# An Analysis of Select Pathogenic Messages in Lesional and Non-Lesional Psoriatic Skin Using Non-Invasive Tape Harvesting

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**We report the use of non-invasive tape stripping to sample psoriatic lesional and non-lesional skin in 96 patients. The procedure was well tolerated with any discomfort described as mild; we did not observe any cases of Koebner phenomena at any non-lesional tape-stripped sites. Tape-harvested epidermis was extracted for RNA, which was profiled by semiquantitative reverse transcriptase-PCR. This analysis revealed that mRNAs for tumor necrosis factor $\alpha$, IFN$\gamma$, Krt-16, CD2, IL-23A, IL-12B, and vascular endothelial growth factor are overexpressed in the "average" psoriatic lesion in a majority of patients. In addition, 10 of these patients were biopsied at lesional and non-lesional sites and the expression data compared to tape-stripping data. This comparison shows that five of seven mRNA are more highly expressed in cells captured by tape stripping than biopsy, suggesting that the upper aspect of a lesion contains cells very active in the disease. The tape-harvesting data reveal that approximately 46% of lesions have at least one pathogenic mRNA within non-lesional skin limits. Data demonstrate that tape stripping reveals mRNA markers not detected in biopsy samples and thus the method may be a useful supplement to biopsy.**

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## INTRODUCTION

Psoriasis is a common inflammatory skin disorder, estimated to affect 1–3% of the world's population and is characterized by lesions exhibiting erythema, induration, and scaling (Peters *et al.*, 2000). At the physiological and molecular level, psoriasis is a complex disease highlighted by hyper-proliferative keratinocytes, defective differentiation and barrier formation, dermal and epidermal infiltration by diverse leukocytes, and profoundly altered gene expression (Lew *et al.*, 2004). Much of our knowledge concerning the pathophysiology of psoriatic skin lesions has been derived from biopsies of lesional and non-lesional control skin (also referred to as uninvolved skin) of a psoriatic patient. While biopsy data have been invaluable in characterizing psoriatic skin, the invasive nature of biopsy makes it much less practical as a method for repetitive clinical investigation of psoriatic lesions. Repetitive non-invasive monitoring of this skin disease would be of considerable value if the information obtained reflected the pathophysiological state of the lesion.

Tape stripping or tape harvesting is a non-invasive method that allows recovery of cells comprising and associated with the upper epidermis (Morhenn *et al.*, 1999; Wong *et al.*, 2004). Recently, it has been demonstrated that the sequential application of four small adhesive tape strips to a normal or inflamed skin site is sufficient to recover a skin sample whose RNA population can be profiled by quantitative reverse transcriptase-PCR or DNA microarray (Wong *et al.*, 2004). The application of this method to dermatological disease – in concert with RNA profiling – could allow for more precise diagnosis and facilitate the pharmacogenomic characterization of skin diseases by allowing large amounts of informative molecular data to be gathered; which because it can be gathered in a non-invasive manner would otherwise be unavailable.

Herein we describe the use of tape harvesting as a method for routinely recovering pathologic information in messenger RNA from the surface of psoriatic and non-lesional skin. We show that the tape harvesting of psoriatic patients gives relatively large quantities of total RNA and that RNA profiles contained within the recovered RNA are consistent with the body of biopsy data in the literature. In a subset of patients, we directly compare biopsy and tape-stripping results. We observe that biopsy and tape-strip RNA samples provide largely similar but also individually unique data suggesting

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*Abbreviations: TNFα, tumor necrosis factor α; VEGF, vascular endothelial growth factor*

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that tape stripping is an important addition to biopsy as a sampling technique.

## RESULTS

### Recovery of RNA from lesional and non-lesional skin

In this study, we tape stripped 96 patients at one lesional site and three non-lesional control sites. From lesions, an average of 116±19.5 ng of RNA was recovered; from the combined three control skin sites an average of 83±17.3 ng of total RNA was isolated (median recoveries were 46.2 and 25.3 ng, respectively). As the control sample was a pool of three separate skin sites (described in Materials and Methods) and the lesion samples were from a single site, it is clear that substantially more RNA was harvested from lesional skin than control skin.

### Gene expression in lesional and non-lesional skin

Gene expression has been well characterized in lesional and non-lesional skin by both immunohistochemistry and quantitative reverse transcriptase-PCR, using incisional skin biopsies to procure samples (Yawalkar *et al*., 1998; Trepicchio *et al*., 1999; Bhawan *et al*., 2004; Lee *et al*., 2004). To determine if tape harvesting could yield data consistent with this body of work, we assayed the samples described above for the mRNAs listed in Table 1. These mRNAs (with the exception of tumor necrosis factor (TNF)α) are known to be elevated in lesional skin and characteristic of psoriatic disease. Table 1 shows the average fold increase of the (gene/β-actin) mRNA ratio in lesion samples relative to non-lesional control skin. The table shows that, on average, all of the mRNAs are significantly (95% confidence interval) overexpressed in lesional skin. This result indicates that RNA recovered using the tape-harvesting method yields data qualitatively identical to biopsy-recovered RNA (Yawalkar *et al*., 1998; Trepicchio *et al*., 1999; Bhawan *et al*., 2004; Lee *et al*., 2004). In later sections, we confirm this observation with analysis of a subset of patients for which we have biopsy samples and address the observation of increased TNFα mRNA in tape samples.

