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Combined Use of Laser Capture Microdissection and cDNA Microarray Analysis Identifies Locally Expressed Disease-Related Genes in Focal Regions of Psoriasis Vulgaris Skin Lesions

Hiroshi Mitsui[1], **Mayte Suárez-Fariñas**[1,2], **Daniel A. Belkin**[3], **Natasha Levenkova**[2,4], **Judilyn Fuentes-Duculan**[1], **Israel Coats**[1], **Hideki Fujita**[1], and **James G. Krueger**[1]

[1]Laboratory for Investigative Dermatology, The Rockefeller University, New York, New York, USA

[2]Center for Clinical and Translational Science, The Rockefeller University, New York, New York, USA

[3]Department of Dermatology, Weill Cornell Medical College, New York, New York, USA

[4]Hospital Informatics, The Rockefeller University, New York, New York, USA, Address: 1230 York Avenue, New York, NY, 10065-6399, USA

Abstract

Psoriasis vulgaris is a complex disease characterized by alterations in growth and differentiation of epidermal keratinocytes as well as marked increase in leukocyte populations. Lesions are known to contain alterations in mRNAs encoding more than 1000 products, but only a very small number of these transcripts have been localized to specific cell types or skin regions. In this study, we used laser capture microdissection (LCM) and gene array analysis to study the gene expression of cells in lesional epidermis and dermis, compared with corresponding non-lesional resions. Using this approach, we detected >1800 differentially expressed gene products in the epidermis or dermis of psoriasis lesions. These results established sets of genes that are differentially expressed between epidermal and dermal compartments, as well as between non-lesional and lesional psoriasis skin. One of our findings involved the local production of CCL19, a lymphoid organizing chemokine, and its receptor CCR7 in psoriatic dermal aggregates, along with the presence of gene products LAMP3/DC-LAMP and CD83, which typify mature DCs. Gene expression patterns obtained with LCM and microarray analysis along with T cell and DC detection by immune staining suggest a possible mechanism for lymphoid organization via CCL19/CCR7 in diseased skin.

Correspondence author: Dr. James G. Krueger, Address: 1230 York Avenue, New York, NY, 10065-6399, USA, Phone: 1-212-327-7730, Fax: 1-212-327-8232, jgk@mail.rockefeller.edu.

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Introduction

Valuable insights into the pathogenic processes of various skin diseases can be obtained by studying differentially expressed genes (DEGs) via cDNA microarray analysis. However, whole-tissue analysis has limitations, including its inability to localize mRNAs to specific cell types. In addition, gene products specific to “minority” cell types may be lost by dilution with mRNAs from more dominant cell types. Our group developed cellular genomic maps of the skin by using in vitro cultured cells of various types to localize in vivo gene changes to specific cell types (Haider et al. 2008). However, there are important differences between cultured cells and their in vivo counterparts. For example, keratinocytes undergo two differentiation programs in vivo - homeostatic growth and regenerative maturation - while cultured keratinocytes reflect predominantly the regenerative phenotype (Mansbridge and Knapp 1987; Kennedy-Crispin et al. 2012). Additionally, cultured keratinocytes are less differentiated than their in vivo counterparts. Inflammatory skin disease like psoriasis and atopic dermatitis also contain several types of dendritic cells (DCs) that cannot be found in normal skin and blood and are distinct from those differentiated from monocytes in vitro with specific cytokines.

Given that psoriasis vulgaris is a complex disease involving distinct cells and tissues (marked alterations in growth, differentiation, and patterning of the epidermis as well as tissue infiltration by T cells, DCs, and other myeloid leukocytes), it would be desirable to localize disease-related genes specifically to the epidermis or immune components. Numerous prior studies have used bulk-tissue homogenates to establish major differences in gene expression between diseased and non-diseased states (Bowcock et al. 2001; Zhou et al. 2003; Yao et al. 2008; Suárez-Fariñas et al. 2010; Gudjonsson et al. 2010). Although specific studies differ in total number of DEGs, the psoriasis transcriptome has consistent alterations in >1000 transcripts between lesional and non-lesional skin. Only a relatively small number of DEGs has been localized to specific cells or regions of psoriasis lesions in prior studies, e.g., by immune staining for protein products.

We therefore sought to expand our understanding of cell/region-specific molecular changes in psoriasis vulgaris through the use of laser capture microdissection (LCM) combined with cDNA microarray analysis. LCM is a technique used to isolate subpopulations of cells from tissue sections under direct microscopic visualization (Epsina et al. 2006), but its application to gene expression analysis has been limited by the difficulty of recovering sufficient amounts of RNA for whole transcriptome analysis (Clément-Ziza et al. 2009). There has also been the concern that multiply amplified mRNA products may not reflect the correct abundance of transcripts as detected by single amplification methods used in whole tissue analysis. We chose to perform regional gene expression analysis on psoriasis, because 1) prior bulk-tissue studies provide a strong reference gene set for comparison to LCM-derived transcripts and 2) distinct cellular and genomic pathways should exist within epidermal and dermal/immune tissue compartments. Our results established marked differences in gene expression between epidermis and dermis of non-lesional psoriasis skin as well as between diseased epidermis and dermis of lesional skin. Specifically, leukocyte-rich dermal inflammatory regions in psoriasis lesions contained high-level expression of a number of genes, as well as corresponding proteins, that direct lymphoid organization and structure.

This suggests a mechanism for the formation and persistence of T cell mediated inflammation in diseased skin. Extensive experiments presented in supplementary results and discussion (SRD) evaluate potential technical limitations of LCM methodology that must be considered before it can be applied widely to other skin diseases.

Results

LCM localizes genes selectively expressed in non-lesional epidermis and dermis

We performed LCM to collect cells in the epidermis (EPI), papillary dermis (PD), reticular dermis (RD), and dermal inflammatory cell aggregates (ICs) in frozen sections of skin, as shown in Figure S1c-d. The potential quality of RNA of our samples and the efficacy of the double amplification are summarized in Figure S1a-b and S1e. We also assessed the linearity of double amplification in Figure S1f. The PCA-plot and heatmap (Figure S2a-b) illustrating unsupervised clustering of expression values for all samples clearly show that there are no outliers and that the major source of variation in the samples is the sample type (i.e. Epidermis, Dermis, and Bulk), even when samples from the same patient are used.

