35 Akiyama M, Sugiyama-Nakagiri Y, Sakai K *et al.* Mutations in lipid transporter ABCA12 in harlequin ichthyosis and functional recovery by corrective gene transfer. *J Clin Invest* 2005; **115**: 1777-84.

36 Sakai K, Akiyama M, Sugiyama-Nakagiri Y *et al.* Localization of ABCA12 from Golgi apparatus to lamellar granules in human upper epidermal keratinocytes. *Exp Dermatol* 2007; **16**: 920-6.

37 Annilo T, Shulenin S, Chen ZQ *et al.* Identification and characterization of a novel ABCA subfamily member, ABCA12, located in the lamellar ichthyosis region on 2q34. *Cytogenet Genome Res* 2002; **98**: 169-76.

38 Peelman F, Labeur C, Vanloo B *et al.* Characterization of the ABCA transporter subfamily: identification of prokaryotic and eukaryotic members, phylogeny and topology. *J Mol Biol* 2003; **325**: 259-74.

39 Heimerl S, Bosserhoff AK, Langmann T *et al.* Mapping ATP-binding cassette transporter gene expression profiles in melanocytes and melanoma cells. *Melanoma Res* 2007; **17**: 265-73.

40 Fletcher JI, Haber M, Henderson MJ *et al.* ABC transporters in cancer: more than just drug efflux pumps. *Nat Rev Cancer* 2010; **10**: 147-56.

41 Smyth I, Hacking DF, Hilton AA *et al.* A mouse model of harlequin ichthyosis delineates a key role for Abca12 in lipid homeostasis. *PLoS Genet* 2008; **4**: e1000192.

42 Yanagi T, Akiyama M, Nishihara H *et al.* Harlequin ichthyosis model mouse reveals alveolar collapse and severe fetal skin barrier defects. *Hum Mol Genet* 2008; **17**: 3075-83.

43 Zuo Y, Zhuang DZ, Han R *et al.* ABCA12 maintains the epidermal lipid permeability barrier by facilitating formation of ceramide linoleic esters. *J Biol Chem* 2008; **283**: 36624-35.

44 Yanagi T, Akiyama M, Nishihara H *et al.* Self-improvement of keratinocyte differentiation defects during skin maturation in ABCA12-deficient harlequin ichthyosis model mice. *Am J Pathol* 2010; **177**: 106-18.

45 Kelsell DP, Norgett EE, Unsworth H *et al.* Mutations in ABCA12 underlie the severe congenital skin disease harlequin ichthyosis. *Am J Hum Genet* 2005; **76**: 794-803.

46 Fischer J. Autosomal recessive congenital ichthyosis. *J Invest Dermatol* 2009; **129**: 1319-21.

47 Herman ML, Farasat S, Steinbach PJ *et al.* Transglutaminase-1 gene mutations in autosomal recessive congenital ichthyosis: summary of mutations (including 23 novel) and modeling of TGase-1. *Hum Mutat* 2009; **30**: 537-47.

48 Kuramoto N, Takizawa T, Matsuki M *et al.* Development of ichthyosiform skin compensates for defective permeability barrier

function in mice lacking transglutaminase 1. *J Clin Invest* 2002; **109**: 243-50.

49 Netherton EW. A unique case of trichorrhexis nodosa; bamboo hairs. *AMA Arch Derm* 1958; **78**: 483-7.

50 Wilkinson RD, Curtis GH, Hawk WA. Netherton's Disease; Trichorrhexis Invaginata (Bamboo Hair), Congenital Ichthyosiform Erythroderma and the Atopic Diathesis. A Histopathologic Study. *Arch Dermatol* 1964; **89**: 46-54.

51 Chavanas S, Bodemer C, Rochat A *et al.* Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat Genet* 2000; **25**: 141-2.

52 Weber F, Fuchs PG, Pfister HJ *et al.* Human papillomavirus infection in Netherton's syndrome. *Br J Dermatol* 2001; **144**: 1044-9.

53 Saghari S, Woolery-Lloyd H, Nouri K. Squamous cell carcinoma in a patient with Netherton's syndrome. *Int J Dermatol* 2002; **41**: 415-6.

54 Krasagakis K, Ioannidou DJ, Stephanidou M *et al.* Early development of multiple epithelial neoplasms in Netherton syndrome. *Dermatology* 2003; **207**: 182-4.

55 Renner ED, Hartl D, Rylaarsdam S *et al.* Comel-Netherton syndrome defined as primary immunodeficiency. *J Allergy Clin Immunol* 2009; **124**: 536-43.

56 Silverman GA, Bartuski AJ, Cataltepe S *et al.* SCCA1 and SCCA2 are proteinase inhibitors that map to the serpin cluster at 18q21.3. *Tumour Biol* 1998; **19**: 480-7.

57 Walz M, Kellermann S, Bylaite M *et al.* Expression of the human Cathepsin L inhibitor hurpin in mice: skin alterations and increased carcinogenesis. *Exp Dermatol* 2007; **16**: 715-23.