Table 1 shows that in our sample of 96 patients, lesional skin contains, on average, elevated levels of pathogenic mRNAs. However, inspection of individual patient data reveals that there is heterogeneity in specific (gene/β-actin) mRNA ratios (data not shown). To reveal this heterogeneity in lesion gene expression we refer to the $\Delta C_t$ value, an experimental measure of the (gene/β-actin) mRNA ratio in a sample (described in Materials and Methods). By comparing individual (gene/actin) mRNA ratios (i.e. $\Delta C_t$ values) to average values from our 96 patients, we can classify a sample as being ''lesion-like'' or ''non-lesion-like''. For reference, the average lesion and non-lesion $\Delta C_t$ data is shown in Table S1.

By comparison of individual patient $\Delta C_t$ values (data not shown) to the average values shown in Table S1, we can determine if individual lesions overexpress each of the mRNAs shown in Table 1. We have carried out such a comparison for all 96 patients and summarize the results in Table 2. The table shows the number of lesion samples with specific gene expression characteristic of non-lesional skin. For instance, of the 95 lesion samples with quantifiable TNFα mRNA, there were five $\Delta C_{t,\ TNF}$ values that were characteristic of average control skin. Similarly, of 92 lesion samples with quantifiable CD2 mRNA, 22 samples were determined to have $\Delta C_{t,\ CD2}$ values characteristic of control skin. Furthermore, if one asks how many of the 96 lesion samples have significant increases in all the mRNAs, we find that 52 samples have increased expression of all mRNAs (K16 is not included in this analysis for reasons discussed later). Thus, 44 of the lesions (46%) have at least one mRNA with expression characteristic of non-lesional skin.

**Table 1. Average fold change in (gene/β-actin) mRNA ratio in lesional relative to non-lesional psoriatic skin**

| Average fold change[1] | | | | | | |
|---|---|---|---|---|---|---|
| **TNFα** | **IFNγ[2]** | **CD2** | **Krt-16** | **IL-12B[2]** | **IL-23A** | **VEGF** |
| 7 (5–7.5) | 6 (1.6–20) | 2.5 (1.8–2.7) | 2 (1.6–2.1) | 6.2 (1–63) | 6.8 (3.5–9.2) | 5 (4–5.3) |

[1]The average fold change in lesional psoriatic skin relative to non-lesional control skin is calculated as described in Materials and Methods using the data in Table S1. The >95% confidence interval is given in parenthesis below the fold change.
[2]These confidence intervals are large because mRNA for these markers could only be detected in two (IFN) and four (IL-12B) control skin samples.

**Table 2. Identification of lesions with levels of pathological mRNA within control skin limits**

| Number of patients displaying a lesion with gene expression characteristic of control skin[1] | | | | | | |
|---|---|---|---|---|---|---|
| **TNFα** | **IFNγ** | **CD2** | **Krt-16** | **IL-12B** | **IL-23A** | **VEGF** |
| 5 (95) | 17 (82) | 22 (93) | 30 (96) | 22 (85) | 10 (93) | 3 (96) |

[1]The number of patients presenting a lesion with mRNA expression characteristic of non-lesional skin. For example, five of 95 lesions with assayable TNFα mRNA had a $\Delta C_{t,\ TNF} > 8.2$ (average control $\Delta C_{t,\ TNF}$ minus 1 SEM; Table S1; see Materials and Methods for a full explanation of how lesions were evaluated), demonstrating that these five lesions do not overexpress TNFα mRNA; one out of the 96 samples could not be evaluated for TNFα because of low RNA yield. The total number of lesions considered is in parentheses; some lesion samples did not have sufficient RNA to be evaluated for some markers.

**Comparison of gene expression in biopsy and tape-strip samples**

In 10 of our 97 patients, we obtained 4 mm punch biopsies from lesional and non-lesional skin directly adjacent to sites which had been tape stripped. The biopsies were further divided into epidermal and dermal sections and RNA prepared from each section. Table 3 shows the comparison of average change in gene expression in lesional relative to control skin in biopsy and tape samples from these patients. In this table, each sample of tape, biopsy-epidermis, and biopsy-dermis from lesional skin is calibrated to the same sample type from non-lesional skin. Thus, the average RNA expression in tape-strip samples of lesions is calibrated to average expression in tape-strip samples of non-lesional skin; lesion epidermis-biopsy samples are calibrated to control epidermis-biopsy samples, etc.