To evaluate the specificity of LCM to localize gene products, we compared gene expression profiles of EPI and RD in non-lesional skin. In EPI, 1151 probe-sets were up-regulated and 1471 were down-regulated. Table 1a-b show the top 25 up- and down-regulated DEGs, respectively. Known keratinocyte-related genes such as KRT1, KRT2, KRT5, DSC1, and DSC3, as well as melanocyte-related genes TYRP1 and DCT, were found in EPI. In RD, we found higher expression of collagens (COL3A1 and COL6A3), immune related genes (CXCL12, IGHA1, and LYZ), and vascular endothelial cell and leukocyte transendothelial migration-related genes (SPARCL1, PECAM1, and VCAM1). We can thus clearly separate gene expression in different regions of the skin combining LCM and cDNA microarray analysis. The complete list of DEGs between EPI and RD of non-lesional skin is shown in Table S1.

LCM localizes disease-related DEGs in psoriasis epidermis

Gene expression profiles between lesional and non-lesional EPI samples were compared. Table 2a-b present the top 25 up- and down-regulated genes in the EPI-related psoriasis transcriptome, respectively. Up-regulated genes include those related to hyperproliferation (KRT6A, KRT6B, and KRT16), epidermal differentiation complex (S100 family proteins and small proline-rich proteins), and proteolysis regulation molecules (SERPINs and PI3). These results are consistent with previous works using bulk-tissue samples, but earlier studies were not able to specify the cells with altered gene expression. Among down-regulated genes, we examined the protein expression of CRIP-1 and CCL27 (Table 2b) using immunohistochemistry (IHC). Both proteins were detected in non-lesional epidermis but were decreased in lesional epidermis, confirming our microarray results (Figure 1a-b).

We also focused our attention on transcription factors (TFs) identified in the EPI transcriptome. TFs that are considered to be involved in keratinocyte proliferation (NFE2L3, MAFF, and EHF) were up-regulated in lesional EPI, whereas those thought to be involved

in keratinocyte differentiation (LASS6, TFAP2B, and GATA3) were down-regulated. The complete gene lists of EPI transcriptome and TFs are found in Tables S2 and S3.

LCM localizes genes selectively expressed in dermal inflammatory cell aggregates of psoriasis lesions

Psoriasis lesions contain dense aggregates of LAMP3/DC-LAMP+ DCs, marking mature DCs, that are intermixed with T cells in the dermis, but gene expression in this “lymphoid” area has not been investigated. Table 3 presents the top 25 up-regulated genes in RD/ICs. Interestingly, six of the 25 were genes encoding chemokines or chemokine receptors, including CCL19 and its receptor CCR7. Genes associated with immune response, such as LAMP3/DC-LAMP, CD28, GZMB, and CD83 are also on this list. The complete list is available in Table S4.

Confirmation by quantitative RT-PCR (qRT-PCR) for EPI and RD transcriptomes were performed on six specific mRNAs as shown in Figure S3a. There is a high correlation between differential expressions detected by the double amplification based microarray and qRT-PCR ($r=0.87$, $p=0.0002$, Figure S3b).

Protein expression of detected genes in dermal aggregates was confirmed by IHC and immunofluorecent staining (IF)

To further confirm the differential expression of certain targets, tissue sections were examined by IHC.

STAT1 stained in the cytosol of cells in non-lesional epidermis and in the nucleus of cells in lesional epidermis. In addition, STAT1+ cells were increased in lesional dermis compared to non-lesional dermis (Figure 1c). CCR7 and CCL19 both stained in dermal aggregates of lesional skin (Figure 1d-e). CCL21, another ligand for CCR7, was not detected by IHC, consistent with our microarray data, where expression of CCL21 was quite low. CXCL13/BCA-1 was detected in lesional dermis (Figure 1g). CXCR5, a receptor for CXCL13/BCA-1, and CD20, B cell marker, were also positive in lesional dermis (Figure 1h-i). Collectively, differences in gene expression of these molecules obtained from microarray analysis using LCM samples correlated very well to differences in their protein expression in tissues.

To further explore the nature of chemokines and relevant receptors in skin tissues, we performed double-label IF. CCR7 and CCL19 were expressed in close proximity in lesional dermal aggregates (Figure 2a). CCR7 co-stained with LAMP3/DC-LAMP and the T cell marker CD3 (Figure 2b-c). CCL19 co-stained with myeloid DC marker CD11c, LAMP3/DC-LAMP, and CD3 (Figure 2d-f). CXCL13/BCA-1 and CXCR5 were also expressed in close proximity in lesional dermal aggregates (Figure 2g). CXCR5+ cells co-stained with CD20 (Figure 2h). These results demonstrate that dermal aggregates in psoriasis, mainly composed of DCs, T cells, and to a lesser degree B cells, were associated with the coordinated expression of lymph node organizing chemokines and their receptors.

LCM detects more DEGs than bulk-tissue analysis

A more detailed discussion can be found in SRD section 2. The psoriasis transcriptome, defined as the DEGs between lesional and non-lesional samples using FCH>2.0 and FDR<0.1 cut-offs, was determined for bulk skin samples (psDEGs-Bulk), laser-captured EPI (psDEGs-EPI), and laser-captured RD (psDEGs-RD), which tends to include ICs in lesional samples. psDEGs-Bulk contained 155/58 (up-/down-regulated) probe-sets (Table S5). psDEGs-EPI contained 815/631 probe-sets (Table S2), and psDEGs-RD contained 309/181 probe-sets (Table S4). The psoriatic transcriptome for PD was not defined because of an insufficient amount of RNA obtained from non-lesional PD (Figure S1c). Scatter-plots show positive correlations between Log2FCH of Bulk and those of EPI and RD (Figure 3a-b). Correlation between Bulk and EPI was higher ($r=0.68$, $p<1\times10^{-16}$) than that between Bulk and RD ($r=0.31$, $p<1\times10^{-16}$), suggesting a greater contribution of EPI-related genes than RD-related genes in Bulk analysis. Importantly, trend lines (red lines in Figure 3a-b) were shifted towards larger FCH in LCM samples compared to Bulk samples. In fact, 76.47% of probe-sets with absolute FCH>2.0 in both EPI and Bulk showed higher FCH in EPI than Bulk, and for RD it was 58.61% (Figure 3c and S2d, see SRD section 2-i). These results may reflect the overall increase in DEGs detected via LCM compared to bulk-tissue analysis (Figure 3d-e). We acknowledge that, as we expected, many fewer genes were detected in our Bulk analysis compared to previously published studies with larger patient cohorts (n=26, Yao et al. 2008; n=15, Suárez-Fariñas et al. 2010; n=58, Gudjonsson et al. 2010, see a discussion of the effect of sample size in SRD section 2-ii). We further compared our uniquely detected genes in EPI (644 up- and 586 down-regulated) and RD (241 up- and 146 down-regulated) to the psoriasis transcriptomes published in the three studies listed above as well as that detected by next generation sequencing (NGS) technology (Jabbari et al, 2012). Approximately 50% of unique genes in EPI and 30% of unique genes in RD were confirmed in at least one out of the four transcriptomes (Figure 3f-g). Furthermore, we performed RT-PCR on the top eight unique down-regulated genes (ABAT, COL4A5, CXCR7, DDB2, ERAP1, NRTN, SRPX, and TCF4) that were not detected in the above four studies and we show that expression of each gene was clearly down-regulated in our bulk-tissue samples (Figure 3h). Our LCM-unique DEGs thus have the potential to provide many new genes that have not previously been associated with the psoriasis transcriptome. The complete list of LCM-unique probe-sets is provided in Table S6.

