Identifying Melanocyte in Pigmented Skin Lesions Based on *In Vivo* Third Harmonic Generation Microscopy

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Poster Session

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Abstract

The identification of melanocytes is important in making diagnosis of melanocytic lesions. However, differentiation of melanocytes and intraepidermal Langerhans cells (ILC) is difficult for the common used in vivo microscopy. In our previous study, we successfully took the advantage of the third harmonic generation (THG)-enhanced nature of melanin, and used in vivo multi-harmonic generation microscopy (HGM) to achieve high diagnostic accuracy in non-melanoma pigmented skin tumors. Thus, the unique THG-identified characteristic of melanocytes is now investigated and introduced to differentiate melanocytes from melanocyte-mimicking ILCs. In the current study we examined several pigmented skin lesions, including the pathological diagnosis of malignant melanoma, pigmented basal cell carcinoma (BCC), seborrheic keratosis (SK), and melanocytic nevus, by using HGM in vivo and ex vivo. Histopathological correlations of the HGM images were made by comparing with hematoxylin and eosin (H&E) and immunohistochemical (IHC) staining with HMB45 and CD1a which indicated the origin of cell with dendritic processes an melanocyte or a ILC, respectively. The result showed that the majority of THG-bright cells with dendritic processes were melanocytes but not ILCs in pigmented skin lesions. Based on the current study, the newly characterized melanocytes identified by THG, which are not able to be identified in traditional histopathological sections, play unique features in various pigmented skin tumors based on our in vivo HGM. This optical diagnostic technique can be beneficially applied to clinical decision and management in the future. This study is sponsored by National Health Research Institute.

Introduction

Melanin

- Protects cells from physical damage and related to cutaneous diseases
- Hypomelanotic disease (vitiligo) may be resulted from impaired melanocyte migration, differentiation or melanosome abnormalities
- Hypermelanotic disorders (melasma), may be caused by abnormal proliferation of melanocytes
- Assist the diagnosis of pigmented skin diseases, including melanoma
- Melanocytes in a pagetoid pattern or spread is an important histopathologic criterion for melanoma diagnosis
- Early diagnosis reduce mortality and morbidity
- The identification of melanocytes are essential in making diagnosis of melanocytic lesions

Previous Study

- Seldom investigated in non-melanocytic pigmented skin tumors
- Reflectance confocal microscopy (RCM) displays bright cells in a pagetoid pattern
- But sometimes represent intraepidermal Langerhans cells (ILC)

Harmonic Generation Microscopy (HGM)

- The third harmonic generation (THG)-enhanced nature of melanin
- *In vivo* HGM achieves high diagnostic accuracy in non-melanoma pigmented skin lesions (basal cell carcinoma, seborrheic keratosis (SK), and nevocellular nevus)
- Submicron spatial resolution could be achieved (lateral <0.5 μm in superficial layers and <0.7 μm at a 270 μm depth)
- The unique THG-identified characteristic of melanocytes is now investigated and introduced to differentiate melanocytes from melanocyte-mimicking ILCs

Materials and Methods

Patients Selection

- 17 patients, 5 ex vivo / 12 in vivo
- 34 to 85 y/o
- melanoma / pigmented BCC / SK / nevus

HGM

- Cr:forsterite laser / 1230 nm / pulse width fs / repetition rate of 110 MHz / output average power of 500 mW
- ≤ 30 min, accumulated energy < 180 J in each volunteer

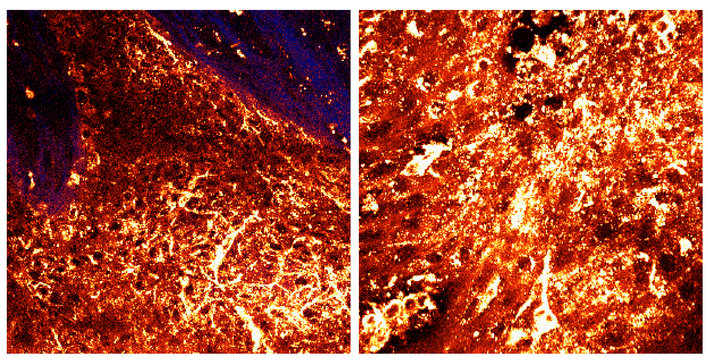
Pathological / IHC study

- H&E staining
- IHC staining - HMB-45 (melanocyte) / CD1a (ILC)
- Suprabasal / basal / dermal layer