In comparison of the average fold expression for all 96 subjects (Table 1) with the subset of 10 tape-strip patients for which we have biopsies (Table 3), we see that, with one exception, all of the (mRNA/actin) mRNA ratios are increased in the subset of 10 tape-strip lesion samples. The sole exception is K16 mRNA, whose average expression is not significantly overexpressed in the subset of 10 tape-strip lesion samples (K16 mRNA is significantly overexpressed in the larger set of 96 samples shown in Table 1). Thus, excepting the K16 data, the 10 tape-strip group is largely representative of the larger sample. In contrast, comparison of gene expression in tape-strip RNA *versus* epidermal- and dermal-biopsy RNA reveals some interesting differences, described below.

Table 3 shows that the (TNF$\alpha$/$\beta$-actin) mRNA ratio in our subset of 10 tape samples of psoriatic lesions is 9-fold elevated relative to control tape samples. In contrast, the epidermis- and dermal-biopsy mRNA ratios are not significantly different in lesional *versus* control samples. Our subset of 10 tape-strip data are in agreement with the larger sample set (Table 1) and our biopsy data are in agreement with published data. Therefore, we conclude that TNF mRNA is overexpressed in cells in the very upper epidermis of a psoriatic lesion; presumably, these cells are diluted out by the majority of other cells captured by a biopsy. This demonstrates that tape stripping is a more sensitive sampling method for assay of TNF$\alpha$ mRNA than is biopsy.

Similar data are revealed for IL-23A expression. Although the average fold increase in expression in lesion *versus* control is 2.7- and 3.9-fold for tape and epidermis-biopsy samples, respectively, inspection of individual $\Delta C_t$ data reveals that tape-strip samples have a much higher (IL-23A/$\beta$-actin) mRNA ratio than do epidermis-biopsy samples. The average $\Delta C_{t,\ IL23A}$ in lesion tape samples was 5.9, while the average $\Delta C_{t,\ IL23A}$ in the epidermal-biopsy fraction was 10.55 (for $\Delta C_t$ values a lower number indicates higher expression); this difference in $\Delta C_t$ values corresponds to a $2^{-(5.9-10.55)}$ or 25-fold higher (IL-23A/actin) mRNA ratio in tape *versus* epidermal-biopsy lesion samples.

For K16 mRNA expression, we see a slight, but not significant 1.3-fold increase in the (K16/actin) mRNA ratio in the subset of 10 tape samples of lesional relative to control skin (Table 3). However, epidermal- and dermal-biopsy lesion samples show, respectively, 71- and 26-fold increases relative to non-lesional skin. If we examine the average $\Delta C_t$ values for tape-strip samples (Table S1; all 96 subjects) and epidermal-biopsy samples from control skin, we find that

**Table 3. Average fold change of inflammatory mRNAs from 10 matched tape and biopsy samples in lesional psoriatic skin relative to control skin**

| | | Sample source and fold change in gene expression | | |
|---|---|---|---|---|
| | | | Biopsy[3] | |
| Gene | Statistic[1] | Tape[2] | Epidermis | Dermis |
| TNF$\alpha$ | Fold change | 9 | 0.8 | 0.7 |
| | 95% CI | (5.4–15) | (0.4–1.5) | (0.4–1.1) |
| | *N* | (10, 4) | (9, 10) | (9, 10) |
| IFN | Fold change | 3.1 | 2 | 1 |
| | 95% CI | — | — | — |
| | *N* | (7, 1) | (9, 1) | (9, 1) |
| CD2 | Fold change | 2.7 | 3.9 | 1.7 |
| | 95% CI | — | (1.8–8.6) | (0.9–3.3) |
| | *N* | (9, 1) | (9, 10) | (9, 10) |
| K16 | Fold change | 1.3 | 71 | 26 |
| | 95% CI | (0.7–2.5) | (18–280) | (8.5–79) |
| | *N* | (10, 9) | (9, 10) | (9, 10) |
| IL-12B | Fold change | 4.6[4] | 9.4 | 3.1 |
| | 95% CI | — | — | — |
| | *N* | (8, 0) | (9, 1) | (8, 1) |
| IL-23A | Fold change | 2.7 | 3.9 | 0.84 |
| | 95% CI | (0.7–11) | — | — |
| | *N* | (10, 2) | (9, 1) | (9, 1) |
| VEGF | Fold change | 2.8 | 1.6 | 1.2 |
| | 95% CI | (1.4–5.6) | (0.91–2.7) | (0.95–1.5) |
| | *N* | (10, 8) | (9, 10) | (9, 10) |

[1]Calculation of fold change and 95% confidence interval (95% CI) is described in Materials and Methods; *N* is the number of lesion and control samples upon which the fold-change calculations are based and are shown as (no. of lesion, no. of control).

[2]Data from the 10 tape-strip samples for which we have accompanying biopsy samples, for fold-change calculations of IFN$\gamma$ and CD2 mRNAs only one of the 10 control tape samples had detectable IFN or CD2 mRNA, thus the fold change is based on that single sample and no 95% CI is presented.

[3]Data for lesion and control biopsy samples split into epidermal and dermal fractions. Data for 10 control samples and nine lesion samples are presented. The average fold change for IFN$\gamma$, IL-12B, and IL-23A (gene/actin) mRNA ratio in lesions is calibrated on the single control sample in which specific mRNA could be assayed (specific mRNA could not be detected in nine control samples), therefore, no 95% CI given for these mRNAs.

