Identifying Active Melanocyte Based on In Vivo Harmonic Generation Microscopy

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[ Ministry of Science and Technology | TAIWAN ]
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Clinical Scenario

- When I was a medical student...

- Stratum corneum
- Stratum granulosum
- Stratum spinosum
- Stratum basale

- Melanosoma
- Keratinocytes
- Melanocyte

www.promocell.com
Clinical Scenario

• When I was a resident in pathology...
Why Active Melanocyte?

• Producing melanin
• Endogenous marker for making diagnosis of melanocytic lesions

Segura. Arch Dermatol. 2007

• Various diseases
  – Hypermelanosis (melasma, solar lentigo)
  – Hypomelanosis (vitiligo, albinism)
  – Melanoma
    • Most fatal skin cancer

ACS Cancer Facts & Figures 2014
Question

- Can I see melanocytes, or even active melanocytes with dendrites and melanin clearly by in vivo skin scanning, but without time-consuming invasive biopsy?
Current Pitfall

• Identification of melanocytes and Langerhans cells (LC)
  – Difficult for the common used *in vivo* microscopy such as confocal microscopy

Why Harmonic Generation?

- THG-enhanced nature of melanin
  Chen *IEEE J Sel Topics Quantum Electron*. 2010

- Melanin quantification
  Tsai. *Focus On Microscopy*. 2014

- HGM has high diagnostic accuracy in non-melanoma pigmented skin tumors
Medical Fact

- Dendritic cells in skin
  - 2 types
  

1. Active melanocyte
   - HMB-45+ in melanocyte
   - Focal area
   - Pagetoid spread in melanoma, nevi (no atypia)
2. Langerhans cell
   – CD1a+ (also in cortical thymocytes)
   – Superficial epidermis
     \textit{Agero. J Am Acad Dermatol. 2006}
   – In stratum spinosum
     \textit{Fitzpatrick's Dermatology in General Medicine. 2008}
Study Protocol

**Case |** 17 patients, 6 ex vivo / 11 in vivo, 34 to 85 y/o melanoma (1) / pigmented BCC (8) / SK (4) / nevus (4)

**HGM |** Cr:forsterite laser / 1230 nm / pulse width 100 fs / repetition rate of 110 MHz / output 500 mW ≤ 30 min, accumulated energy < 180 J in each volunteer

2-3 sections with the most **dendritic cell-like** signal

**Tissue Prove |** By IHC staining
HMB-45 (active melanocyte) / CD1a (LC)
Observe suprabasal / basal layer and lesion

**Interpretation and Analysis |** By two doctors
HGM vs. IHC / statistical analysis
• Agreement between 2 observers
  – Dermatologist and pathologist
  – Near 100% in more than 700 training images
  – Inconsistent data ➔ Discard
Dendritic Cell Identification

- Dermis
- Basal layer
- Spinous layer
- Basal layer
Evaluation of Special Staining

Melanocyte
HMB-45

Langerhan’s cell
CD1a
Summary of Results

• Dendritic cell-like signal in THG
  – Suprabasal 17.6%, basal and lesion 58.8%
  – 100% in basal layer and lesion of BCC

• HMB-45 (melanocyte)
  – Suprabasal 11.8%, basal and lesion 64.7%
  – 100% in basal layer and lesion of BCC

• CD1a (Langerhan’s cell)
  – Suprabasal 100%, basal and lesion 17.6%
Suprabasal Layer

Basal Layer

Dermal Layer
Good Consistency

- Consistency test for reliability
  - Dendritic cell signal vs. active melanocyte
    - Kappa statistic = 0.807 → Good agreement
  - Dendritic cell signal vs. Langerhan’s cell
    - Kappa statistic = -0.339 → Poor agreement

- Dendritic cells in HGM are more related to active melanocyte but not Langerhans’ cell
High Sensitivity and Specificity

- **Statistics**
  - Dendritic cell signal vs. active melanocyte
    - Accuracy = 90.9%
    - Sensitivity = 95.0%
    - Specificity = 84.6%
    - PPV = 90.5%
    - NPV = 91.7%

- THGM has high accuracy to detect active melanocytes
Discussion

• First study of *in vivo* imaging demonstrate **active melanocyte** clearly by THGM
  ➔ Potential clinical application (Teledermatology / Telemedicine)
### Comparison

<table>
<thead>
<tr>
<th></th>
<th>Melanocyte</th>
<th>Langerhan’s cell</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Third Harmonic Generation</strong></td>
<td>Active – clear!</td>
<td>No</td>
</tr>
<tr>
<td><strong>Reflectance Confocal</strong></td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Segura. *Arch Dermatol.* 2007
Limitation

- Small case numbers
- Histopathological validation by melasma, vitiligo or albinism (usually no biopsy)
Conclusion

Thank you for your attention!

Active Melanocyte

Dendritic-like THG bright cells in THGM image

THG enhancement nature of melanin

Histopathological and IHC proved

Potential of making differential diagnosis of cutaneous lesions

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Sun Lab | Molecular Imaging Center | National Taiwan University | Taiwan

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Langerhan’s cell
CD1a
**Harmonic Generation**