58 Yang T, Liang D, Koch PJ *et al.* Epidermal detachment, desmosomal dissociation, and destabilization of corneodesmosin in Spink5-/- mice. *Genes Dev* 2004; **18**: 2354-8.

59 Descargues P, Deraison C, Bonnart C *et al.* Spink5-deficient mice mimic Netherton syndrome through degradation of desmoglein 1 by epidermal protease hyperactivity. *Nat Genet* 2005; **37**: 56-65.

60 Hewett DR, Simons AL, Mangan NE *et al.* Lethal, neonatal ichthyosis with increased proteolytic processing of filaggrin in a mouse model of Netherton syndrome. *Hum Mol Genet* 2005; **14**: 335-46.

61 Curth HO, Macklin MT. The genetic basis of various types of ichthyosis in a family group. *Am J Hum Genet* 1954; **6**: 371-82.

62 Capetanakis J, Stratigos J, Tsambaos D *et al.* Ichthyosis hystrix of 'porcupine man' type. Report of a case. *Dermatologica* 1975; **151**: 177-83.

63 Judge MR, McGibbon DH. Ichthyosis hystrix and skin cancer. *Clin Exp Dermatol* 1994; **19**: 240-2.

64 Hendrix JD, Jr., Patterson JW, Greer KE. Skin cancer associated with ichthyosis: the MAUIE syndrome. *J Am Acad Dermatol* 1997; **37**: 1000-2.

65 Cheng J, Syder AJ, Yu QC *et al.* The genetic basis of epidermolytic hyperkeratosis: a disorder of differentiation-specific epidermal keratin genes. *Cell* 1992; **70**: 811-9.

66 Chipev CC, Korge BP, Markova N *et al.* A leucine----proline mutation in the H1 subdomain of keratin 1 causes epidermolytic hyperkeratosis. *Cell* 1992; **70**: 821-8.

67 Rothnagel JA, Dominey AM, Dempsey LD *et al.* Mutations in the rod domains of keratins 1 and 10 in epidermolytic hyperkeratosis. *Science* 1992; **257**: 1128-30.

68 Edwards JM, Cooper MA, Bannerjee S. Congenital epidermolytic hyperkeratosis associated with multiple malignancies. *Br J Dermatol* 1989; **120**: 141-4.

69 Happle R, Koch H, Lenz W. The CHILD syndrome. Congenital hemidysplasia with ichthyosiform erythroderma and limb defects. *Eur J Pediatr* 1980; **134**: 27-33.

70 Konig A, Happle R, Bornholdt D *et al.* Mutations in the NSDHL gene, encoding a 3beta-hydroxysteroid dehydrogenase, cause CHILD syndrome. *Am J Med Genet* 2000; **90**: 339-46.

71 Jacyk WK, La Cock A. Squamous cell carcinoma arising in CHILD syndrome. *J Eur Acad Dermatol Venereol* 2006; **20**: 311-3.

72 Smith FJ, Irvine AD, Terron-Kwiatkowski A *et al.* Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 2006; **38**: 337-42.

73 Palmer CN, Irvine AD, Terron-Kwiatkowski A *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006; **38**: 441-6.

74 Hagstromer L, Ye W, Nyren O *et al.* Incidence of cancer among patients with atopic dermatitis. *Arch Dermatol* 2005; **141**: 1123-7.

75 Arana A, Wentworth CE, Fernandez-Vidaurre C *et al.* Incidence of cancer in the general population and in patients with or without atopic dermatitis in the U.K. *Br J Dermatol* 2010; **163**: 1036-43.

76 Ming ME, Levy R, Hoffstad O *et al.* The lack of a relationship between atopic dermatitis and nonmelanoma skin cancers. *J Am Acad Dermatol* 2004; **50**: 357-62.

77 Naylor M, Elmets C, Jaracz E *et al.* Non-melanoma skin cancer in patients with atopic dermatitis treated with topical tacrolimus. *J Dermatolog Treat* 2005; **16**: 149-53.

78 Ames BN, Profet M, Gold LS. Nature's chemicals and synthetic chemicals: comparative toxicology. *Proc Natl Acad Sci U S A* 1990; **87**: 7782-6.

79 Milstone LM. Epidermal desquamation. *J Dermatol Sci* 2004; **36**: 131-40.

80 Chen J, Roop DR. Genetically engineered mouse models for skin research: taking the next step. *J Dermatol Sci* 2008; **52**: 1-12.

Clinico-pathologic Analysis of 66 Japanese Thin Melanomas with Metastasis of Sentinel or Regional Lymph Node

running title: Clinico-pathologic analysis of thin melanoma

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Background: Assessment of sentinel lymph node metastasis (LNM) is commonly performed for cutaneous melanomas. However, there are no definite guidelines for thin melanomas with Breslow's tumor thickness <1.0 mm, in part because thin melanomas are relatively infrequently positive for LNM.