[4]In this subset of 10 tape-strip samples of control skin IL-12B mRNA could not be detected, therefore in order to calculate a fold change for IL-12B mRNA we used the average $\Delta C_{t,\ IL12B}$ value of the four of 96 tape samples where IL-12B mRNA could be assayed (taken from Table S1).

tape samples have a $2^{-(-1.46-3.81)}$ or 38-fold higher (K16/actin) mRNA ratio than epidermal-biopsy samples (Table S1). Thus in non-lesional skin, there is active K16 expression in the very upper epidermis (revealed by tape stripping) that is not seen in the epidermal-biopsy sample (or the dermal sample). We also observe this high expression of K16 mRNA in tape-strip samples from normal healthy individuals (data not shown).

In lesion samples, the activity of K16 expression in tape, epidermis, and dermis RNA samples is approximately equal (Table S1). Therefore, the large changes in K16 mRNA expression induced by psoriasis are occurring deeper in the skin, in layers of a lesion not sampled by tape stripping.

For the remaining mRNAs, the average fold-change data in Table 3 show that changes revealed in tape-strip lesion samples are qualitatively mirrored by similar changes in epidermal-biopsy lesion samples. The data in Table 3 also show that in general the large changes in mRNA expression are restricted to the epidermis, since the dermal sections show few significant changes in lesional compared to control dermis, the sole exception being K16 expression which, as might be expected because of the acanthotic epidermis, is increased in psoriatic dermis compared to the control dermis.

## DISCUSSION

In this study, we demonstrate that mRNA can be recovered from the surface of lesional and non-lesional skin of psoriatics using a non-invasive tape-stripping method. Further, we have shown this RNA can be quantitatively assayed for specific mRNAs. This work expands the work of Wong *et al*. (2004) who have generated similar data for normal skin and sodium lauryl sulfate-inflamed skin.

In the course of tape-harvesting the superficial epidermis from lesional and non-lesional skin of 96 subjects with psoriasis, we observed no adverse events, including any evidence of the Koebner phenomenon. Previously, we reviewed the literature, reported our experience and noted that $\approx$30% of psoriatic subjects will develop psoriasis in areas receiving significant trauma (Eyre and Krueger, 1991). In this review, it was noted that ''deep'' tape stripping has been reported as inducing the ''Koebner reaction.'' The fact that we have not seen psoriasis develop in any of the three control sites in the 96 subjects we report here nor in the over 300 patients we have treated since (data not presented) argues favorably for this method of sample collection as being minimally invasive and easily tolerated.

There is mounting evidence that psoriasis is an immune-mediated disease, where activated T cells and activated dendritic cells are the principle effectors (Lew *et al*., 2004; Lowes *et al*., 2005) and a hyperproliferative, poorly differentiated epidermis is the result. The mRNAs we have chosen to assay (Table 1) are well described in the literature with direct involvement in the pathogenesis of psoriasis, being markers of general inflammation, activated T cells or hyperproliferative keratinocytes. Our gene expression data from tape-harvested and biopsied lesions are – excepting K16 and TNF$\alpha$ mRNA expression – qualitatively in compete agreement with known gene expression profiles describing the ''average'' psoriatic lesion, data which is all based on biopsies (Yawalkar *et al*., 1998; Trepicchio *et al*., 1999; Bowcock *et al*., 2001; Oestreicher *et al*., 2001; Lee *et al*., 2004).

In our comparison of lesion *versus* control skin gene expression in tape strip, epidermal-, and dermal-biopsy RNA samples, we have observed a general trend of higher expression in tape and epidermis-biopsy lesion samples and modest or no change in dermis-biopsy samples. Thus, generally speaking tape-strip RNA and epidermal-biopsy RNA gave equivalent results. Exceptions to these observations were the already noted changes in TNF$\alpha$ and K16 mRNA expression.

While it is well established that TNF$\alpha$ protein is increased in psoriatic lesions, increases in TNF$\alpha$ mRNA have not been demonstrated (Zhou *et al*., 2003; Johansen *et al*., 2006). The lack of increased TNF$\alpha$ mRNA in lesional skin has led to various post-translational TNF-activating mechanisms to account for the increases in protein (Kawaguchi *et al*., 2005; Johansen *et al*., 2006). We also did not observe increased TNF$\alpha$ mRNA in lesional skin biopsies (Table 3) but our tape-stripping data clearly show a significant increase in TNF$\alpha$ mRNA.

As we see significant increases in TNF$\alpha$ mRNA in 90 of 95 lesion tape-strip samples (Table 2) there is little doubt that tape stripping is recovering cells with increased TNF$\alpha$ mRNA transcription. We hypothesize that these cells are in the very upper aspect of lesions and in a biopsy sample contribute little to the overall mRNA pool. Similar observations have been reported by Wong *et al*. (2004) for IL-8 mRNA expression in tape *versus* biopsy samples of normal and irritated skin.

