Non-invasive tape sampling reveals a type I interferon RNA signature in cutaneous lupus erythematosus that distinguishes affected from unaffected and healthy volunteer skin

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Introduction

Type I interferon genes (IFN-I) are upregulated in skin lesions and blood from patients with cutaneous lupus erythematosus (CLE)[1-3]. Punch biopsy, the standard diagnostic procedure, impedes patient recruitment and follow up due to risk of infection, discomfort and cosmetic scarring. This study assessed the feasibility of an adhesive tape device from DermTech, Inc. to collect RNA from affected lesions and unaffected skin (Fig. 1) in subjects with CLE, atopic dermatitis (AD) or healthy volunteers (HV), and its potential to detect gene expression differences between groups.

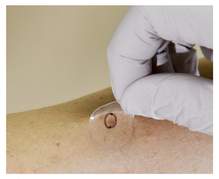


Figure 1: Sampling with the DermTech, Inc. device.

Hypothesis

A non-invasive adhesive tape device can be used to sample IFN-I and other genes from keratinocytes and immune cells associated with CLE pathogenesis to distinguish CLE affected (lesional) from unaffected (non-lesional) and from healthy volunteer skin.

Methods

- **Subjects enrolled**: Adult subjects with active discoid lupus erythematosus (DLE; n=9) or subacute CLE (SCLE; n=1) with or without systemic LE, active AD (n=4) and HV (n=10) were enrolled. The study was approved by the local IRBs.
- **Biomarkers sampled**: Whole blood (WB), skin tape from affected (CLE-A) and unaffected (CLE-U) skin, and punch biopsy from affected (CLE-A) skin.
- **RNA amplification and protein quantification**: RNA was extracted from tape using standard DermTech, Inc protocols. Gene expression was quantified by qPCR on the OpenArray platform. Immunohistochemistry (IHC) for Myxovirus protein A (MXA), an interferon-regulated protein, was conducted on formalin fixed paraffin embedded punch biopsies. MXA protein was quantified with custom-designed algorithms in Visiopharm, Inc. software.
- **Statistics**: Descriptive statistics were calculated for demographics and disease characteristics by disease groups. To accommodate for missing RNA data the following steps were taken: 1. bridging step using samples run in 2 separate assay batches; 2. Linear mixed effects model to predict gene set score under a missing at random assumption; and 3. Gene-wise single-imputation via multivariate, unsupervised random forests. Gene set scores were computed as a mean of log expression (-ddCt). Housekeeping genes and the mean of dCt results for HV subjects were used as ddCt calibrators. Linear mixed models were fitted to log2 transformation gene set scores with subjects as random effects. P-values from multiple comparisons were adjusted using Tukey approach. Pearson's correlations and associated p values were calculated to determine the associations of biomarkers from skin tape RNA and IHC based on skin biopsy.

Gene set	Genes amplified from RNA sampled with tape and whole blood (WB, abbreviated IFN-I set only)
IFN-I	*IFI44, IFI6, RSAD2, USP18, MX1, IFI27, OAS1, OAS2, XAF1, EPSTI1, CMPK2, SIGLEC1, IFI44L, OAS3, ISG15, LYE6E, TRANK1, HERC5, PLSCR1, IFIT1, RTP4, IFIT3, DDX58, GALM, IFITM3, TRIM38, TRIM56, MOV10, SPATS2L*
IFN-I Abbreviated[1]	*IFI44, RSAD2, MX1, USP18, IFI27, IFI6, CMPK2, OAS1, EPSTI1, OAS3, HERC5, IFIT1, ISG15, LYE6E, PLSCR1, IFIT3, RTP4, DDX58, UBE2L6, LAMP3, LIPA, TIMM10*
CD8 CTL[2]	*FAS, GZMB, PRF1*
CLE upregulated	*OASL, GNLY, AIM2, CD163, CXCL13, KLRK1, IL10RA, CXCL10, IGJ, ICAM1, MMP1, VCAM1, IL1B, MR1, KRT6A, HLA-DRB1, S100A7*
pDC[3]-related	*STAT1, CD123/IL3RA, CD69, IL15, CD86, CD207, KLRD1, CMKLR1, TCF4, LILRA4*
pan T-cell	*CCL2, IL7R, CD8A, CD3D, SERPINB3, CASP1*
Keratinocyte	*SERPINB4, S100A8, S100A9*