Methods: We analyzed the clinico-pathologic relationship among tumor thickness, mitotic rate, tumor infiltrating lymphocytes (TILs), tumor size, regional LNM, and prognosis in 66 Japanese patients with thin melanomas. Immunohistochemical evaluations for TILs were also performed.

Results: Thirty-one of the 66 melanomas were Clark's level I without LNM (0/31, 0%). In tumors of Clark's level II or higher (35/66), there were five (14%) regional LNMs. Melanomas with two or more mitoses in 1 mm^2 per high-power fields showed higher frequencies of LNM (2/3, 67%), compared to those with fewer than two mitoses (3/32, 9%). Tumors with intensive TILs that partially or completely surrounded the tumor revealed higher frequencies of LNM (5/28, 18%), compared to those with none or slight TILs (0/7, 0%). The main components of TILs were CD8-positive T-lymphocytes. No metastasized tumors were under 2.0 cm^2.

Conclusions: The presence of mitotic activity, large tumor size and an intense lymphocytic infiltrate should prompt sentinel lymph node biopsy in thin melanomas.

Introduction

Sentinel lymph node biopsy (SLNB) for cutaneous melanomas of intermediate Breslow's tumor thickness (1.0–4.0 mm) has been widely accepted as a useful technique to determine the possibility of further metastasis and predict the prognosis.[1-3] However, there are no clearly defined indications for thin melanomas (tumor thickness ≤1.0 mm), because nodal metastasis in this category is relatively uncommon.[4-8] The incidence of positive sentinel lymph node (SLN) metastasis in melanomas of less than 1.0 mm thickness has been reported to range from 0% to nearly 8%.[1] Several reports showed that melanomas of less than 0.75 mm tumor thickness exhibited less positivity for SLN metastasis than those of 0.75–1.00 mm thickness. For thin melanomas with atypical features such as ulceration, regression and mitoses, SLNB is indicated.[1,4,8] The 7th edition of the American Joint Committee on Cancer (AJCC) melanoma staging system has recently declared that thin melanomas with mitotic activity are associated with a higher frequency of SLN metastasis; this statement is largely based on melanoma data in patient populations that are predominantly Caucasian. No similar evaluation has been performed in populations of Asian patients with thin melanoma. Therefore, the probability of SLN metastasis and the validity of SLNB for thin melanomas in Asian people remain controversial, because the prevalence and the predominant primary sites of melanomas differ between Asians and Caucasians. We studied Japanese thin melanoma cases to clarify the correlations among the histopathological findings, the rate of lymph node (LN) metastasis, and the prognosis and to discuss the validity of SLNB for thin melanoma.

Materials and methods

Patients

A database prospectively maintained at the Department of Dermatology of Nagoya University Hospital was queried for all patients with thin melanoma. A total of 66 patients, 28 males (28/66, 42%) and 38 females (38/66, 58%), with thin melanomas (≤1.0 mm thickness) including *in situ* melanomas (Clark's level I, 31 cases) were identified from 1998 to 2008, as shown in Tables 1 (*in situ*) and 2 (invasive). SLNB was performed in 21 cases (32%). The average age of the patients was 61 years old. The average observation period for the patients' progress was 55 months. Tumor size was computed by multiplying the major axis and the minor axis of the tumor plane.

There were seven cases that had SLNB even in Clark's level I, treated before 2007. In Japan, SLNB used to be carried out in suspicious cases of LN metastasis, including Clark's level I, because the indication of SLNB has not been clearly defined in acral lentiginous melanoma (ALM), the dominant melanomas in Japanese patients.

Sentinel lymph node biopsy

Until 2002, patients received only an SLNB procedure with blue dye injection. Since April 2002, preoperative lymphoscintigraphy, intraoperative blue dye, and a handheld gamma probe have been

used for all patients. In the SLNB procedure, ^{99m}Tc-Fytate colloid was injected into the dermis surrounding the primary melanoma 2–6 hours before the operation, and the location of the SLNs was determined by lymphoscintigrams 10 and 30 minutes after the injection. In the operating room, patients received a cutaneous injection of 2% patent blue around the tumor, and the SLNs stained with blue dye and with high concentration of the radioisotope colloid were easily identified intraoperatively by visual inspection and the handheld gamma probe, respectively. The removed SLNs were fixed in 10% formalin for pathological evaluation.[9,10] In each case, the pathological evaluation of the SLNs was performed using one H&E-stained slide of the maximum tumor slice and immunohistochemical staining of HMB45, S-100 and MART-1.

Microscopical analyses

The mitotic rate was defined as the number of mitoses in 1 mm^2, observed in the high-power field (HPF) of a light microscope. After counting the mitoses in the area with the most mitoses, the count is extended to adjacent fields until an area corresponding to 1 mm^2 is assessed. If no area can be clearly identified as having the most mitoses, and mitoses are sparse and randomly scattered throughout the lesion, then a representative mitosis is chosen; beginning with that field, the count is then extended to adjacent fields until an area corresponding to 1 mm^2 is assessed. In practice, the evaluation was performed as recommended in the 7th edition of AJCC melanoma staging system.[11,12]

Tumor infiltrating lymphocytes (TILs) are usually evaluated in the vertical growth phase (VGP) of melanoma using the terms "brisk", "non-brisk" and "absent". Because the degree of lymphocytic infiltrate was evaluated in the radial growth phase (RGP) in most cases, we used the following terminology: "intensely infiltrated type", or 2+, indicating lymphocytes infiltrating all around the tumor; "non-intensely infiltrated type", 1+, indicating lymphocytes infiltrating in a portion of the tumor; and –, indicating no or slight lymphocyte infiltration.