Discussion

By combining LCM and cDNA microarray analysis, we sought to define gene expression within different regions of human skin, as altered by psoriasis vulgaris. We focused on the cellular aggregates found in psoriatic lesional dermis, where LAMP3/DC-LAMP+ DCs have been identified along with T cells (Zaba et al. 2009). The importance of this aggregate structure in the pathogenesis of psoriasis is suggested by the observations that it is not identified in non-lesional skin and that effective treatment leads to its disappearance (Zaba et al. 2007).

Psoriasis vulgaris contains at least two distinct subsets of myeloid DCs: BDCA1- and BDCA1+ DCs. The BDCA1- subset includes immature/inflammatory TNFα and iNOS-

producing DCs and their distribution is rather scattered (Lowes et al. 2005). BDCA1+ DCs are immature and do not express LAMP3/DC-LAMP in normal skin. However, psoriasis lesions contain clusters of LAMP3/DC-LAMP+ DCs bearing BDCA1. We have previously compared the gene expression profiles of BDCA1- and BDCA1+ DCs sorted by FACS from psoriasis skin (Zaba et al. 2010). Although BDCA1+ DCs are phenotypically matured compared to BDCA1- DCs based on much greater expression of CD83, CD86, and HLA-DR by flow cytometric analysis (Zaba et al. 2009), these molecules were not recognized as DEGs in that study (Zaba et al. 2010). In contrast, we were able to identify CD83, CD86, and LAMP3/DC-LAMP as up-regulated DEGs in lesional RD/ICs. Because LCM permits us to identify mature DCs, we are thus able to study genes associated with the mature DC subsets of psoriasis.

Using LCM, we established the ectopic expression of CCL19 and its receptor CCR7 in the dermal cellular aggregates of lesional skin. Using bulk tissue extracts, Zhou et al. (2003) also detected the up-regulation of CCL19 and CCL21 mRNA in psoriasis skin. However, to date, the cells producing these key lymphoid-organizing chemokines have not been localized in vivo. We show that CCL19 production occurs selectively within perivascular T cell and DC aggregates and that it likely acts to recruit CCR7+ (self-antigen-specific) T cells and DCs into this focus. Approximately 80% skin resident T cells are thought to be CCR7- effector-memory T cells (T_{EM}) (Clark et al, 2006) and there is a concept that CCR7+ central-memory T cells (T_{CM}) circulate from blood to draining lymph node in order to be activated by DCs (Clark, 2010). Our results, however, propose that the organized aggregates in psoriatic skin contain a sizable number of CCR7+ T cells, which would be T_{CM} that are normally circulating between blood and lymph nodes. Hence skin lesion may create the same environment for expansion of T_{EM} from T_{CM}. The concept of lymphoid organization in lesions is supported by a previous report showing in transgenic mice that ectopic expression of CCL19 alone could organize functional lymphoid structures within the pancreas (Luther et al. 2002). With regard to DC maturation, Gillet's group recently published that self-RNA-LL37 complexes could activate myeloid DCs through TLR8 in vitro. Moreover, these complexes were co-localized with LAMP3/DC-LAMP in psoriatic lesional dermis (Ganguly et al. 2009). Together with our data presented here, this suggests that DC maturation could reflect both chemoattraction of precursors from blood and local activation by self-RNA-LL37 complexes.

Many genes altered in the psoriatic epidermis have been studied previously with bulk gene-sets. We showed a heavy representation of epidermis-related genes in Bulk analysis. However, many DEGs detected in prior studies have not been localized to defined skin regions or specific cell types. With LCM, we were able to successfully localize to EPI or RD 935 probe-sets that were detected in at least one of the three cited studies analyzing bulk-tissues (Figure S5a-b, see SRD section 2-ii). For example, CRIP-1, which is the second largest down-regulated gene in lesional EPI, has not previously been localized to the epidermis, although the down-regulation of this gene is consistent across earlier bulk studies. We also explored major differences in the expression of transcription factors (TFs) between lesional and non-lesional EPI. NFE2L3 and MAFF were up-regulated in lesional EPI. NFE2L3 is a member of the “cap‘n’collar” family of TFs that dimerize with other leucine zipper proteins, such as small Maf proteins (Kobayashi et al. 1999). NFE2L2,

another TF of this family, was shown to be involved in the proliferation and KRT6 expression of forestomach keratinocytes in mice when it dimerizes with MAFF (Motohashi et al. 2004). Although NFE2L3 but not NFE2L2 appeared on our EPI list, NFE2L3 is known to partially compensate for the role of NFE2L2 in mice keratinocytes during re-epithelialization in wound healing (Bauns et al. 2002). These results suggest the possible involvement of NFE2L3 together with MAFF in the proliferation of keratinocytes in psoriasis. In contrast, LASS6, TFAP2B, and GATA3 were down-regulated in lesional EPI. LASS6 is involved in ceramide synthesis in mice (Mizutani et al. 2005). TFAP2B increases cystatin A expression in normal human keratinocytes (Takahashi et al. 2000). GATA3 is a master TF for the differentiation of Th2 cells (Ho et al. 2009), but it also transactivates the lipid acyltransferase gene AGPAT5, leading to the formation of epidermal barrier (de Guzman Strong et al. 2006). These genes are therefore involved in terminal differentiation of keratinocytes. This TF gene list thus induces the key genes involved in keratinocyte proliferation and differentiation and reflects hyper-proliferation of keratinocytes and impaired differentiation in psoriatic epidermis.