1. 22-Gene abbreviated set used in Figure 2; 2. CTL, cytotoxic T-cell; 3. pDC, plasmacytoid dendritic cell

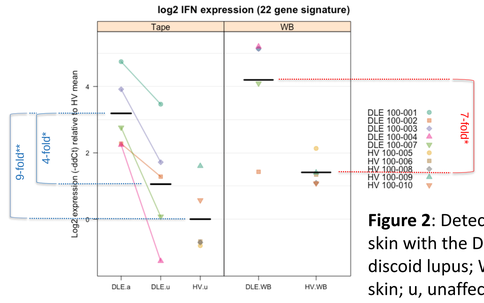
RESULTS

Table 1: Subject Characteristics

	HV	CLE		AD
		DLE	SCLE	
Subject Number	10	9	1	4
Age mean (SD)	55.7 (5.4)	56.0 (12.0)	58.0	47.5 (15.9)
Gender n (%)				
Female	5 (50)	7 (78)	1 (100)	3 (75)
Race n (%)				
American Indian/Alaskan Native	1 (10)	0	0	0
Asian	0	0	0	0
Black or African American	0	3 (33)	0	3 (75)
White	9 (90)	6 (67)	1 (100)	1 (25)
Concomitant SLE per ACR criteria n	N/A	3	0	N/A
Concomitant medication n (%)	0	7 (78)	1 (100)	1 (25)
Hydroxychloroquine	0	5 (56)	1 (100)	0
Prednisone	0	2 (22)	0	1 (25)
Corticosteroid NOS	0	3 (33)	0	0
Clobetasol propionate	0	2 (22)	0	0
Fluocinonide	0	1 (11)	0	0
Hydrocortisone	0	0	0	1 (25)
Mycophenolate mofetil	0	1 (11)	1 (100)	0
Tacrolimus (topical)	0	0	1 (100)	0
Disease Activity				
PGA mm Mean	N/A	13.3	20	N/A
CLASI-A Mean	N/A	11.8	11	N/A

AD: Atopic Dermatitis; CLASI-A: Cutaneous Lupus Area and Severity Index – Activity; CLE: Cutaneous Lupus Erythematosus; DLE: Discoid Lupus Erythematosus; PGA: Physician Global Assessment; N/A: Not applicable

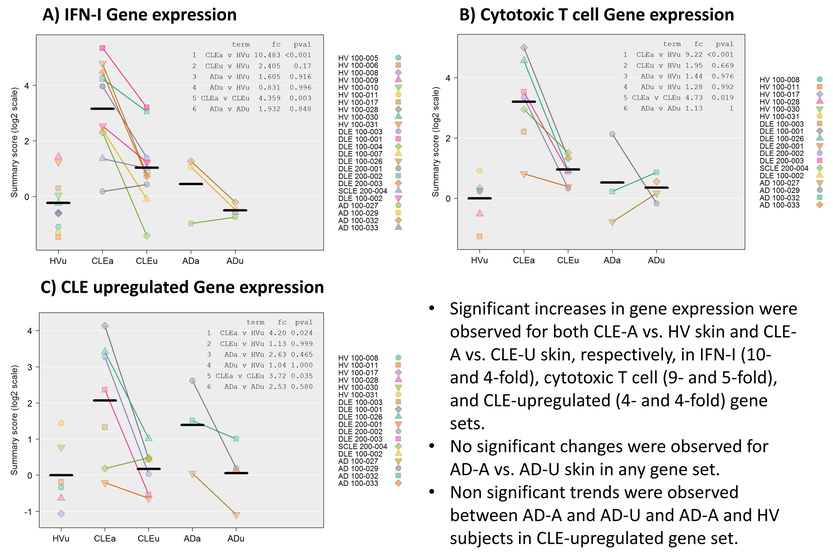
Differential expression of IFN-I genes, detected with the DermTech tape device, is more pronounced in skin than in whole blood