Histopathological regression was determined if a tumor had an area of epidermis without recognizable tumor in or adjacent to areas of obvious melanoma. Deep beneath the tumor-free epidermis, the papillary dermis is also free of tumor and usually widened because of delicate fibrous tissue with increased vascularity and scattered lymphocytes and melanophages.[13]

Immunostaining

In 16 of 35 cases in which the Clark's level was II or higher, immunohistochemical staining of CD3, CD4, CD5, CD8, CD20, CD56, TIA-1, Perforin, and FOXP3 was performed. TILs stained immunohistochemically with every marker were classified as 2+, +, or –, indicating that the percentage of positively stained cells was >60%, 30–60%, or <30%, respectively. These percentages were each compared with the non-stained control. All histopathologic evaluations were made by Dr. M.M. and Dr. M.S.

Statistical analyses

The relationship between each factor and LN metastasis was examined by Fisher's exact test (age,

sex, tumor site, melanoma subtype, Clark's level, mitoses, ulceration and TILs), by logistic regression analysis (tumor thickness) and by Wilcoxon signed-rank test (tumor size). All *P* values were considered statistically significant if the *p* value was less than 0.05. All statistical analyses were performed by using the SPSS software package (SPSS Inc, Chicago, IL).

Results

Tumor subtypes and metastasis

The numbers of patients with melanoma subtypes of acral lentiginous melanoma (ALM), superficial spreading melanoma (SSM), and lentigo maligna melanoma (LMM) were 39 (59%), 18 (27%) and nine (14%), respectively.

ALM, SSM and LMM with Clark's level II or higher revealed metastasis in two of 16 cases (13%), three of 16 cases (19%), and none of three cases (0%), respectively (Table 2). There was no statistical difference among the three subtypes ($P > 0.05$).

Tumor invasion and metastasis

In terms of the various histopathological levels of tumor invasion, at Clark's level I (*in situ*), II, III and IV, there were 31 (47%), 23 (35%, mean thickness 0.40 mm), nine (14%, mean thickness 0.77 mm) and three cases (5%, mean thickness 0.77 mm), respectively.

Ulceration is a microscopic attribute that has been correlated with the development of metastasis. Among all 66 tumors in this study, ulcer formation was observed in only one case (No. 66), which had no metastasis. We observed no cases of histopathological regression.

Five of the 35 patients with invasive melanoma developed metastatic melanoma. Four were diagnosed with clinically evident regional lymph node metastases shortly after diagnosis, and underwent complete therapeutic lymph node dissection rather than SLNB. One of the 14 patients with invasive melanoma who received SLNB had SLN metastasis (Figs. 1, 2). The patient with positive SLNB had a primary tumor thickness of 0.6 mm; the other four had thicknesses of 0.25, 0.3, 0.6 and 0.9 mm. All five primary tumors had 1–2+ lymphoid infiltrate. Mitoses were not identified in the primary tumors of the two patients who died (Table 2).

Tumor mitosis and metastasis

In patients with Clark's level II or higher, two (No. 33 and 35) out of 27 patients (2/27, 7%) with 0 mitosis/mm^2 showed LN metastasis. In cases with 1 mitosis/mm^2, one (No. 36) of five patients (1/5, 20%) exhibited metastasis. In cases with ≥2 mitoses/mm^2, two (No. 32 and 34) of three patients (2/3, 67%) exhibited metastasis. These data are summarized in Table 2. There was a significant difference in the metastasis rate between patients with <2 mitoses/mm^2 and those with ≥2 mitoses/mm^2 ($P < 0.01$).

Lymphocyte infiltration and metastasis

There were seven cases classified as TILs(−), out of 35 cases in which no LN metastasis was

recognized. On the other hand, two (No. 32 and 36) of 13 cases (2/13, 15%) classified as TILs(+)(non-intensely infiltrated type) had LN metastasis. In TILs(2+) cases (intensely infiltrated type), three (No. 33, 34 and 35) of 15 cases (3/15, 20%) had LN metastasis and two of them died of the disease (Table 2). There was a significant difference in the rate of metastasis between patients classified as TILs negative(−) and TILs positive(+ and 2+) ($P < 0.01$).

Variable factors and metastasis

The presence of intense TILs was associated with Clark's level (level II, P=0.01; level IV, borderline at P=0.07), with tumor thickness ($P=9\times10^{-6}$), and with mitotic rate (borderline, P=0.07).