Two concerns have been raised regarding applying LCM to global gene expression analysis. The first is that “extreme” gene amplification from small mRNA amounts obtained by LCM may bias the set of genes amplified such that it is not representative of gene pools amplified by conventional one-cycle method (Wang et al. 2007). The second, which comes from a variety of cancer analyses, is that it may be difficult to derive normal cells by LCM for comparison to pathological counterparts (Klee et al. 2009). Both issues have been addressed in this study, and relevant data and discussion are presented in SRD section 1. In contrast to prior work, we found an extremely high concordance between genes detected in psoriasis tissue using single and double amplification methods ($r=0.93$, $p<1\times10^{-16}$, Figure S4d). In this sense, our study was more comparable to that using high quality control mRNA pools (Singh et al. 2005) than to those doing gene detection in biopsies of human cancers (Luzzi et al. 2003; de Bruin et al. 2005; Klee et al. 2009). The second concern regarding the comparison of pathological to normal cell counterparts is well obviated by using skin, where tissue structure makes it possible to derive intrinsically valid comparisons. We therefore did not detect limitations of the LCM method compared to bulk-tissue analysis. We found it instead to be advantageous, providing us the ability to localize and increase disease-related gene products to specific cells/skin regions. Though our Bulk comparison identified a smaller DEG set than previously published studies, we established through statistical simulations that this was due mainly to our study's much smaller sample size (n=3) and consequent weaker statistical power (see SRD section 2-ii). Using LCM we identified several hundred new DEG products in psoriatic EPI+RD that were not detected in the three large studies, suggesting greater sensitivity for detection of some gene products. Still, the greatest advantage of this technique is the ability to study defined cellular regions of the epidermis or dermis that could not otherwise be separated from intact skin by enzymatic or other physical methods (Clemmensen et al. 2009).

In conclusion, we have established the validity of the combined use of LCM and cDNA microarray technologies. We demonstrated that this approach, while not replacing conventional bulk-tissue analysis, complements it through 1) localization of transcripts to specific cells or regions, and 2) more sensitive detection of transcripts within small regions

of tissue, particularly where dilution of transcripts associated with “minority” cell populations may occur. It would be interesting to apply this method to other conditions in which focal cellular processes may differ; to explore, for example, cells specific to the invasive edge of a cancer or cells of certain appendages in the skin.

Materials and Methods

The detailed protocols and statistical analysis are described in supplemental materials and methods.

Patients and Samples

Institutional review board (The Rockefeller University) and written informed consent were obtained before enrolling patients to participate in this study. The study was performed in adherence with the Declaration of Helsinki Principals. Paired lesional and non-lesional samples from 7 psoriatic patients were used. Samples from the first four patients were used in the single vs. double amplification comparison. Samples from the remaining three patients were subjected to LCM.

LCM

LCM was performed according to the manufacturer's protocol for the CellCut system (Molecular Machines and Industries).

RNA extraction

Total RNA was extracted from bulk tissue homogenates, sliced frozen tissue sections, and microdissected samples using RNeasy Kit (QIAGEN).

cDNA microarray analysis

Target amplification and labeling was performed according to the Affymetrix protocols for one-cycle or two-cycle cDNA synthesis. Two-cycle cDNA synthesis was slightly modified according to a previous report (Kube et al, 2007). Affymetrix HGU133A2.0 arrays were used. The data has been deposited at the Gene Expression Omnibus repository (GSE26866).

qRT-PCR on LCM samples

First-strand cDNA synthesis was performed using SuperScriptIII and Random Primers (Invitrogen). The resulting cDNA was used for quantitative PCR reaction. All data were normalized to RPLP0/hARP (Wingens et al. 1998). Primers and probes used in this experiment are listed in Table S12.

IHC and IF

Frozen skin sections were prepared and standard procedures were used. Antibodies used in this experiment are listed in Table S13.

Statistical analysis

Microarray data was analyzed using GeneSpring GX version10.0 software (Agilent) and R/Bioconductor packages. The Harshlight package (Suárez-Fariñas et al. 2005) was used to scan Affymetrix chips for spatial artifacts. Expression values were obtained using the RMA procedure. Expression values were modeled using the mixed-effect framework of Bioconductor's *limma* package. Genes with FDR<0.1 and FCH>2.0 were considered as DEGs. Probe-set distance to the 3′ end of the transcriptome was compared between single and double amplified samples according to a previous report (Klee et al, 2009).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used in this manuscript

LCM	Laser capture microdissection
DEGs	differentially expressed genes
qRT-PCR	quantitative reverse-transcribe polymerase chain reaction
FCH	fold change
FDR	false discovery rate
EPI	epidermis
PD	papillary dermis
RD	reticular dermis
ICs	dermal inflammatory cell aggregates
psDEGs	psoriatic transcriptome
IHC	immunohistochemistry
IF	immunofluorecent staining
DC	dendritic cell
TF	transcription factor
NGS	next generation sequencing
SRD	supplemental results and discussion
T_{EM}	effector-memory T cells
T_{CM}	central-memory T cells