- Dendritic cell-like THG signal presents

<table>
<thead>
<tr>
<th></th>
<th>Suprabasal</th>
<th>Basal Lesional</th>
<th>Dermis</th>
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<tbody>
<tr>
<td>Melanoma</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td>BCC</td>
<td>2/8 (25%)</td>
<td>8/8 (100%)</td>
<td>0/8 (0%)</td>
</tr>
<tr>
<td>SK</td>
<td>0/4 (0%)</td>
<td>0/4 (0%)</td>
<td>0/4 (0%)</td>
</tr>
<tr>
<td>Nevus</td>
<td>0/4 (0%)</td>
<td>1/4 (25%)</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td></td>
<td>Suprabasal</td>
<td>Basal Lesional</td>
<td></td>
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<td>----------------</td>
<td>------------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td><strong>Melanoma</strong></td>
<td>1/1 (100%)</td>
<td>1/1 (100%)</td>
<td></td>
</tr>
<tr>
<td><strong>BCC</strong></td>
<td>1/8 (12.5%)</td>
<td>8/8 (100%)</td>
<td></td>
</tr>
<tr>
<td><strong>SK</strong></td>
<td>0/4 (0%)</td>
<td>0/4 (0%)</td>
<td></td>
</tr>
<tr>
<td><strong>Nevus</strong></td>
<td>0/4 (0%)</td>
<td>2/4 (50%)</td>
<td></td>
</tr>
</tbody>
</table>

HMB-45 (Melanocyte)
<table>
<thead>
<tr>
<th></th>
<th>Suprabasal</th>
<th>Basal Lesional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>1/1 (100%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>BCC</td>
<td>8/8 (100%)</td>
<td>2/8 (25%)</td>
</tr>
<tr>
<td>SK</td>
<td>4/4 (100%)</td>
<td>0/4 (0%)</td>
</tr>
<tr>
<td>Nevus</td>
<td>4/4 (100%)</td>
<td>1/4 (25%)</td>
</tr>
</tbody>
</table>
Look into 8 BCC Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>THG</th>
<th>HMB-45</th>
<th>CD1a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SB</td>
<td>B/L</td>
<td>SB</td>
</tr>
<tr>
<td>1 (S01)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2 (S02)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3 (S03)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4 (S11)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5 (S12)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6 (P13)</td>
<td>few</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7 (P33)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8 (P39)</td>
<td>-</td>
<td>+</td>
<td>-</td>
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</table>
## Other Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>THG</th>
<th>HMB-45</th>
<th>CD1a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SB</td>
<td>B/L</td>
<td>SB</td>
</tr>
<tr>
<td>9 (S04)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10 (P07)</td>
<td>-</td>
<td>-</td>
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<tr>
<td>11 (P34)</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12 (P42)</td>
<td>-</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>13 (P43)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14 (P17)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15 (P31)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16 (P36)</td>
<td>-</td>
<td>-</td>
<td>1+</td>
</tr>
<tr>
<td>17 (P38)</td>
<td>-</td>
<td>-</td>
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</tr>
</tbody>
</table>
Case of Vitiligo
Melanin Quantification

- **Melanosome in melanocyte**
  - Membrane-bound organelle where melanin biosynthesis takes place
  - Eumelanosomes and pheomelanosomes
  - Eumelanin or pheomelanin

- **Eumelanin**
  - Dominant component of human epidermal melanin
  - More than 90% of total epidermal melanin

Alaluf 2002
Melanin Quantification by HGM

1. THG and TPEF *in vivo* imaging of mouse melanocytes
   - Correlation between THG and TPEF

2. Imaging different concentration of melanin solution by 2PF
   - Correlation between melanin concentration and TPEF
   - TPEF = 68 * melanin concentration + 390.8

3. Bridging two correlations and get the empiric correlation between melanin concentration and THG enhancement ratio

Liu 2013
## HGM criteria for differential diagnosis of non-melanoma pigmented skin lesions

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity / CI (%)</th>
<th>Specificity / CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basal cell carcinoma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral palisading cells</td>
<td>95 (84-99)</td>
<td>100 (96-100)</td>
</tr>
<tr>
<td>Proliferation of polymorphous basaloid cells</td>
<td>98 (88-100)</td>
<td>90 (82-95)</td>
</tr>
<tr>
<td>Elongated cells/nuclei</td>
<td>88 (75-95)</td>
<td>92 (85-96)</td>
</tr>
<tr>
<td>Collagen changes</td>
<td>67 (52-79)</td>
<td>98 (92-99)</td>
</tr>
<tr>
<td><strong>Nevus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monomorphous cell nests</td>
<td>98 (90-100)</td>
<td>96 (89-99)</td>
</tr>
<tr>
<td>Normal epithelial stratification</td>
<td>98 (90-100)</td>
<td>88 (80-94)</td>
</tr>
<tr>
<td>Elongation of rete ridges</td>
<td>74 (61-84)</td>
<td>81 (71-88)</td>
</tr>
<tr>
<td><strong>Seborrheic keratosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proliferation of monomorphous melanocytic cells</td>
<td>97 (83-99)</td>
<td>98 (93-99)</td>
</tr>
<tr>
<td>Acanthotic epidermis</td>
<td>97 (83-99)</td>
<td>100 (96-100)</td>
</tr>
</tbody>
</table>
Sensitivity and specificity for differential diagnosis of three kinds of pigmented tumors

- Based on these HGM features with histopathological diagnostic scales
  - Overall sensitivity is 92% and specificity is 96% for direct judgment
  - Overall sensitivity is 94% and Specificity is 100% for presence of 2 criteria

<table>
<thead>
<tr>
<th></th>
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<th>Specificity / CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Direct judgment</td>
<td>Presence of 2 criteria*</td>
</tr>
<tr>
<td>BCC</td>
<td>95 (72-99)</td>
<td>93 (69-98)</td>
</tr>
<tr>
<td>Nevi</td>
<td>93 (72-98)</td>
<td>96 (77-99)</td>
</tr>
<tr>
<td>SK</td>
<td>87 (58-94)</td>
<td>93 (64-99)</td>
</tr>
</tbody>
</table>

*Two criteria are with the highest sensitivity and specificity*