In Table 3, we report P values of the relationship between each factor and LN metastasis, which were analyzed by Fisher's exact test (age, sex, tumor site, melanoma subtype, Clark's level, mitoses, ulceration and TILs), by logistic regression analysis (tumor thickness), and by Wilcoxon signed-rank test (tumor size). These findings demonstrate that mitotic rate and tumor size are more strongly associated with LN metastasis than TILs are. Tumor thickness and tumor site (trunk) showed borderline significance. Other factors (age, sex, melanoma subtypes, Clark's levels and ulceration) showed no statistically significant relationship with LN metastasis.

Immunostaining

Immunohistochemical staining for CD3, CD4, CD5, CD8, CD20, CD56, TIA-1, Perforin, and FOXP3 was further performed for TILs in 16 cases (Table 4). As shown in Table 5, the rate of positivity for T-cell markers CD3, CD5, and CD8 was dominant in both the LN metastasis positive case (No. 36) and negative cases.

TIA-1, which is expressed in the intracytoplasmic microsomes of cytotoxic T cells (CTL), was also dominantly stained in both groups (Tables 4, 5). Thus, no apparent statistical difference was recognized in TILs by immunohistochemical staining between cases with and without LN metastasis ($P > 0.05$).

Discussion

It is well known that melanomas in Asian and Caucasian populations differ in prevalence and other characteristics; in Asian patients, melanomas are relatively rare and highly ALM dominant.[14]

In 2010 AJCC melanoma staging system, mitotic rate replaced level of invasion as a primary criterion for defining T1b melanomas. Balch et al.[11] reported that in patients with localized melanoma, tumor thickness, mitotic rate (histopathologically defined as mitoses/mm^2), and ulceration were the most dominant prognostic factors. Nevertheless, these factors have been established from data derived from predominantly Caucasian populations. In this study, we evaluated these factors in thin melanomas in Japanese subjects, yielding results that could have greater practical importance for the treatment of early melanomas in Asian people.

Several studies have reported recurrences and fatal outcomes at various rates in patients with

melanomas ≤0.75 mm in thickness.[15,16] In 2002, AJCC revised the 6th edition of the melanoma staging system, with the definition of "thin melanoma" changing from lesions ≤0.75 mm to lesions ≤1.00 mm.[2] As we described in the results (Table 3), there were only borderline statistical differences between tumor thickness and LN metastasis in thin melanoma. This is likely because the precision of measuring tumor thicknesses less than 1.0 mm is limited by practical considerations. Therefore, it would be difficult to clearly determine that patients with thin melanoma of 1 mm or less in thickness would not require SLNB.

SLN metastasis at Clark's level II (≤1.0 mm) or higher occurred in one case among all SLNBs performed, a rate of 7% (1/14). This rate of LN metastasis positivity was almost the same as that reported by Wong et al.[17] in 2006.

On the contrary, mitotic activity was well correlated with LN metastasis (Table 3). When many mitoses are observed it is better to perform SLNB even in thin melanoma cases (≤1.0 mm). Several past studies reported that a high mitotic rate was an independent predictor of SLN metastasis[18-20], and our results support those of previous studies.[18-20] In addition, high mitotic activity is associated with an increased rate of melanoma recurrence.[21-25] Moreover, Scolyer et al.[26] and Gimotty et al.[27] emphasized that dermal or VGP mitotic rate was an important factor in localized primary cutaneous melanomas.

The five patients with LN metastasis in our study showed no ulcers. Thus, we cannot confirm the previous report that ulcer formation was a risk factor for metastasis in this time.

In this study, we could evaluate the factors of primary tumor site, tumor size and TILs in thin melanomas that would affect the prognosis of thin melanomas. Primary melanomas in the trunk showed higher association with LN metastasis than those in the finger, arm and toe (Table 3); however, there were no significant differences in this study. We expect that a larger study would help to reveal any relationship between tumor site and LN metastasis.

The median tumor size in non-metastatic tumors was 1.5 cm^2, vs. that in metastatic tumors, 9.0 cm^2. There were no metastasized tumors under 2.0 cm^2. Tumor size exhibited a stronger relationship with LN metastasis (Table 3). We expect that clinicians may be able to use these outcomes to decide about performing SLNB.

As shown in Table 2, all cases with LN metastasis had positive TILs in their primary tumors. The intensely infiltrated type with infiltration of TILs all around the tumor showed a little higher rate of LN metastasis (3/15, 20%) compared to the non-intensely infiltrated type (2/13, 15%).

The presence of intense TILs was significantly associated with Clark's level (level II is P=0.01) and tumor thickness ($P=9\times10^{-6}$). In our results, TILs were not statistically independent and seem to provide an overlapping prognostic factor.

