Welfare, aiming at the first gene therapy for melanoma in Japan.

Key words: Dermoscopy; Sentinel lymph node biopsy; Peptide vaccine therapy; Gene therapy

Introduction

Melanoma is the malignancy of melanocytes (pigment cells) that produce the melanin pigment. Since the 1970s, the incidence of melanoma has doubled in Europe and the U.S., and this increase has become a serious social concern. Changes in lifestyle and the increase in UV radiation due to ozone depletion have been identified as causes that contribute to this increase.

On the other hand, the incidence of melanoma in Japan has been 1/10 to 1/20 of that in Europe and the U.S. While about 40% of melanoma cases in Japan used to have lesions in the plantar area, which is not associated with UV radiation, recent cases show an increase in lesions on the trunk and lower limbs that are exposed to sunlight. This trend is alarming.

Melanoma tends to undergo lymph node
metastasis from the relatively early stages. The prognosis after progression is extremely poor since melanoma does not respond to most chemotherapeutic agents and has low sensitivity to radiation therapy. It is urgently necessary to develop methods for the accurate diagnosis of early-stage lesions and therapies for the advanced stages of melanoma.

This article explains the progress of diagnosis and treatment based on recent achievements in melanoma studies.

**Progress in Diagnosis**

1. Dermoscopy

Dermoscopy is a technique in which the affected skin is coated with a gel or another appropriate medium and observed with a dermatoscope or video microscope. A magnification of 10× is attained by the former, and 20× to 100× by the latter. Because the coating prevents the diffuse reflection of light and improves the transparency of the horny layer and the epidermis, this method is effective for the observation of skin down to the upper layer of the dermis. For this reason, dermoscopy is used for the diagnosis of pigmented skin lesions. Melanomas often develop in the plantar area, and about 1 in 10 people has melanocytic nevus in this area. It is important to establish differential diagnosis of these two types of lesion.

Characteristic features of plantar melanocytic nevus can be classified into 3 patterns as follows.1) (1) A parallel pattern along skin furrows: pigmentation occurs in parallel lines corresponding to skin furrows (Fig. 1). (2) Lattice pattern: a lattice is formed by a parallel pattern along skin furrows plus linear pigmentation perpendicular to it. (3) Filamentous pattern: linear pigmentation crosses skin furrows and skin ridges perpendicularly or obliquely.

In contrast with the parallel pattern along skin furrows seen in melanocytic nevus, early melanoma lesions characteristically show a parallel pattern along skin ridges, in which...
tion of black spots of varying sizes in the lesion borders.

Because dermoscopy is a non-invasive and simple technique, it is useful for the diagnosis of pigmented lesions, particularly melanomas in the plantar area, which often present difficulty in clinical diagnosis.

2. Comparative genomic hybridization (CGH) method

Spitz nevus, a type of malanocytic nevus, is also called juvenile melanoma although it is a benign disease. It is important to differentiate this disease from melanoma, but differential diagnosis is difficult in many cases not only by clinical diagnosis but also by histopathological study. Some patients receive unnecessary treatment as a result of this difficulty, while some others do not receive the treatment needed, with tragic outcomes through relapse and metastasis. Hence, a method of differentiating these diseases has long been awaited. Recently, the CGH method is attracting a lot of attention.

CGH is a technique for analyzing alteration in the number of DNA copies in all chromosomes at a time. A DNA sample from cancer tissues and that from normal tissues are labeled with different fluorescent dyes. These are allowed to bind competitively to human metaphase chromosomes, and changes in the number of DNA copies are detected based on the relative intensity of the fluorescence.

In most cases of melanoma, this method detects alteration in the number of DNA copies in more than 1 chromosome. On the other hand, Spitz nevus either does not show alteration in the number of DNA copies or shows alteration only at a particular site (the short arm of chromosome 11). Because melanoma does not exhibit anomaly in the short arm of chromosome 11, this method can differentiate between the two diseases. Although this method is currently used at research level, it will be used widely in the future, as assay can be performed in paraffin-embedded specimens.

Progress in Treatment

1. Sentinel lymph node biopsy

It is an important problem to decide whether or not prophylactic lymph node dissection should be performed when there is no swelling of the regional lymph nodes. In Japan, prophylactic lymph node dissection is usually performed when the thickness of the primary tumor is 3 mm or more or when ulceration is observed. However, metastasis actually occurs in 20 to 30% of cases with thicknesses of 1.5 to 4 mm, and micrometastasis is detected in a few percent of cases with a thickness of about 1 mm. If the presence or absence of micrometastasis to lymph nodes could be determined preoperatively, this information would greatly assist the decision on the necessity of lymph node dissection. A method invented for this purpose examines the sentinel lymph node, which is the first regional lymph node in the course of lymphatic migration of tumor cells from the primary lesion.

