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Identification of Novel Gene Classifiers to Non-Invasively Diagnose Non-Melanoma Skin Cancer

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Abstract

Non-melanoma skin cancers (NMSC) are the most common types of skin cancer and include both basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). NMSC primarily form on sun exposed skin including the head, face, neck, arms, and hands. BCC accounts for >75% of NMSC cases; however, SCC is more aggressive and may occur in other locations as well. Combined, BCC and SCC are responsible for >15,000 deaths each year in the US alone, which exceed deaths due to melanoma. Current diagnosis of NMSC relies on an in-depth visual assessment of the lesion in question followed by a surgical skin biopsy for histopathologic review. This analysis investigated whether the non-invasive collection of skin tissue with 'smart stickers' and subsequent genomic analysis could properly classify NMSC. Adhesive skin collections kits were used to collect the lesional skin from 58 patients with BCC, 41 patients with SCC, and 42 patients with non-cancerous skin diseases. Whole transcriptomic analysis was conducted on each sample and differentially expressed genes were determined by comparing BCC and/or SCC with non-cancerous skin disease (other) using multiple comparisons. Eighteen genes were significantly (fold change >1.5; p<0.1) increased in BCC compared to other skin diseases while 14 genes were increased in SCC (fold change >1.5; p<0.1). Further analysis identified 12 genes that were differentially expressed in both lesional BCC and lesional SCC compared to other skin diseases. These results require further investigation but suggest that "smart sticker" enabled non-invasive skin sampling and genomic analysis may provide an opportunity to identify patients with NMSC earlier and without the need for surgical biopsy.

Methods

Subjects

Subjects at least 18 years of age with clinically suspected basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) lesions were enrolled in this study. Non-invasive skin samples were collected from all enrolled subjects using the DermTech adhesive skin collection kit (DermTech, Inc.; La Jolla, CA) as described below. Additionally, skin biopsies were collected from all enrolled subjects for histopathological confirmation. The study was reviewed and approved by Aspire IRB (Santee, CA). All subjects provided written consent prior to enrollment. The breakdown of histopathologically confirmed subjects is provided in Table 1.

Table 1. Breakdown of Histopathologically Confirmed Subjects

Basal Cell Carcinoma	Squamous Cell Carcinoma	Other*
N=58	N=41	N=42

*Subjects were classified as "other" if histopathology confirmed their diagnosis as a non-cancerous skin disease including but not limited to seborrheic keratosis, actinic keratosis, verruca vulgaris, and plaque psoriasis

Skin Sampling

The DermTech adhesive skin collection kit (DermTech, La Jolla, CA) was used to collect skin samples from lesional skin and nearby non-lesional skin. Prior to application of the Smart Sticker™ (Figure 1), the target skin was prepped with an alcohol pad to remove oils and then dry wicked with a gauze pad to remove any remaining moisture. Each kit contains a total of 4 Smart Stickers™ for sample collection. Smart Stickers™ were applied individually to the designated area and 5 circular motions with the thumb were used to ensure adhesion to the targeted skin. Using a pen, small marks were made on the skin after placement of the first tape-strip to ensure consistent placement of each subsequent tape-strip. Smart Stickers™ were removed slowly using standard precautions to prevent folding and eliminate potential sources of contamination. Once removed, the Smart Sticker™ was then placed onto the tri-fold sample collector. This process was repeated with the 2nd, 3rd, and 4th Smart Sticker™ on the same lesional/non-lesional skin. Once all 4 Smart Stickers™ were in the tri-fold collector, the collector was carefully folded and placed in a re-sealable bag for overnight shipment (the Smart Sticker™ and tri-fold collector are stable for 10 days at room temperature). Upon arrival at DermTech, samples were stored at -70ºC or colder until RNA extraction.

RNA Extraction and Gene Expression Analysis

RNA was extracted from Smart Stickers™ using a closed-tube, bead-based method. Briefly, Smart Stickers™ were enzymatically digested to extract the genomic material from the adhesive and nucleic acid isolated using magnetic beads on a kingfisher flex instrument (ThermoFisher Scientific). The whole transcriptomic analysis library was prepared in house using the 'SMART-Seq® Stranded Kit' (Takara Bio), and all sequencing was performed on the Illumina HiSeq x Ten.

Statistical Analysis

The identification of differentially expressed genes among the disease types was performed by the R package "DeSeq2". P values were adjusted for multiple comparisons by the Benjamini-Hochberg method. Among the 54k genes analyzed, using an adjusted P-value threshold of 0.1. Top genes with chosen based on sorted adjusted P values. Dotplots and heatmaps were based on the top genes.