In the original concept of TILs in invasive melanomas, when there is a brisk lymphocytic response[13], TILs are present throughout the VGP or are present across the entire base of the VGP. When there is

a non-brisk lymphocytic response[13], TILs are present in one or more foci of the VGP. Based on the concept of briskness, brisk-type TILs should be present at VGP. VGP tended to be recognized when tumor thickness was >0.76 mm, according to a report by Clark et al.[13] In our study, >0.76 mm thick invasive melanomas appeared in only seven cases. Because we rarely observed VGP in invasive thin melanoma, we referred to invasive thin melanomas with lymphocytes infiltrated all around the tumor as "intensely infiltrated type". Interestingly, patients with the intensely infiltrated type in the RGP in our study showed the highest rate of LN metastasis and death, although Clemente et al.[28] reported that patients with the brisk type in the VGP of advanced melanoma had the highest five-year survival rate. Our results suggest that TILs play different roles for the prognosis between RGP and VGP in invasive melanoma.

Our immunohistochemical staining of the specimens from 16 cases showed that the main cells in TILs were cytotoxic CD8(+) T lymphocytes (Tables 4, 5). We could not clarify further by comparing T-cell subtype in TILs between cases with and without LN metastasis, because we only had paraffin-embedded tumor specimen from one patient with LN metastasis. Immunohistochemical staining of TILs has rarely been employed in previous studies of thin melanoma, but further studies can be expected to clarify the role of TILs.

The population of melanoma subtypes in this study was clearly different from those in previous studies of thin melanomas in Caucasians, because ALM was predominant in Japanese thin melanomas. To date, evaluation of thin melanoma in Asian patients has rarely been performed. Therefore, we report here our preliminary study showing a significant association between mitotic rates and LN metastasis. These findings support the 2010 AJCC melanoma staging system.

In this study, both patients who died also had intense RGP infiltrates, and neither of these patients had undergone SLNB.

In conclusion, the presence of mitotic activity, large tumor size and an intense lymphocytic infiltrate in the RGP should prompt the consideration of SLNB in Japanese patients with thin melanomas.

References

1. Warycha MA, Zakrzewski J, Ni Q, et al. Meta-analysis of sentinel lymph node positivity in thin melanoma(≤1mm). Cancer 2009; 115: 869.
2. Balch CM, Buzaid AC, Soong SJ, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol 2001; 19: 3635.
3. Bleicher RJ, Essner R, Foshag LJ, Wanek LA, Morton DL. Role of sentinel lymphadenectomy in thin invasive cutaneous melamomas. J Clin Oncol 2003; 21: 1326.
4. Nahabedian MY, Tufaro AP, Manson PN. Sentinel lymph node biopsy for the T1(thin) melanoma: Is it necessary? Ann Plast Surg 2003; 50: 601.
5. Frahm SO, Schubert C, Parwaresch R, Rudolph P. High proliferative activity may predict early metastasis of thin melanomas. Hum Pathol 2001; 32: 1376.
6. Pontikes LA, Temple WJ, Cassar SL, et al. Influence of level and depth on recurrence rate in thin melanomas. Am J Surg 1993; 165: 225.
7. Büttner P, Garbe C, Bertz J, et al. Primary cutaneous melanoma. Optimized cutoff points of tumor thickness and importance of Clark's level for prognostic classification. Cancer 1995; 75: 2499.
8. McMasters KM, Reintgen DS, Ross MI, et al. Sentinel lymph node biopsy for melanoma: controversy despite widespread agreement. J Clin Oncol 2001; 19: 2851.
9. Tsutsumida A, Furukawa H, Yamamoto Y, et al. Sentinel node biopsy versus elective lymph node dissection in patients with cutaneous melanoma in a Japanese population. Int J Clin Oncol 2007; 12: 245.
10. Tsutsumida A, Furukawa H, Hata S, Saito A, Yamamoto Y. Prediction of metastases in melanoma patients with positive sentinel node: histological and molecular approach. J Dermatol 2007; 34: 31.
11. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 2009; 27: 6199.
12. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A 3rd, eds. AJCC Cancer Staging Manual, 7th edn. New York: Springer, 2009; 325.
13. Clark WH Jr., Elder DE, Guerry D 4th, et al. Model predicting survival in stage I melanoma based on tumor progression. J Natl Cancer Inst 1989; 81: 1893.
14. Nakano J, Muto M, Shimizu T, Hirota T, Ichimiya M, Asagami C. Ganglioside expression in melanomas from Japanese individuals: unusual pattern in two patients with metastatic lesions of acral lentiginous melanomas. Pigment Cell Res 1997; 10: 201.
15. Woods JE, Soule EH, Creagan ET. Metastasis and death in patients with thin melanomas (less than 0.76mm). Ann Surg 1983; 198: 63.
16. Slingluff CL Jr, Vollmer RT, Reintgen DS, Seigler HF. Lethal "thin" malignant melanoma.