The potential for tape samples to enrich for surface cells compared to biopsy samples is created by the much larger surface area of the tape relative to the 4 mm punch biopsy, resulting in a many-fold advantage for tape (discussed in Wong *et al*., 2004). We conclude that TNF$\alpha$ mRNA is increased in lesional tissue, largely in the immature stratum corneum. This result supports the idea that a thorough analysis of tape-strip samples of lesions may reveal differentially expressed mRNAs that are not detected in biopsy samples, which, if true will lead to a reanalysis of the role this modified barrier might have on the expression and maintenance of disease.

Inspection of the (gene/$\beta$-actin) mRNA ratio (i.e. $\Delta C_t$ values; Table S1) for IFN$\gamma$, IL-12B, IL-23A, and vascular endothelial growth factor (VEGF) also reveals that the (gene/actin) mRNA ratios for these genes are higher in tape-strip samples than in epidermis or dermis samples. Thus, five of the seven mRNA we have assayed in tape and biopsy samples have higher expression in mRNA samples obtained by tape strippings.

In contrast to our discussion above on increased mRNA expression in tape samples relative to deeper biopsy samples, K16 mRNA expression shows a different pattern. Tape-strip samples of lesional skin shows a slight but significant increase (2-fold; Table 1) relative to non-lesional skin. In contrast, biopsy samples of lesions display a large increase in K16

mRNA expression relative to non-lesional skin (both epidermal and dermal fractions; Table 3). If one compares the (K16/$\beta$-actin) mRNA ratio in tape-strip RNA from non-lesional samples to that same ratio in lesion epidermal- or dermal-biopsy samples we observe that this ratio is approximately equal (i.e. compare the $\Delta C_{t,\ K16}$ values; −1.46 (non-lesional skin, tape) *vs* −2.35 (lesional skin epidermis) *vs* −2.49 (lesional skin, dermis); Table S1). These data indicate that tape stripping recovers a cell population from non-lesional skin that is actively transcribing K16 mRNA almost to the extent of psoriatic tissue (we observe similar data in tape-stripping skin from normal, healthy individuals (data not shown)). In a psoriatic lesion, this active transcription extends to the epidermis and dermis and is virtually equal (Table S1). The modest change in K16 mRNA expression in lesions revealed by tape stripping make K16 mRNA expression a poor biomarker in a tape-stripping assay.

We have used our $\Delta C_t$ data (Table S1) for samples recovered by tape-stripping non-lesional skin to establish average control values for each mRNA. By comparison of these average control $\Delta C_t$ values to individual lesion $\Delta C_t$ values we can determine if an individual lesion overexpresses a specific mRNA without reference to that particular patient's control skin. The result has been the demonstration that some lesions have mRNA levels characteristic of ''average'' non-lesional skin. Thus, while the average psoriatic lesion has increased quantities of the mRNAs shown in Table 1, only 54% of the lesions had elevated levels of all TNF$\alpha$, IFN$\gamma$, CD2, IL-12B, IL-23A, and VEGF mRNAs, the remaining 46% of lesions had at least one mRNA within control skin limits (K16 expression is not considered in this analysis).

Intriguing in this analysis were six patients whose lesions had normal levels of at least four of TNF$\alpha$, IFN$\gamma$, CD2, IL-12B, IL-23A, and VEGF mRNAs. All six of these patients had control levels of IL-23A and IL-12B, while five of six had control levels of IFN$\gamma$ and CD2 mRNAs. Four of the six patients had control levels of TNF$\alpha$ mRNA. In these six patients, the remaining mRNAs which were not within control values, were very close to that limit, suggesting that the molecular physiology of these lesions was quite different than that of the typical psoriatic lesion.

While the significance of these observations remains to be elucidated it harmonizes with the widely held belief that the disease waxes and wanes. Thus, it would be reasonable to conjecture that quantitation of markers of disease activity of lesional and non-lesional skin would have expression levels that overlap. Over 20 years ago Krueger *et al*. noted that while lesions of psoriasis have increased rates (2.3 times) of epidermal proliferation, about 10% of subjects had average proliferation rates from five lesional and five non-lesional sites from similar anatomical locations that were nearly identical (Krueger, 1981). Our findings of a cohort of lesions where molecular markers of disease are similar to those seen in non-lesional skin support a molecular basis for the waxing and waning that seems inherent to this disease. The analyses of message in this study further reinforce this early work and Table 2 quantifies this concept.

The demonstration of heterogeneity in lesion mRNA expression has precedent in the literature. Oestreicher *et al*. (2001) analyzed lesions from eight patients by DNA microarray and showed a wide variation in the patient-to-patient values of differentially expressed genes compared to control skin. Trepicchio *et al*. (1999) have suggested that heterogeneity in K16 and IL-8 expression is correlated with response or lack of response to human recombinant IL-11 therapy. In their study of IL-23 p19 and p40 subunit mRNA expression, Lee *et al*. (2004) show individual patient data revealing that some lesion mRNA expression is close to the average non-lesional mRNA expression level.