- Differential expression for an abbreviated (22-gene) IFN-I gene set between DLE-A and HV was more pronounced in skin (9-fold) than in whole blood (7-fold).
- In this comparison, 1/5 subjects had concurrent SLE.

Figure 2: Detection of IFN-I gene expression in skin with the DermTech, Inc. tape device. DLE, discoid lupus; WB, whole blood; a, affected skin; u, unaffected skin; HV, healthy volunteer.

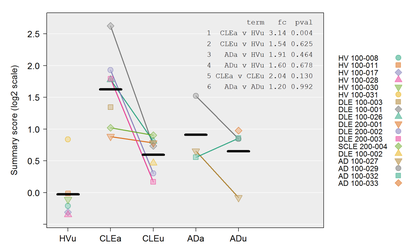
Increased gene expression in CLE-A vs. HV and CLE-A vs. CLE-U in IFN-I, cytotoxic T cell and CLE-upregulated gene sets



- Significant increases in gene expression were observed for both CLE-A vs. HV skin and CLE-A vs. CLE-U skin, respectively, in IFN-I (10- and 4-fold), cytotoxic T cell (9- and 5-fold), and CLE-upregulated (4- and 4-fold) gene sets.
- No significant changes were observed for AD-A vs. AD-U skin in any gene set.
- Non significant trends were observed between AD-A and AD-U and AD-A and HV subjects in CLE-upregulated gene set.

Figure 3: Gene expression in CLE-A, CLE-U, AD and HV for gene set in A) IFN, B) Cytotoxic T cell and C) CLE upregulated gene sets.

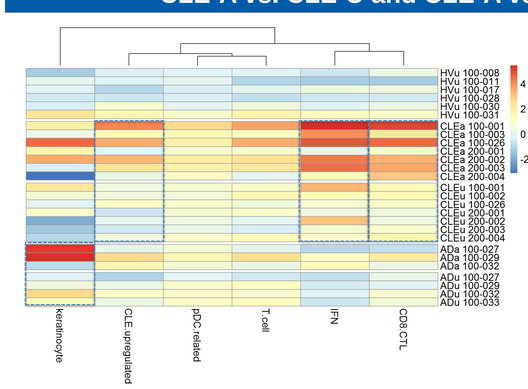
Upregulated gene expression in CLE-A vs. HV in pDC- related gene set



- Plasmacytoid dendritic cell related genes were upregulated (3-fold) for CLE-A vs. HV.
- Non significant trends were observed for AD vs. HV.

Figure 4: Gene expression in CLE-A, CLE-U, AD and HV for plasmacytoid dendritic cell related gene set.

IFN-I, cytotoxic T cell and CLE-upregulated gene sets allow the greatest discrimination between CLE-A vs. CLE-U and CLE-A vs. HV



CLE-A vs CLE-U:
- Relatively strong differential signals in IFN-I and CD8 cytotoxic T cell gene sets.
- Intermediate signal in CLE-upregulated gene set.

AD-A vs AD-U:
- Differential expression for AD is strongest in the keratinocyte gene set.

Figure 5: Heatmap of each gene set showing differential expression relative to the mean of HV.