Identifying patients at risk. Ann Surg 1988; 208: 150.
17. Wong SL, Brady MS, Busam KJ, Coit DG. Results of sentinel lymph node biopsy in patients with thin melanoma. Ann Surg Oncol 2006; 13: 302.
18. Kesmodel SB, Karakousis GC, Botbyl JD, et al. Mitotic rate as a predictor of sentinel lymph node positivity in patients with thin melanomas. Ann Surg Oncol 2005; 12: 1.
19. Ra JH, McMasters KM, Spitz FR. Should all melanoma patients undergo sentinel lymph node biopsy? Curr Opin Oncol 2006; 18: 185.
20. Thompson JF, Shaw HM. Is sentinel lymph node biopsy appropriate in patients with thin melanomas: too early to tell? Ann Surg Oncol 2006; 13: 279.
21. Mansson-Brahme E, Carstensen J, Erhardt K, Lagerlöf B, Ringborg U, Rutqvist LE. Prognostic factors in thin cutaneous malignant melanoma. Cancer 1994; 73: 2324.
22. Wagner JD, Gordon MS, Chuang TY, et al. Predicting sentinel and residual lymph node basin disease after sentinel lymph node biopsy for melanoma. Cancer 2000; 89: 453.
23. Schmid-Wendtner MH, Baumert J, Schmidt M, Plewig G, Volkenandt M, Hölzel D. Prognostic index for cutaneous melanoma: an analysis after follow-up of 2715 patients. Melanoma Res 2001; 11: 619.
24. Azzola MF, Shaw HM, Thompson JF, et al. Tumor mitotic rate is a more powerful prognostic indicator than ulceration in patients with primary cutaneous melanoma: an analysis of 3661 patients from a single center. Cancer 2003; 97: 1488.
25. Ranieri JM, Wagner JD, Wenck S, Johnson CS, Coleman JJ 3rd. The prognostic importance of sentinel lymph node biopsy in thin melanoma. Ann Surg Oncol 2006; 13: 927.
26. Scolyer RA, Thompson JF, Shaw HM, McCarthy SW. The importance of mitotic rate as a prognostic factor for localized primary cutaneous melanoma. J Cutan Pathol 2006; 33: 395.
27. Gimotty PA, Elder DE, Franker DL, et al. Identification of high-risk patients among those diagnosed with thin cutaneous melanomas. J Clin Oncol 2007; 25: 1129.
28. Clemente CG, Mihm MC Jr., Bufalino R, Zurrida S, Collini P, Cascinelli N. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer 1996; 77: 1303.

Table 1. Summary of 31 patients with *in situ* (Clark's level I) melanomas.

No	sex	age	tumor site	melanoma subtype	mitosis	SLNB	SLNM	F/U
1	M	46	trunk	SSM	0	No		107
2	F	54	face	LMM	0	No		129
3	M	52	finger	ALM	0	No		84
4	M	67	eyelid	LMM	0	No		2
5	M	71	toenail	ALM	0	Yes	−	59
6	M	66	sole	ALM	0	No		80
7	F	66	fingernail	ALM	0	No		24
8	F	77	sole	ALM	0	Yes	−	27
9	F	69	finger	ALM	0	No		69
10	M	72	toenail	ALM	0	No		61
11	F	28	fingernail	ALM	0	No		60
12	F	66	lip	LMM	0	No		58
13	F	80	fingernail	ALM	1	No		58
14	F	47	palm	ALM	0	No		48
15	F	51	face	LMM	0	No		11
16	F	65	sole	ALM	0	No		53
17	F	68	vulva	LMM	0	No		66
18	F	66	sole	ALM	1	Yes	−	29
19	M	67	toe	ALM	0	Yes	−	62
20	M	67	sole	ALM	0	No		58
21	M	56	sole	ALM	0	Yes	−	36
22	F	35	fingernail	ALM	0	No		36
23	M	57	trunk	SSM	0	No		41
24	M	24	sole	ALM	0	No		46
25	M	79	sole	ALM	0	No		25
26	M	63	sole	ALM	0	No		24
27	F	60	face	LMM	0	No		23
28	F	89	toenail	ALM	0	Yes	−	22
29	F	58	fingernail	ALM	0	No		22
30	F	44	toenail	ALM	0	Yes	−	21
31	F	71	sole	ALM	0	No		18

SSM, superficial spreading melanoma; ALM, acral lentiginous melanoma; LMM, lentigo maligna melanoma; mitosis, number of mitoses in $1mm^2$; SLNB, sentinel lymph node biopsy; SLNM, sentinel lymph node metastasis; F/U, follow-up period (months).

Table 2. Summary of the 35 patients with invasive (Clark' level II to IV) melanomas.