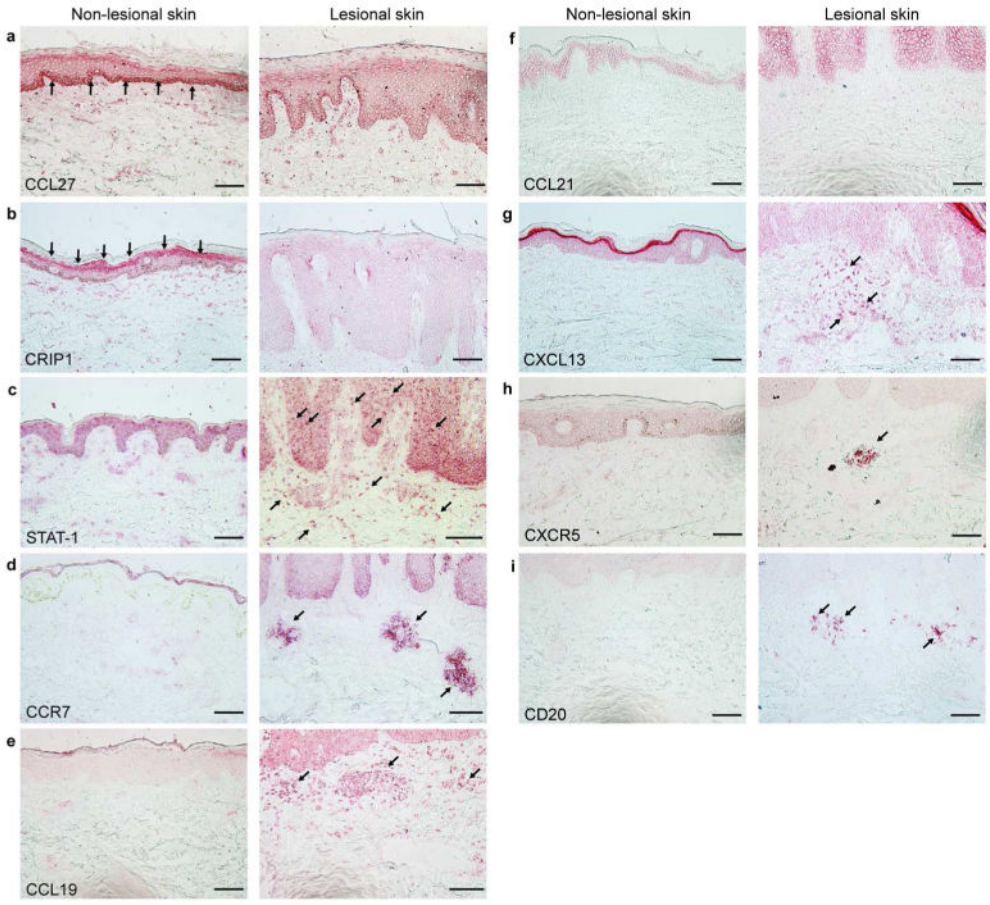

Figure 1. Immunohistochemical staining patterns were correlated with cDNA microarray data

(a) CCL27 was expressed in the basal layer of non-lesional epidermis. (b) CRIP1 was expressed in the granular layer of non-lesional epidermis. (c) STAT-1 stained in the cytosol of cells in non-lesional epidermis, whereas it stained in the nuclei of cells in lesional epidermis and dermis. (d-f) CCR7 and CCL19 were positive in dermal aggregates in lesional skin, whereas CCL21 was detected in neither non-lesional nor lesional skin. (g-h) CXCL13 (3 out of 5 samples) and CXCR5 (6 out of 8 samples) were detected in lesional dermis. (i) CD20+ cells (3 out of 5 samples) were also found in lesional dermis. Arrows indicate the positive layers and cells. Bar=100μm.

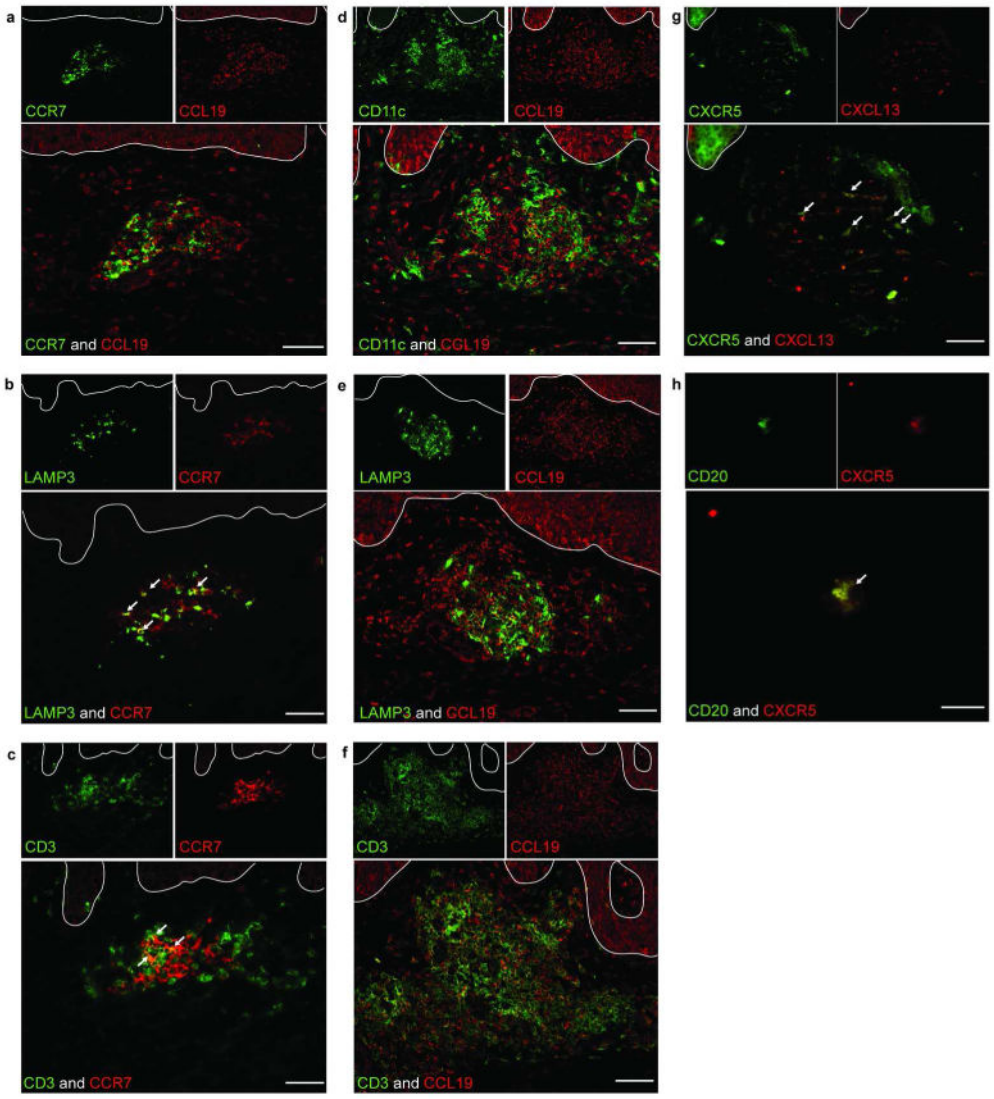

Figure 2. Characterization of lymphoid tissue like structures in lesional skin

(a) Both CCR7 and CCL19 were localized in dermal aggregates of the lesional skin. (b) LAMP3/DC-LAMP was co-expressed with CCR7. (c) CD3 was also co-expressed with CCR7. (d-f) CCL19 expression was detected on CD11c+ (d) and LAMP3/DC-LAMP+ DCs (e) as well as CD3+ T cells (f). We also stained CXCR5 in combination with CXCL13 and CD20. (g-h) Both CXCL13 and CXCR5 stained in dermal aggregates of lesional skin (g) and CXCR5 co-stained with CD20 (h). White arrows indicate the double positive cells. White lines represent epidermal dermal junctions. Bar=100μm.