The data in Table 2 invokes some provocative questions. For instance, of the five patients with control values of lesion TNF$\alpha$ mRNA levels, would an immunomodulatory agent designed to decrease TNF$\alpha$ levels have a therapeutic effect? Likewise, would the 24% of patients with control values of CD2 mRNA in lesions respond to a therapy targeting CD2 protein? The answers to such questions have implications for the potential efficacy of a therapeutic regime. Answering these questions is beyond the scope of this report; however, tracking RNA profiles via tape strips during clinical trials and correlating them with clinical outcome would answer the question as well as and do so without the downsides of invasive biopsies.

We have recently shown that the method can be used to sample patients undergoing treatment and we have observed gene expression changes in lesions before clinical effect (N. Benson, G. Krueger, unpublished observations). In conclusion, our work demonstrates the utility of non-invasive tape stripping as a means of collecting RNA from lesional and non-lesional skin of psoriatic subjects. The nature of the method allows it to be used repeatedly at the same site over time or on many sites during a single visit, a quality that is unique to this approach. We demonstrate that it is feasible to sample psoriatic plaques and obtain valuable molecular data with minimal discomfort to the patient, although clearly tape stripping cannot provide samples of cells from the deep epidermis or dermis. In addition, we have shown that tape harvesting has identified a source of TNF$\alpha$ mRNA not revealed by biopsy, suggesting that other markers of the disease may have gone unnoticed in biopsies, thus demonstrating that tape stripping could be a valuable supplement to biopsy. This method may also be of value in non-life-threatening diseases where a biopsy would not be an option for a routine diagnostic test.

## MATERIALS AND METHODS

### Clinical protocol

This study was IRB approved and all patients signed informed consent. The study was conducted according to the Declaration of Helsinki Principles. Research subjects participating in tape stripping were taken from a population of subjects enrolled in the Utah Psoriasis Initiative (UPI, Salt Lake City, UT). During the initial UPI examination, psoriasis severity was established using the National Psoriasis Foundation Psoriasis Score (Carlin *et al.*, 2003) for the evaluation of erythema, induration, and scale, while a body surface

area measurement was determined using the estimation of one palm's area of affected skin representing one percent body surface area involvement.

Following the medical history and physical examination, those subjects who showed an National Psoriasis Foundation Psoriasis Score plaque induration of $\geqslant 3$ ($\approx 0.75$ mm) underwent tape stripping of lesional and non-lesional sites. The choice of an induration (thickness of plaque) score $\geqslant 3$ was based on a frequency analysis of induration of a large ($\sim 200$) group of untreated subjects using the National Psoriasis Foundation Psoriasis Score Card. This card is embossed with six levels of elevation which increase at 0.25 mm intervals where a score of 0 is no elevation and a score of 5 corresponds to an elevation of $\geqslant 1.25$ mm. This simple device was developed to provide a consistent comparison of the induration of lesions. The frequency analysis (unpublished data) showed that this assessment of induration has a Gaussian distribution where thickness scores of 3 were observed most frequently and where 1 and 5 were observed least commonly. The psoriatic plaque chosen for tape stripping was one that the investigator felt was representative of the subject's overall level of severity. Tape strips were handled with gloves so as not to contaminate the samples with the investigator's RNA. A circular tape disc was placed on the lesion and, using the rounded end of a pen, Eppendorf tube or the investigator's thumb, moderate to hard pressure was applied to the disc and moved in a circular fashion to cover the surface of the tape for 20 cycles or for approximately 20 seconds. The tape disc was then removed and placed into its storage bag. This process was repeated at precisely the same site with an additional three tape discs. Collection of non-lesional skin took place in a similar manner, with four discs applied sequentially to each of three non-lesional sites. Thus, each subject had a total of 12 tape discs collected from non-lesional sites and four tape discs from lesional skin. The three sites used for the collection of non-lesional skin were: the deltoid and sites bilaterally on the back at the level of the mid trapezius; as the project proceeded the mastoid process was substituted for deltoid sites. If these areas had nearby disease, defined as $<5$ cm from the proposed site, clinically normal skin from other, non-extensor sites above the elbow and knee were used as the non-lesional site; intertriginous, facial, scalp, and genital skin sites were not used. Immediately after placing the tape discs into their storage bags the samples were moved to a freezer and stored at $-70$°C. At the time of transport, the samples were placed in dry ice and delivered overnight to DermTech International for RNA isolation.