Top Genes Differentiating Basal Cell Carcinoma From Other Skin Diseases

Figure 1. Principal component analysis of samples collected from lesional skin of BCC (n=58) and Other non-cancerous skin diseases (n=42)

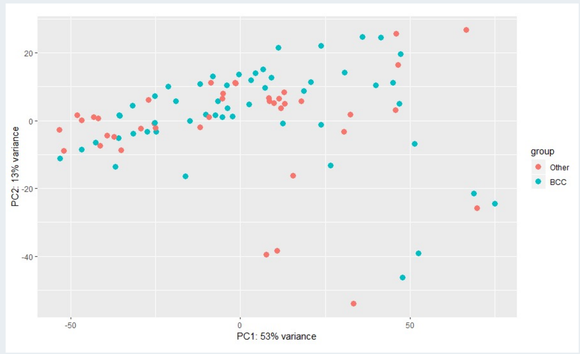


Figure 2. Heat map of gene expression of BCC (n=58) compared with other non-cancerous skin diseases (n=42) .

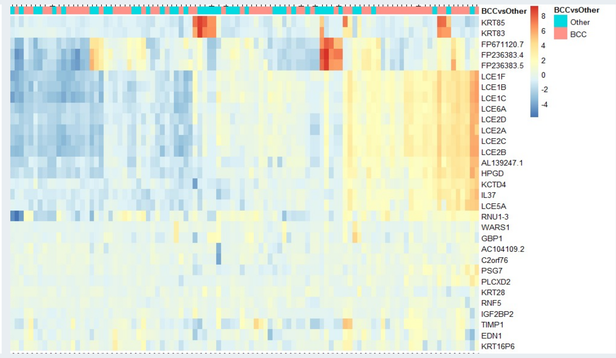
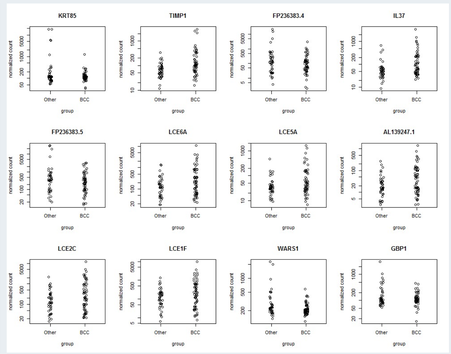


Figure 3. Top 12 genes capable of classifying BCC versus non-cancerous skin disease



Top Genes Differentiating Squamous Cell Carcinoma From Other Skin Diseases

Figure 4. Principal component analysis of samples collected from lesional skin of SCC (n=41) and Other non-cancerous skin diseases (n=42)

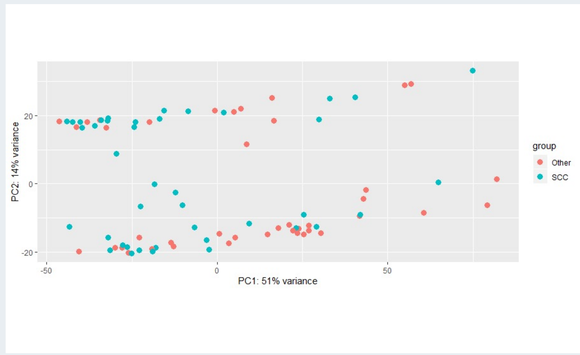


Figure 5. Heat map of gene expression of SCC (n=58) compared with other non-cancerous skin diseases (n=42) .

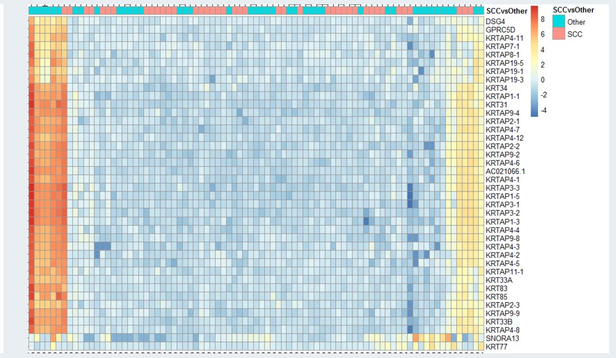
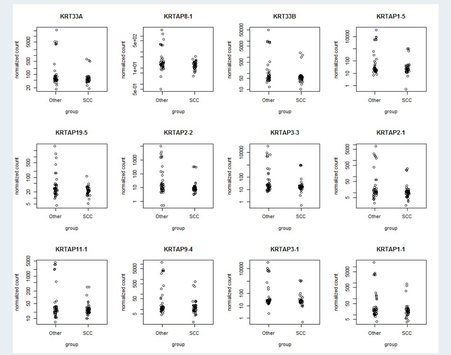


Figure 6. Top 12 genes capable of classifying SCC versus non-cancerous skin disease



Conclusions

- **Approximately 1/3 of subjects enrolled in this study were incorrectly clinically diagnosed as BCC or SCC, suggesting the importance of additional objective assessments in ruling out non-melanoma skin cancer prior to a surgical biopsy**
- **Non-invasively assessing BCC or SCC lesions identified gene signatures capable of distinguishing non-melanoma skin cancer from non-cancer skin inflammation**
- **Additional studies would provide further characterization of BCC and/or SCC lesions to aid in diagnosis while refining biomarker signatures**

Disclosures

C Adase, J McGhee, T Holscher, M Kim, Z Yao, J Rock, B Jansen, and MD Howell are employees and shareholders at DermTech, Inc. M. Walker and J Shi are biostatistical consultants for DermTech have nothing additional to disclose.

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