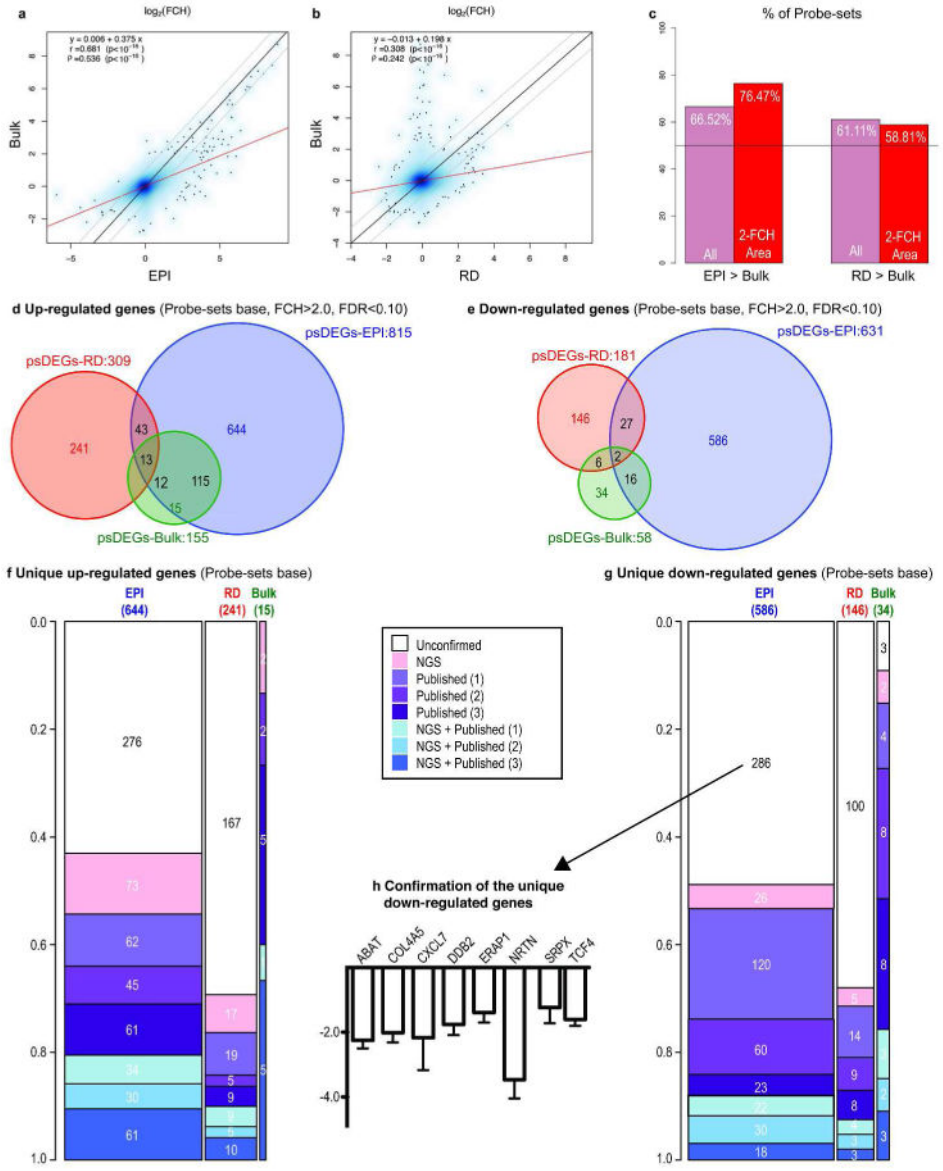

Figure 3. Unique detection of DEGs by LCM samples compared to Bulk skin sample

(a-b) Scatterplots of Log2FCH of Bulk vs. EPI and RD. Black lines: identity lines; gray lines: ±2-FCH; red lines: robust linear regression estimates. (c) Percentage of probe-sets with larger absolute FCH in LCM samples than in Bulk. Pink bars: all probe-sets; red bars: probe-sets considering |FCH|>2.0 (d-e) Venn-Diagrams of Bulk, EPI and RD psoriasis transcriptome. (f-g) Proportion of unique EPI-, RD-, and Bulk-related probe-sets that appear in 1, 2 or 3 of the published Affymetrix studies (considering HU133A2.0 probe-sets) or in the NGS-transcriptome. The width of each bar depicts the number in each category. (h) RT-PCR fold changes of the top eight unconfirmed down-regulated genes using bulk tissue samples (n=9).

Table 1

Short lists of DEGs in psoriasis non-lesional EPI vs. non-lesional RD (LCM)*

a. Top 25 Up-regulated genes in psoriasis non-lesional EPI vs. non-lesional RD				
Probe Set ID	**FCH**	**FDR**	**Symbol**	**Description**
205900_at	252.28	<0.01	KRT1	keratin 1
205694_at	244.41	<0.01	TYRP1	tyrosinase-related protein 1
207908_at	243.36	<0.01	KRT2	keratin 2
206400_at	153.37	0.01	LGALS7 / 7B	lectin, galactoside-binding, soluble, 7 / 7B
206032_at	139.60	0.02	DSC3	desmocollin 3
207324_s_at	130.69	<0.01	DSC1	desmocollin 1
204379_s_at	92.21	<0.01	FGFR3	fibroblast growth factor receptor 3
207955_at	91.10	<0.01	CCL27	chemokine (C-C motif) ligand 27
213506_at	88.21	<0.01	F2RL1	coagulation factor II (thrombin) receptor-like 1
201131_s_at	82.44	0.01	CDH1	cadherin 1, type 1, E-cadherin (epithelial)
207109_at	81.63	<0.01	POU2F3	POU class 2 homeobox 3
204455_at	78.56	0.01	DST	dystonin
221854_at	76.68	<0.01	PKP1	plakophilin 1
213929_at	70.43	0.01	EXPH5	exophilin 5
206276_at	66.07	<0.01	LY6D	lymphocyte antigen 6 complex, locus D
205337_at	64.29	0.01	DCT	dopachrome tautomerase
209570_s at	63.15	<0.01	D4S234E	DNA segment on chr 4 - 234 expressed sequence
206642_at	58.40	0.01	DSG1	desmoglein 1
205807_s_at	57.30	<0.01	TUFT1	tuftelin 1
219995_s_at	55.76	<0.01	ZNF750	zinc finger protein 750
204469_at	50.94	0.01	PTPRZ1	protein tyrosine phosphatase, receptor-type, Z polypeptide 1
204653_at	49.93	0.01	TFAP2A	transcription factor AP-2 α
217528_at	49.02	0.08	CLCA2	chloride channel accessory 2
217744_s_at	47.24	0.03	PERP	PERP, TP53 apoptosis effector
201820_at	46.26	0.03	KRT5	keratin 5