The biopsy sites for the 10 subjects donating both tape strips and biopsies were from the same general locations as the tape strips. Biopsy and tape-strip sites were within 2 cm of each other for both the lesional and non-lesional skin. Local anesthesia, 1% xylocaine, was used for each of the 4 mm punch biopsies collected. Biopsies were immediately placed in RNA Later at 4°C. After 96 hours the upper layer (which our experience shows contains most to all of the epidermis) was manually sliced away from the remainder (mostly dermis) of the biopsy with a thin double-edged razor blade while holding the dermal component with a forceps. The thickness of the biopsy was measured with a micrometer that accurately measures to 1 $\mu$m; the upper, mostly epidermal component, of non-lesional biopsies ranged in thickness from 100 to 120 $\mu$m and that of lesional skin ranged from 110 to 150 $\mu$m. Storage in RNA Later partially fixes the skin which makes it easier to identify the boundary of the epidermis and dermis and the firmness facilitates the manual separation of epidermis from the dermis. Each component was returned to clean RNA Later, and stored at $-70$°C until shipped on dry ice to DermTech International. Care was taken, throughout the process – from taking the biopsy to separation of the skin into the two layers – to avoid contaminating the specimens with RNA from the operator by using gloves, clean slides, and instruments that had been placed in a flame until red hot. Data for only nine psoriatic lesion biopsies are presented because one sample was lost during handling.

### Materials and reagents

The tape used was purchased from Adhesives Research (PN 90068-991; Glen Rock, PA) in bulk rolls. These rolls were custom fabricated into small circular discs, 17 mm in diameter, by Diagnostic Laminations Engineering (Oceanside, CA). The adhesive on this tape is a synthetic rubber and was chosen because it seems to recover more RNA from normal skin than acrylic-based tapes (data not shown). In comparison of this tape to D-squame (CuDerm, Dallas, TX) and several acrylic adhesives provided by Adhesives Research we found that the 90068 adhesive outperformed acrylic adhesives for the amount of RNA recovered with four tapes applied to a site on the upper back (data not shown). In addition, we found that the 90068 adhesive mounted on a polyurethane (soft and flexible) backing outperformed the same adhesive on a polyester (rigid) backing (data not shown). However, it is likely that less-efficient tapes will recover RNA, but will require higher numbers of tape applications to do so. Total spleen RNA was purchased from Ambion (Austin, TX). RNeasy RNA extraction kit and Sensiscript Reverse Transcriptase kit were purchased from Qiagen (Valencia, CA). PCR primers and probes (TaqMan Pre-Developed Assay Reagents) and TaqMan Universal Master Mix, which included all buffers and enzymes necessary for the amplification and fluorescent detection of specific cDNAs, were purchased from Applied Biosystems (Foster City, CA).

### Isolation of RNA

The RNA within skin cells adherent to the four tapes used to harvest a site was pooled by simultaneously extracting the tapes in a volume of buffer RLT as previously described (Wong *et al.*, 2004). In addition to this procedure, we have recently developed an alternative protocol that speeds processing considerably; this is our current preferred method. This procedure uses a novel pressure cycling technology to lyse skin cells (Garrett *et al.*, 2002; Schumacher *et al.*, 2002). Tapes are extracted in pairs by insertion into a PULSE™ tube (Pressure Biosciences, Gaithersburg, MD) with 1.2 ml of buffer RLT (supplied in the Qiagen RNeasy kit). The buffer used to extract the first two tapes in a set of four was used to extract the second pair of tapes in order to pool the contents of all four tapes. The PULSE™ tube is inserted into the pressure cycling technology-NEP2017 pressure cycler and the sample is pressure extracted using the following parameters: room temperature, five pressure cycles of 35 Kpsi with pressure held for 20 seconds at the top and bottom of each cycle. After pressure extraction the buffer is removed and processed according to the standard Qiagen RNeasy protocol. For control samples, which involved pooling three sites (12 tapes), each set of four tapes was extracted and treated separately until the column purification stage, at which point all three control samples were adsorbed onto the same column. We have found that purification protocols which dissolve the adhesive (e.g., phenol/chloroform)

interfere with the recovery of RNA with Qiagen column purification kits, this was true even when purified RNA was spiked onto a fresh tape before phenol extraction (data not shown). For biopsy samples, epidermal and dermal fractions (described above) were extracted using the pressure cycling technology procedure in 1.2 ml of buffer RLT and RNA purified with the RNeasy kit.

### Reverse transcription and amplification/detection

Of RNA, 10 $\mu$l was reverse transcribed into cDNA with the Sensiscript Reverse Transcriptase kit using random hexamer primers in a final volume of 20 $\mu$l according to the manufacturer's directions. The reaction was diluted 5-fold with sterile, nuclease-free water for use in the subsequent amplification/detection reaction. For each specific mRNA detection, three replicate $RT^+$ reactions and one $RT^-$ (no reverse transcriptase; negative control) reaction were performed. Two amplification/detection reactions were carried out on each $RT^+$ reaction to yield a total of six independent determinations of the threshold value ($C_t$; discussed below). Amplification and detection assays were performed using TaqMan Pre-Developed Assay Reagents ($\beta$-actin, 4326315E; TNF$\alpha$, Hs99999043_m1; IFN$\gamma$, Hs99999041_m1; Keratin 16, Hs00373910_g1; CD2, Hs00233515_m1; IL-12B (p40), Hs00233688_m1; IL-23A (p19), Hs00372324_m1; VEGF, Hs00173626_m1; Applied Biosystems, Foster City, CA) or Assay on Demand reagents on an Applied Biosystems 7900HT Sequence Detection System as previously described (Wong *et al.*, 2004). All $RT^-$ reactions were amplified using two replicates and were negative (data not shown).