Data Analysis

- Retrospectively analyzed by two independent physicians
- Blinded to participant name, sex, age, and diagnosis
- HGM data were compared with the H&E and IHC standard

Result



HGM Feature of Melanocyte

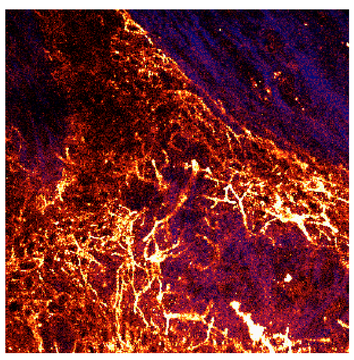
Elongated cells with long dendritic processes, with extremely strong THG signal, enlarged cell volume, irregular shape with several dendritic processes, the cells can be distinguished from surrounded keratinocytes

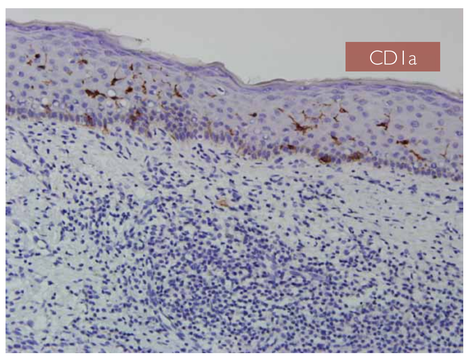
Results of Comparison

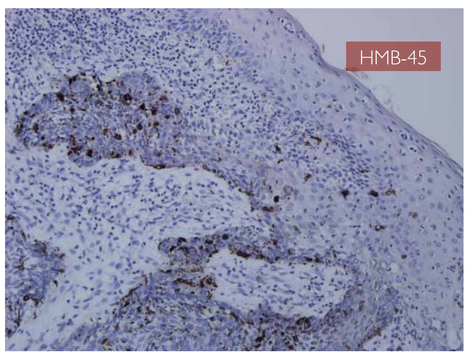
A total of 17 pigmented lesions from 17 patients were evaluated by HGM and histopathologic examination. Clinical, histopathologic, IHC and HGM results of lesions are shown in the Table.

	Suprabasal	Basal	Dermis
Melanoma	1/1(100%)	1/1(100%)	1/1(100%)
BCC	2-3/8 (25-37.5%)	8/8 (100%)	0/6 (0%)
SK	0/4 (0%)	0/4 (0%)	0/4 (0%)
Nevus	0/4 (0%)	1/4 (25%)	2/4 (50%)

	HGM		HMB-45		CD1a	
Case	Suprabasal	Basal	Suprabasal	Basal	Suprabasal	Basal
1	+	+	+/-	2+	1+	1+
2	+/-	+	+/-	2+	2+	2+
3	-	+	-	1+	2+	-
4	-	+	-	1+	1+	-
5	-	+	-	2+	1+	-
6	+/-	+	-	2+	1+	-
7	-	+	-	2+	1+	-
8	-	+	-	2+	2+	1+







Complications

- No erythema, pigmentation, or blister formation on the examined skin was found
- No evidence of photodamage, such as coagulation necrosis, was found under pathological examination

Conclusion

The result showed that the majority of THG-bright cells with dendritic processes were melanocytes rather than ILC under the comparison of HGM images, H&E, and IHC staining in the cases of malignant melanoma, pigmented BCC, SK and melanocytic nevus. Thus the newly characterized melanocytes identified by THG, which are not able to be identified in traditional histopathological sections, play unique features in various pigmented skin tumors based on our in vivo HGM.

Reference

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