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

Wei-Hung Weng, M.D.1,2, Ming-Rung Tsai1, Yi-Hua Liao, M.D.1,2, and Chi-Kuang Sun1,3,4

1 Molecular Imaging Center, National Taiwan University, Taipei 10617, Taiwan.  
2 Department of Dermatology, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei 10002, Taiwan  
3 Department of Electrical Engineering and Graduate Institute of Photonics and Optoelectronics, National Taiwan University, Taipei 10617, Taiwan.  
4 Institute of Physics and Research Center for Applied Sciences, Academia Sinica, Taipei 11529, Taiwan

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 as a 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.

Materials and Methods

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
- HGM data were compared with the H&E and IHC standard

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.

<table>
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<tr>
<th>Patients Selection</th>
<th>17 patients, 5 ex vivo / 12 in vivo</th>
<th>34 to 85 y/o</th>
<th>melanoma / pigmented BCC / SK / nevus</th>
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| 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 |

| 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