In this method, 1 ml of a dye solution (1–2% patent blue) is administered into the skin near the primary lesion. When the dye has reached the sentinel lymph node about 15 min after injection, a skin incision is made. The lymph vessels and lymph node stained blue are confirmed visually and excised. If the result of histopathological study is positive for metastasis, staged lymph node dissection is performed. If it is negative, no lymph node dissection is performed because the probability of metastasis to other lymph nodes is considered extremely low. A primary lesion located in the central part of the trunk presents difficulty in deciding the location of regional lymph nodes using the dye method as these may be inguinal or axillary lymph nodes on the right or left side. In addition, there may be more than one sentinel lymph nodes for a primary lesion. In these cases, the use of isotopes such as 99mTc-labeled tin colloid is effective for the preoperative identification of the accurate location and number of regional lymph nodes and sentinel
lymph nodes. A gamma probe can be used intraoperatively to identify sentinel lymph nodes through direct contact with lymph nodes.

Thus, it is desirable to combine preoperative or intraoperative isotope study and intraoperative dye study. The reliability of this combination is high, with a less than 1% occurrence of pseudo-negative results (i.e., the sentinel lymph nodes are negative and other lymph nodes are positive). This examination is essential in deciding whether prophylactic lymph node dissection should be conducted, and is expected to contribute to improvement in the patient’s QOL.

2. Peptide vaccine therapy

Recently, many melanoma-associated peptides recognized by T cells have been identified. These are human leukocyte antigen (HLA)-restricted, and most of them are presented by HLA class I to cytotoxic T lymphocytes (CTLs). The clinical application of the hypodermal administration of these peptides with adjuvants has already been introduced in Europe and the U.S. A key to the success of this peptide therapy is the effective induction of CTLs. For this reason, attention is being directed to the use of dendritic cells, which are a type of antigen-presenting cell that plays the most important role in T cell activation.

Researchers at Geneva University induced dendritic cells from peripheral blood, allowed them to take up multiple melanoma-associated peptides with keyhole limpet hemocyanin (KLH), and directly injected the cells into lymph nodes. As a result, tumor regression was observed in 5 of the 16 cases tested. This method has been tested repeatedly in various countries.

Because patients with melanoma in Japan and those in Europe and the U.S. have different HLA types, we need to select peptides that are suitable for Japanese patients. Under the leadership of the National Cancer Center, a clinical study using 5 types of peptide and dendritic cells has been conducted on patients with HLA A2 or A24. The results of this study are awaited with interest.

On the other hand, the effectiveness of peptide therapy and other immunotherapies aiming at CTL induction depends on the ability of CTLs to recognize the peptides presented by HLA class I in melanoma cells. However, in some cases of advanced melanoma, the tumor cells may lack tumor-associated peptides and HLA class I, and treatment can be ineffective despite success in CTL induction. Overcoming this problem is a major challenge in the clinical use of T cell based immunotherapy.

3. Gene therapy

Like all cancers, genetic aberrations in melanoma are complicated. It is difficult to restore all causative genes by gene therapy. For this reason, the current focus is on immunogene therapy aiming to enhance immunity against cancer cells through gene transfer.

The authors conducted a study and the development of gene therapy for melanoma using the interferon (IFN) β gene embedded in positively charged multilayer liposomes, which were developed by Jun Yoshida at the Department of Neurosurgery, the Nagoya University School of Medicine. We have clarified the following facts:

1. The effectiveness of gene transfer in cultured human melanoma cells is about 10%.
2. Melanoma cells with transferred IFN-β gene produce IFN-β.
3. The produced IFN-β exhibits not only a growth inhibition effect but also a cytotoxic effect, and this eventually results in the complete extinction of melanoma cells. The administration of IFN-β in itself shows a growth inhibition effect, but does not show a cytotoxic effect. The IFN-β produced by melanoma cells after gene transfer exerts a stronger antitumor effect than IFN-β per se.
4. Human melanoma cells transplanted into the hypodermis of nude mice die out after
30 to 60 days. A single local dose of liposomes containing the IFN-β gene after tumor formation results in the arrest of tumor growth for 30 days. Six repeated doses result in the complete disappearance of tumor after 40 days. The action mechanism involves the direct effect of IFN-β and the induction of apoptosis.

In inbred mice, the effectiveness is also seen in tumors other than those in the administration site, and the induction of NK cells and CTLs is observed.

Liposomes containing the IFN-β gene have already been tested in a clinical trial on patients with glioma at Nagoya University, and the treatment was found to be effective without any adverse reactions. The clinical study of gene therapy for melanoma was approved by the Ministry of Health, Labor and Welfare in July 2003, and the first case of gene therapy for melanoma in Japan has just begun. (The clinical study of gene therapy is conducted in cooperation with the Department of Dermatology, Shinshu University; the Department of Neurosurgery, Nagoya University Postgraduate School; and the Department of Gene Therapy, Nagoya University Postgraduate School.)

**Conclusion**

This paper outlines new methods for the diagnosis and treatment of melanoma. Dermoscopy, with the advantage of simplicity of procedure, is expected to be used widely in primary care settings and to facilitate the early detection of patients with melanoma. With respect to surgery, the routine use of sentinel lymph node biopsy is expected to improve the patient’s QOL. The clinical use of leading-edge therapies for advanced cases, such as peptide therapies and gene therapies based on achievements in immunological and molecular biological studies, is becoming a reality. It is hoped that the results of basic study will be increasingly applied to clinical treatment.

**REFERENCES**