b. Top 25 Down-regulated genes in psoriasis non-lesional EPI vs. non-lesional RD				
Probe Set ID	**FCH**	**FDR**	**Symbol**	**Description**
209687_at	-250.00	<0.01	CXCL12	chemokine (C-X-C motif) ligand 12
217022_s_at	-250.00	<0.01	IGHA1	immunoglobulin heavy constant alpha 1
200795_at	-200.00	<0.01	SPARCL1	SPARC-like 1 (hevin)
215388_s_at	-166.67	<0.01	CFH / CFHR1	complement factor H /complement factor H-related 1
213975_s_at	-166.67	<0.01	LYZ	lysozyme (renal amyloidosis)
217757_at	-142.86	<0.01	A2M	alpha-2-macroglobulin
215076_s_at	-125.00	0.01	COL3A1	collagen, type III, α 1
208982_at	-125.00	<0.01	PECAM1	platelet/endothelial cell adhesion molecule

b. Top 25 Down-regulated genes in psoriasis non-lesional EPI vs. non-lesional RD				
Probe Set ID	**FCH**	**FDR**	**Symbol**	**Description**
209613_s_at	-111.11	<0.01	ADH1B	alcohol dehydrogenase 1B(class I), β polypeptide
213800_at	-111.11	<0.01	CFH	complement factor H
206201_s_at	-111.11	<0.01	MEOX2	mesenchyme homeobox 2
208335_s_at	-90.91	<0.01	DARC	Duffy blood group, chemokine receptor
222043_at	-83.33	<0.01	CLU	clusterin
202291_s_at	-83.33	<0.01	MGP	matrix Gla protein
219777_at	-76.92	<0.01	GIMAP6	GTPase, IMAP family member 6
201744_s_at	-76.92	0.01	LUM	lumican
212950_at	-71.43	<0.01	GPR116	G protein-coupled receptor 116
204115_at	-62.50	<0.01	GNG11	guanine nucleotide binding protein (G protein), γ 11
218353_at	-62.50	<0.01	RGS5	regulator of G-protein signaling 5
203868_s_at	-62.50	<0.01	VCAM1	vascular cell adhesion molecule 1
221731_x_at	-62.50	<0.01	VCAN	versican
201438_at	-58.82	<0.01	COL6A3	collagen, type VI, α 3
217028_at	-58.82	<0.01	CXCR4	chemokine (C-X-C motif) receptor 4
202404_s_at	-55.56	0.01	COL1A2	collagen, type I, α 2
202404_s_at	-55.56	0.01	NR2F2	nuclear receptor subfamily 2, group F, member 2

*The Probe Set ID whose fold change was largest was listed when multiple probe sets were available for same gene. Un-annotated genes were excluded from the top 25 gene list.

Table 2

Short lists of DEGs in psoriasis lesional EPI vs. non-lesional EPI (LCM)*

a. Top 25 Up-regulated genes in psoriasis lesional EPI vs. non-lesional EPI						
Probe Set ID	**Symbol**	**EPI (LCM)**		**Bulk**		**Description**
		FCH	**FDR**	**FCH**	**FDR**	
211906_s_at	SERPINB4	**470.72**	**<0.01**	419.60	<0.01	serpin peptidase inhibitor, clade B member 4
209720_s_at	SERPINB3	**366.10**	**<0.01**	171.61	<0.01	serpin peptidase inhibitor, clade B member 3
203535_at	S100A9	**136.52**	**<0.01**	106.56	<0.01	S100 calcium binding protein A9
203691_at	PI3	**128.03**	**<0.01**	67.67	<0.01	peptidase inhibitor 3, skin-derived
207356_at	DEFB4	**114.24**	**<0.01**	69.88	<0.01	defensin, beta 4
205916_at	S100A7	**84.37**	**<0.01**	10.67	0.21	S100 calcium binding protein A7 (psoriasin)
213680_at	KRT6B	**67.99**	**<0.01**	3.61	0.54	keratin 6B
205863_at	S100A12	**57.07**	**<0.01**	32.81	<0.01	S100 calcium binding protein A12
209125_at	KRT6A	**55.71**	**<0.01**	18.77	0.02	keratin 6A
217272_s_at	SERPINB13	**55.50**	**<0.01**	8.13	<0.01	serpin peptidase inhibitor, clade B member 13
220322_at	IL1F9	**55.34**	**<0.01**	19.15	<0.01	interleukin 1 family, member 9 (IL-36 gamma)
206488_s_at	CD36	**53.72**	**<0.01**	1.80	0.30	CD36 molecule
202917_s_at	S100A8	**50.84**	**0.03**	53.07	0.14	S100 calcium binding protein A8
205513_at	TCN1	**50.11**	**<0.01**	39.15	<0.01	transcobalamin I
219795_at	SLC6A14	**47.10**	**<0.01**	5.84	0.13	solute carrier family 6 member 14
206177_s_at	ARG1	**46.24**	**<0.01**	3.57	0.07	arginase, liver
220664_at	SPRR2C	**45.97**	**<0.01**	32.99	<0.01	small proline-rich protein 2C
213796_at	SPRR1A	**35.17**	**<0.01**	6.03	0.24	small proline-rich protein 1A
208650_s_at	CD24	**29.72**	**<0.01**	7.49	<0.01	CD24 molecule
206643_at	HAL	**24.28**	**<0.01**	5.42	<0.01	histidine ammonia-lyase
207381_at	ALOX12B	**22.66**	**<0.01**	4.59	<0.01	arachidonate 12-lipoxygenase, 12R type
209800_at	KRT16	**21.75**	**<0.01**	16.48	0.02	keratin 16
201242_s_at	ATP1B1	**21.33**	**<0.01**	2.46	0.57	ATPase, Na+/K+ transporting, β 1 polypeptide
206561_s_at	AKR1B10	**20.04**	**<0.01**	20.14	<0.01	aldo-keto reductase family 1, member B10

a. Top 25 Up-regulated genes in psoriasis lesional EPI vs. non-lesional EPI						
Probe Set ID	**Symbol**	**EPI (LCM)**		**Bulk**		**Description**
		FCH	**FDR**	**FCH**	**FDR**	
213060_s_at	CHI3L2	**19.97**	**<0.01**	5.46	<0.01	chitinase 3-like 2

