Rapid visualization of nonmelanoma skin cancer

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Background: Mohs micrographic surgery examines all margins of the resected sample and has a 99% cure rate. However, many nonmelanoma skin cancers (NMSCs) are not readily amenable to Mohs micrographic surgery. This defines an unmet clinical need to assess the completeness of non-Mohs micrographic surgery resections during surgery to prevent re-excision/recurrence.

Objective: We sought to examine the utility of quenched activity-based probe imaging to discriminate cancerous versus normal-appearing skin tissue.

Methods: The quenched activity-based probe GB119 was applied to NMSC excised from 68 patients. We validated activation of the probe for hematoxylin-eosin–confirmed cancerous tissue versus normal-appearing skin tissue.

Results: Topical application of the probe differentiated basal cell carcinoma and squamous cell carcinoma from normal-appearing skin with overall estimated sensitivity and specificity of 0.989 (95% confidence interval 0.940-1.00) and 0.894 (95% confidence interval 0.769-0.965), respectively. Probe activation accurately defined peripheral margins of NMSC as compared with conventional hematoxylin-eosin–based pathology.

Limitations: This study only examined NMSC debulking excision specimens. The sensitivity and specificity for this approach using final NMSC excision margins will be clinically important.

Conclusions: These findings merit further studies to determine whether quenched activity-based probe technology may enable cost-effective increased cure rates for patients with NMSC by reducing re-excision and recurrence rates with a rapid and easily interpretable technological advance. (J Am Acad Dermatol 2017;76:209-16.)

Key words: cathepsin-B; cathepsin-L; molecular optical imaging; nonmelanoma skin cancer; quenched activity-based probe; re-excision rate; topical application.

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This study was supported by an R21 (5R21CA183160-02) and P30 AR039750 award (Dr Basilion) and a subcontract to Dr Basilion from 5R21CA183160-02 awarded to Dr Straight of Akrotome Imaging Inc. The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

Disclosure: Drs Basilion and Bogyo are both board members and co-founders of Akrotome Imaging Inc. Dr Straight is the chief executive officer of Akrotome Imaging Inc. Drs Walker, Mann, Honda, Vidimos, Schluchter, and Popkin have no conflicts of interest to declare.

The abstract of this study was presented orally at the World Molecular Imaging Congress; Honolulu, Hawaii; September 2-5, 2015.

eFigures and eTable are available at http://www.jaad.org.

Accepted for publication September 2, 2016.

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Published online November 19, 2016.

0190-9622/$36.00

http://dx.doi.org/10.1016/j.jaad.2016.09.008

Nonmelanoma skin cancer (NMSC) is the most common form of human cancer with approximately 3.5 million cases diagnosed in the United States in 2006 and approximately 4 million diagnosed each year hence.[1,2] Approximately 80% of all NMSC are basal cell carcinomas (BCC) with most of the remaining being squamous cell carcinomas (SCC).[3] Several approaches to ensure complete removal of diseased tissue with minimal resection of normal tissue have been developed, including Mohs micrographic surgery (MMS)[4,5] that checks margins during surgery and has the highest cure rate of all approaches (99%). However, conventional or non-MMS resection is the most common surgical approach for skin cancers representing approximately 75% of surgical procedures for BCC and SCC[1] and has much lower cure rates than MMS. Well-differentiated SCC cure rates approach 81% using non-MMS procedures but decreases to 46% for poorly differentiated lesions.[6] Resections of BCC fare slightly better with approximately 93% cure rates.[7] Because rates are related to the size of margins that the dermatologist removes, conventional resections generally result in larger resections of normal tissue to ensure clear margins, which are determined by conventional pathology several days after the surgery.[8,9] Here we describe an easily interpretable methodology that could be cost-effectively deployed and rapidly executed to enable surgeons to better assess peripheral surgical margins, thus reducing the amount of normal tissue resected during conventional NMSC excisions.

The study presented exploits molecular imaging technology[10,11] to target overexpressed skin cancer-associated cathepsin proteases.[12-16] The technique uses a cysteine cathepsin selective class of near-infrared fluorescent quenched activity-based probes (QABPs)[17] effective for imaging tumor-expressed cathepsins, in vitro and in vivo,[18-22] which are hydrophobic and readily penetrate the skin. Interaction of QABPs with target proteases results in de-quenching and production of a fluorescent signal. Here, GB119 is used to target cancer cysteine cathepsin enzymes to discriminate NMSC from normal-appearing skin ex vivo creating a 2-dimensional (2D) fluorescent map for each sample. Specifically, ex vivo topical administration of this probe is used to develop a standardized method to differentiate cancer from normal-appearing skin in excised human skin cancer specimens.

CAPSULE SUMMARY

- Quenched activity-based probes have been used to localize brain cancer.
- Rapid fluorescent quenched activity-based probe visualization technique highlights areas positive for hematoxylin-eosin–defined nonmelanoma skin cancer with great accuracy.
- Quenched activity-based probes may rapidly and easily identify residual nonmelanoma skin cancers during excision.

METHODS

Collection of human skin specimens

This study was approved by the University Hospitals Case Medical Center Institutional Review Board (protocol #12-05-17). Discarded skin tissues containing previously diagnosed BCC or SCC were taken during debulking for MMS (eTable I) for this prospective case series. All BCC/SCC debulk specimens greater than or equal to 1 × 1 cm were processed, imaged, and analyzed.

Ex vivo imaging of human skin specimens

Freshly removed debulk specimens were covered with saline-soaked gauze and delivered to the laboratory 90 to 120 minutes after surgery. Samples were rinsed with saline, blotted dry with gauze, followed by baseline imaging (0 minutes). Probe GB119 (10 μmol/L in dimethyl sulfoxide) (Sigma-Aldrich, St Louis, MO) was applied to the dermal side. After 5 minutes, excess probe was rinsed from the sample with saline and the dried sample was imaged in the Maestro imaging system (PerkinElmer, Waltham, MA) with a near-infrared filter set. Two sectioning approaches were used (Graphical abstract, Step 4). In the breadloaf section, ink was used to mark the location of fluorescence before signals sectioning.

Analysis of Cy5-fluorescence

Fluorescence was measured and pseudo-color images were derived using Maestro and supplied software 3.0.1.2 (PerkinElmer). Levels of autofluorescence (0 minutes) were subtracted from fluorescence at 5 minutes with GB119 and remaining signal intensity was pseudo-colored. Signal was mapped onto the surface of the samples and inked in some cases. Samples were immersed in optimal cutting temperature compound, snap-frozen, and kept at −80°C for further study.

Histologic and immunofluorescent analysis of human skin samples

Frozen blocks were sectioned (10 μm) in a breadloaf manner at −30°C (Leica-CM3050S).