No	sex	age	tumor site	melanoma subtype	level	thick	mitosis	TILs *	SLNB	SLNM	RLM	DISM	F/U	status	tumor size (cm²)
32	M	76	finger	ALM	2	0.6	≥2	+	No		+		41	alive	2.25(1.5×1.5)
33	M	48	arm	SSM	2	0.25	0	2+	No		+	+	7	DOD	6.25(2.5×2.5)
34	F	55	trunk	SSM	3	0.6	≥2	2+	Yes	+			39	alive	20.0(5.0×4.0)
35	F	59	trunk	SSM	4	0.9	0	2+	No		+	+	5	DOD	9.0(3.0×3.0)
36	M	63	toe	ALM	2	0.3	1	+	No		+		122	alive	9.0(3.0×3.0)
37	F	60	neck	SSM	2	0.2	0	2+	No				3	Dead:cerebral hemorrhage	0.413(0.75×0.55), 0.25(0.5×0.5)
38	M	69	sole	ALM	2	0.25	0	2+	Yes	−			82	alive	4.0(2.0×2.0)
39	F	83	leg	SSM	2	0.3	0	+	No				94	alive	2.25(1.5×1.5)
40	F	56	arm	SSM	2	0.3	0	+	No				56	alive	0.96(1.2×0.8)
41	M	69	eyelid	LMM	2	0.4	0	+	No				82	alive	3.0(2.0×1.5)
42	F	58	leg	SSM	2	0.4	0	2+	No				79	alive	4.0(2.0×2.0)
43	F	80	face	SSM	2	0.4	0	+	No				61	alive	0.16(0.4×0.4)
44	F	62	sole	ALM	2	0.45	1	−	Yes	−			109	alive	1.5(1.5×1.0)
45	F	44	leg	SSM	2	0.5	0	2+	No				120	alive	3.0(2.0×1.5)
46	M	71	head	SSM	2	0.5	0	+	Yes	−			56	alive	1.0(1.0×1.0)
47	M	41	arm	SSM	4	0.5	0	2+	No				117	alive	1.0(1.0×1.0)
48	F	55	trunk	SSM	3	0.6	0	2+	No				88	alive	2.25(1.5×1.5)
49	F	43	leg	SSM	3	0.8	1	2+	No				118	alive	1.0(1.0×1.0)
50	M	35	arm	SSM	3	0.8	0	2+	No				25	alive	1.44(1.2×1.2)
51	F	49	arm	SSM	4	0.9	0	2+	No				118	alive	1.8(1.2×1.5)
52	M	65	finger	ALM	2	0.25	0	+	Yes	−			69	alive	2.0(2.0×1.0)
53	F	53	sole	ALM	2	0.3	0	+	No				15	Dead: lung cancer	9.0(3.0×3.0)
54	M	69	sole	ALM	2	0.35	0	2+	Yes	−			107	alive	6.25(2.5×2.5)
55	M	61	sole	ALM	2	0.35	0	−	Yes	−			70	alive	10.88(3.4×3.2)
56	F	74	sole	ALM	2	0.4	0	+	Yes	−			24	alive	0.5(1.0×0.5)
57	M	56	trunk	SSM	2	0.4	0	+	Yes	−			22	alive	3.0(2.0×1.5)
58	F	75	sole	ALM	2	0.5	0	−	Yes	−			67	alive	5.0(2.5×2.0)
59	F	66	face	LMM	2	0.55	0	−	No				51	alive	0.6(1.0×0.6)
60	M	66	finger	ALM	2	0.6	≥2	+	No				27	alive	3.52(2.2×1.6)
61	M	57	face	LMM	3	0.65	0	−	No				38	alive	1.0(1.0×1.0)
62	F	74	sole	ALM	3	0.7	0	+	Yes	−			22	alive	10.0(5.0×2.0)
63	F	60	fingernail	ALM	2	0.75	1	−	No				93	alive	0.4(0.8×0.5)
64	F	49	sole	ALM	3	0.8	1	2+	Yes	−			62	alive	0.72(0.6×1.2)
65	M	72	toenail	ALM	3	0.95	0	−	Yes	−			33	alive	1.0(1.0×1.0)
66	F	84	sole	ALM	3	1	0	2+	Yes	−			51	alive	1.5(1.0×1.5)

SSM, superficial spreading melanoma; ALM, acral lentiginous melanoma;
LMM, lentigo maligna melanoma; level, Clark's pathological level in primary tumor; thick, Breslow's tumor thickness (mm); mitosis, number of mitoses in $1mm^2$.
* TILs, tumor infiltrating lymphocytes; 2+, intensely infiltrated type (lymphocytes infiltrate all around the tumor.); +, non-intensely infiltrated type (lymphocytes infiltrate in a portion of the tumor.); −, none or slight lymphocytes.
SLNB, sentinel lymph node biopsy; SLNM, sentinel lymph node metastasis;
RLM, regional lymph node metastasis; DISM, distant metastasis;
F/U, follow-up period (months); DOD, dead of disease.

Table 3. Statistical relationships between various factors and lymph node metastasis.

Factors	*P* value
Age	0.86
Sex	
Male	0.64
Female	0.64
Tumor site	
Finger	0.33
Arm	0.33
Trunk	0.06
Toe	0.14
Melanoma subtype	
ALM	0.39
SSM	0.12
LMM	1
Clark's level	
II	0.33
III	0.53
IV	0.21
Mitoses	0.005
Ulceration	1
TILs	0.01
Tumor thickness	0.07
Tumor size	<0.001

ALM, acral lentiginous melanoma; SSM, superficial spreading melanoma; LMM, lentigo maligna melanoma; TILs, tumor infiltrating lymphocytes.