b. Top 25 Down-regulated genes in psoriasis lesional EPI vs. non-lesional EPI						
Probe Set ID	**Symbol**	**EPI (LCM)**		**Bulk**		**Description**
		FCH	**FDR**	**FCH**	**FDR**	
210809_s_at	POSTN	**-62.50**	**<0.01**	-1.44	0.35	periostin, osteoblast specific factor
205081_at	CRIP1	**-20.41**	**<0.01**	-2.48	0.17	cysteine-rich protein 1 (intestinal)
214598_at	CLDN8	**-15.38**	**<0.01**	-4.57	0.08	claudin 8
201540_at	FHL1	**-9.80**	**<0.01**	-1.88	0.35	four and a half LIM domains 1
219087_at	ASPN	**-9.52**	**<0.01**	-1.79	0.50	asporin
215516_at	LAMB4	**-9.17**	**<0.01**	-1.98	<0.01	laminin, beta 4
201843_s_at	EFEMP1	**-8.85**	**<0.01**	-1.89	0.22	EGFcontaining fibulin-like extracellular matrix protein1
213369_at	PCDH21	**-8.26**	**<0.01**	-2.26	0.07	protocadherin 21
202746_at	ITM2A	**-7.87**	**<0.01**	-2.35	0.03	integral membrane protein 2A
206170_at	ADRB2	**-7.52**	**<0.01**	-2.95	0.01	adrenergic, beta-2-, receptor, surface
209292_at	ID4	**-7.41**	**<0.01**	-3.06	0.06	inhibitor of DNA binding 4
207955_at	CCL27	**-7.25**	**0.01**	-3.38	0.21	chemokine (C-C motif) ligand 27
202973_x_at	FAM13A	**-7.25**	**<0.01**	-1.76	0.07	family with sequence similarity 13, member A
201506_at	TGFBI	**-7.04**	**<0.01**	-1.72	0.13	transforming growth factor, β-induced, 68kDa
215239_x_at	ZNF273	**-6.85**	**<0.01**	-2.29	0.28	zinc finger protein 273
218804_at	ANO1	**-6.80**	**<0.01**	-1.58	0.57	anoctamin 1
208096_s_at	COL21A1	**-6.71**	**<0.01**	-1.85	0.14	collagen, type XXI, α 1
218820_at	C14orf132	**-6.62**	**<0.01**	-2.47	0.12	chromosome 14 open reading frame 132
217627_at	ZNF573	**-6.25**	**<0.01**	-2.41	0.13	zinc finger protein 573
209335_at	DCN	**-6.21**	**<0.01**	-1.54	0.26	decorin
221645_s_at	ZNF83	**-5.65**	**<0.01**	-1.78	0.12	zinc finger protein 83
214723_x_at	ANKRD36	**-5.49**	**<0.01**	-1.53	0.58	ankyrin repeat domain 36
220940_at	ANKRD36B	**-5.49**	**<0.01**	-1.54	0.49	ankyrin repeat domain 36B

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b. Top 25 Down-regulated genes in psoriasis lesional EPI vs. non-lesional EPI						
Probe Set ID	**Symbol**	**EPI (LCM)**		**Bulk**		**Description**
		FCH	**FDR**	**FCH**	**FDR**	
203881_s_at	DMD	**-5.46**	**0.02**	-2.10	0.54	dystrophin
206030_at	ASPA	**-5.41**	**<0.01**	-1.51	0.74	aspartoacylase

* The Probe Set ID whose fold change was largest was listed when multiple probe sets were available for same gene. Un-annotated genes were excluded from the top 25 gene list.

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Table 3

A short list of DEGs in psoriasis lesional RD/ICs vs. non-lesional RD (LCM)*

Top 25 Up-regulated genes in psoriasis lesional RD/ICs vs. non-lesional RD						
Probe Set ID	**Symbol**	**RD/ICs (LCM)**		**Bulk**		**Description**
		FCH	**FDR**	**FCH**	**FDR**	
216834_at	RGS1	**27.12**	**0.02**	11.53	0.08	regulator of G-protein signaling 1
201890_at	RRM2	**10.66**	**0.04**	4.29	0.23	ribonucleotide reductase M2
205569_at	LAMP3	**10.58**	**0.01**	4.38	0.08	lysosomal-associated membrane protein 3 (CD208)
204470_at	CXCL1	**8.02**	**0.08**	3.88	0.36	chemokine (C-X-C motif) ligand 1
203559_s_at	ABP1	**7.70**	**0.02**	1.45	0.82	amiloride binding protein 1
209774_x_at	CXCL2	**7.22**	**<0.01**	1.62	0.23	chemokine (C-X-C motif) ligand 2
213241_at	PLXNC1	**7.14**	**<0.01**	-1.08	0.96	plexin C1
220330_s_at	SAMSN1	**7.14**	**0.02**	2.82	0.21	SAM domain, SH3 domain and nuclear localization signals 1
206337_at	CCR7	**7.10**	**0.01**	1.75	0.38	chemokine (C-C motif) receptor 7
209969_s_at	STAT1	**6.93**	**0.01**	3.49	0.10	signal transducer and activator of transcription 1
210072_at	CCL19	**6.83**	**<0.01**	4.10	0.02	chemokine (C-C motif) ligand 19
207165_at	HMMR	**6.63**	**0.02**	2.59	0.26	hyaluronan-mediated motility receptor
206545_at	CD28	**6.03**	**0.09**	1.39	0.93	CD28 molecule
219648_at	MREG	**5.94**	**0.04**	2.15	0.49	melanoregulin
210164_at	GZMB	**5.83**	**0.03**	3.00	0.21	granzyme B
204440_at	CD83	**5.58**	**<0.01**	2.10	0.03	CD83 molecule
207861_at	CCL22	**5.41**	**0.04**	2.36	0.35	chemokine (C-C motif) ligand 22
206134_at	ADAMDEC1	**5.33**	**0.01**	3.54	0.04	ADAM-like, decysin 1
204026_s_at	ZWINT	**5.05**	**0.03**	2.37	0.26	ZW10 interactor
204714_s_at	F5	**5.05**	**0.01**	-1.29	0.84	coagulation factor V
205242_at	CXCL13	**4.99**	**0.01**	2.40	0.14	chemokine (C-X-C motif) ligand 13
214023_x_at	TUBB2B	**4.93**	**0.06**	-1.20	0.96	tubulin, beta 2B
208103_s_at	ANP32E	**4.57**	**0.02**	1.33	0.79	acidic nuclear phosphoprotein 32 family, member E
207957_s_at	PRKCB	**4.53**	**0.04**	-1.34	0.86	protein kinase C, β

Top 25 Up-regulated genes in psoriasis lesional RD/ICs vs. non-lesional RD						
Probe Set ID	**Symbol**	**RD/ICs (LCM)**		**Bulk**		**Description**
		FCH	**FDR**	**FCH**	**FDR**	
204932_at	TNFRSF11B	**4.46**	**0.02**	1.06	0.98	tumor necrosis factor receptor superfamily member 11b

* The Probe Set ID whose fold change was largest was listed when multiple probe sets were available for same gene. Un-annotated genes were excluded from the top 25 gene list.