### Quantitation of RNA and determination of fold change

Quantitation of RNA mass was performed as previously described (Wong *et al.*, 2004). Briefly, RNA mass recovered from tapes is determined by using quantitative reverse transcriptase-PCR with reference to a standard curve ($C_{t,actin}$ *vs* log[RNA]; AppliedBiosystems 2001) created from commercially purchased human spleen total RNA. Amplification and detection of unknowns was accomplished using $\beta$-actin mRNA as the quantified marker. The average of six replicate $C_{t,actin}$ values was used to calculate the concentration of RNA in a sample with reference to the standard curve. The calculation of change in gene expression was determined using the comparative or $\Delta\Delta C_t$ method as described by Wong *et al.* (2004). In this method (5′-nuclease assay, "real-time" PCR) experimental data is reported as the number of PCR cycles required to achieve a threshold fluorescence (the "$C_t$" value; Gibson *et al.*, 1996; Heid *et al.*, 1996; AppliedBiosystems 2001). Each reported $C_t$ is the mean of six replicate assays (described above) and is directly related to the starting concentration of specific cDNA in the sample. In order to compensate for samples with different total RNA masses, individual mRNAs are normalized to $\beta$-actin mRNA in each sample. Normalization is accomplished by separate assay of $\beta$-actin mRNA and subsequent determination of the $\Delta C_{t,gene}$ value which is equal to $C_{t,gene} - C_{t,actin}$. The $\Delta C_t$ value is a measure of specific mRNA relative to $\beta$-actin mRNA in a sample (*Z*) and is related to the (gene/$\beta$-actin) mRNA ratio by the equation:

$$\left(\frac{\mathrm{mRNA}_X}{\mathrm{mRNA}_{\beta-\mathrm{actin}}}\right)_{\mathrm{Sample}Z} = k2^{-\Delta C_{t,X}} = k2^{-(C_{t,X} - C_{t,\mathrm{actin}})}$$

In practice, "*k*" is an unknown constant related to the PCR conditions and the fluorescent probe; because *k* is unknown, the absolute ratio in a sample is also unknown; therefore, in order to determine changes in gene expression using this method, experimental samples must be calibrated to a control sample, which in this work is typically from uninvolved skin. Thus, given two samples (psoriatic lesion and control skin), the change in the (TNF$\alpha$/$\beta$-actin) mRNA ratio in the lesion relative to the control is given by the equation below:

$$\left(\frac{\mathrm{mRNA}_{\mathrm{TNF}}}{\mathrm{mRNA}_{\mathrm{actin}}}\right)_{\mathrm{lesion}} \Big/ \left(\frac{\mathrm{mRNA}_{\mathrm{TNF}}}{\mathrm{mRNA}_{\mathrm{actin}}}\right)_{\mathrm{control}} = 2^{-\Delta\Delta C_{t,\mathrm{TNF}}^{\mathrm{lesion}}}.$$

Where $\Delta\Delta C_{t,TNF}^{lesion} = \Delta C_{t,TNF}^{lesion} - \Delta C_{t,control}^{control}$ and $\Delta C_t$ is defined above. All normalized gene expression values ($\Delta C_t$ values) were calculated from $C_t$ values of the gene of interest and $\beta$-actin, which were determined during the same amplification/detection assay. In this work, we typically refer to changes in the (gene/actin) mRNA ratio relative to a control ratio to infer a change in the mRNA of interest; occasionally refer to changes in specific gene expression, however, it is to be understood that all changes in gene expression are inferred by reference to the (gene/actin) mRNA ratio and the assumption that $\beta$-actin expression is unchanged between the experimental and control samples, this assumption was verified for sodium lauryl sulfate-irritated skin (Wong *et al.*, 2004) and we believe accurate for psoriatic skin (N. Benson, unpublished observations).

### Classification of psoriatic and control mRNA expression levels

Statistical analysis of average $\Delta C_t$ data in Table S1 demonstrates that these values are significantly different for lesional and control skin. To determine if a particular $\Delta C_t$ value is more characteristic of lesional or control skin we assign it to one or the other category if the $\Delta C_t$ is within 1 SEM of either the average lesion $\Delta C_t$ or control $\Delta C_t$. We have used this strategy to define whether lesion $\Delta C_t$ values are within non-lesional limits (Table 2). To classify a lesion $\Delta C_t$ value as within non-lesional limits we have taken the average non-lesional $\Delta C_t$ value for a specific mRNA and added one SEM. If a lesion sample has a $\Delta C_t$ greater than or equal to this value, that $\Delta C_t$ (hence that lesion) is classified as within control limits.

## CONFLICT OF INTEREST

N.R.B., R.W., and V.T. are employees of DermTech International; G.G.K. is a member of the DermTech International Scientific Advisory Board.

## SUPPLEMENTARY MATERIAL

**Table S1.** Average $\Delta C_t$ values for lesional and control skin in tape and biopsy samples.

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