MXA, an interferon regulated protein, is increased in skin of CLE but not AD subjects

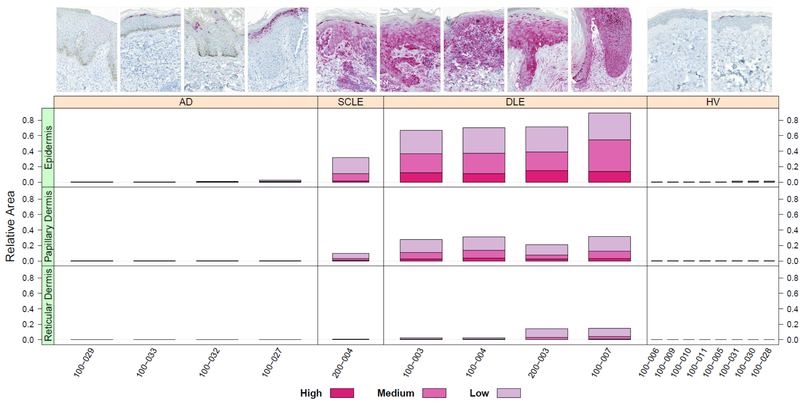
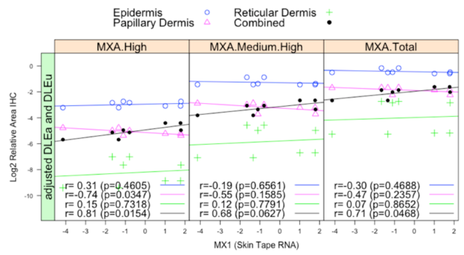


Figure 6: MXA IHC in skin biopsies of AD, SCLE, DLE and HV. MXA immunoreactivity per skin region is measured in three intensity bins using color saturation thresholding; high, medium and low. Y-axis, % area MXA immunoreactivity; X-axis, subject number.

IHC MXA protein correlates with *MXA* RNA measured by tape sampling and amplification



- *MXA* RNA expression from affected and unaffected DLE skin tape samples correlates with high intensity and total MXA protein by IHC when epidermal and dermal skin regions are combined.

Figure 7: Correlation of *MXA* RNA with MXA protein expression from affected and unaffected DLE skin tape samples. A single SCLE subject is excluded as its inclusion of this subject results in a non-significant correlation (data not shown). RNA amplified from skin tape is scale-adjusted by normalizing to distance from mean of CLE-A or CLE-U, respectively. r: Pearson correlation; Y axis: IHC values for MXA in affected skin, MXA.High: highest bin based on colorimetric thresholding, MXA.Medium.High: medium and high bins, MXA.Total: low, medium and high bins; X axis: RNA expression from affected and unaffected skin.

Conclusions

- RNA from the skin surface distinguishes CLE-A from HV skin and CLE-A from CLE-U skin with robust fold changes in genes implicated in CLE pathogenesis including cytotoxic T cells and IFN-I.
- Differential expression of genes expressed by cytotoxic T/NK cells (*GZMB*, *GNLY*), macrophages/monocytes (*SIGLEC1*) and pDCs/B cells/monocytes (*IRF7*) indicates that signals from inflammatory cells, in addition to those from keratinocytes, can be detected with the dermtech tape device.
- Gene sets related to pan T cells or keratinocytes were unchanged in CLE (data not shown).
- No significant changes were observed for AD skin.
- *MXA* RNA, sampled by tape, correlates with MXA protein measured in combined epidermis and dermis in punch biopsies.
- This non-invasive technique offers promise for the diagnosis, stratification and monitoring of patients with lupus-specific dermatologic disease.

Limitations

- Statistical power was hampered by challenges with enrollment, particularity of ACLE/SCLE subjects.
- RNA sample quality was variable such that 2 samples were omitted from analysis and 18/36 remaining samples were missing at least one important IFN gene. Statistical techniques implemented to accommodate for missing data are described in the methods section.

References

1. A. Jabbari et al. (2014) *J Invest Derm*. **134**: 87-95. 2. R. Dey-Rao & A.A. Sinha (2015) *Genomics.* **105**:90-100. 3. A.A. Sinha & R. Dey-Rao (2017) *J Invest Derm.* **18**:S75-S80.

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Conflict of Interest Disclosures

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