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Regulated genes in psoriatic skin during treatment with fumaric acid esters

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H.B.T. and E.P.P. share senior authorship.

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Summary

Background Fumaric acid esters (FAEs) are widely used in Europe for the treatment of psoriasis because of their clinical efficacy and favourable safety profile. However, the mechanisms of action by which FAEs improve psoriasis remain largely unknown.

Objectives To identify pathways and mechanisms affected by FAE treatment and to compare these with pathways affected by treatment with the antitumour necrosis factor (anti-TNF)-α biologic etanercept.

Methods In a prospective cohort study, 50 patients with plaque psoriasis were treated with FAEs for 20 weeks. Nine patients were randomly selected for gene expression profiling of plaque biopsies from week 0 and week 12. The groups consisted of FAE responders [> Psoriasis Area and Severity Index (PASI)-75 improvement] and nonresponders (< PASI-50 improvement). Changes in gene expression profiles were analysed using Ingenuity Pathway Analysis (IPA) and the outcome was compared with gene expression affected by etanercept.

Results Response to FAE treatment was associated with a ≥ 2-fold change (P < 0.05) in the expression of 458 genes. In FAE responders the role of interleukin-17A in the psoriasis pathway was most significantly activated. Glutathione and Nrf2 pathway molecules were specifically induced by FAE treatment and not by etanercept treatment, representing an FAE-specific effect in psoriatic skin. In addition, FAE treatment specifically induced the transcription factors PTTG1, NR3C1, GATA3 and NFκBIZ in responding patients.

Conclusions FAE treatment induces glutathione and Nrf2 pathway genes in lesional skin of patients with psoriasis. In responders, FAEs specifically regulate the transcription factors PTTG1, NR3C1, GATA3 and NFκBIZ, which are important in normal cutaneous development, and the T-helper (Th)2 and Th17 pathways, respectively.

What’s already known about this topic?

- Fumaric acid esters (FAEs) are used in the treatment of psoriasis, but the mechanisms of action are poorly known.
- In vitro actions of FAEs include inhibition of keratinocyte proliferation and inhibition of dendritic cell maturation.

What does this study add?

- FAE treatment of patients with psoriasis specifically induces activation of the Nrf2 and glutathione pathways in psoriatic skin.
Psoriasis is a common chronic inflammatory skin disease, characterized by hyperproliferation of keratinocytes and an increased dermal infiltration by immune cells, notably neutrophils and T helper (Th)1/Th17 cells. Most patients with moderate-to-severe disease require long-term systemic treatment to control their psoriasis. Fumaric acid esters (FAEs) are small molecules that have been used as oral treatment in psoriasis for more than 25 years, mainly in Western Europe. Of patients with psoriasis have been used as oral treatment in psoriasis for more than 25 years, mainly in Western Europe. Of patients with psoriasis treated with FAEs, 50–70% show a clinical improvement of at least 75% following 16 weeks of treatment. This treatment response is comparable to the efficacy of first-generation anti-tumour necrosis factor (TNF-α) biologics, but at a fraction of the costs. Data from long-term observational studies on treatment of patients with psoriasis with FAEs indicate a favourable safety profile without evidence for an increased risk of infections or malignancies. In vitro, FAEs inhibit dendritic cell maturation and keratinocyte proliferation.

Anti-TNF-α biologics, including etanercept, are commonly used effective systemic treatments for moderate-to-severe psoriasis. In recent years, gene expression profiling studies have provided insights into the mechanisms of action and the signalling pathways by which anti-TNF-α biologics improve psoriasis. However, the molecular pathways by which FAEs improve psoriasis remain largely unknown. FAEs have not been studied by gene expression profiling nor have they been compared with anti-TNF-α biologics.

In this study we investigated pathways and mechanisms targeted by FAE treatment, and assessed whether successful FAE treatment invoked different molecules and pathways from etanercept treatment. Gene expression profiling was performed on RNA derived from biopsies of psoriatic plaques taken before and after 12 weeks of FAE treatment. We then compared the molecules and pathways that were differentially affected by FAE treatment with those affected by etanercept treatment.

Materials and methods

Study design and skin biopsies

In a prospective, single-centre clinical study, 50 patients with a Psoriasis Area and Severity Index (PASI) ≥ 10 were treated with oral FAE for 20 weeks. In the Netherlands, the import of Fumaderm tablets from Germany is often not reimbursed by insurance companies. We therefore used a Dutch FAE formulation with enteric-coated tablets containing 105 mg FAEs (30 mg dimethylfumarate, 75 mg calcium monoethylfumarate) and 215 mg FAEs (120 mg dimethylfumarate, 95 mg calcium monoethylfumarate). The short-term efficacy of this FAE formulation is comparable with that of oral methotrexate 15 mg weekly.

Eligible patients were at least 18 years of age, had a diagnosis of plaque psoriasis for at least 6 months, and were candidates for phototherapy or systemic therapy. Patients were excluded when they had received systemic psoriasis therapy or phototherapy within the previous 4 weeks, or had received topical psoriasis treatment within 2 weeks. All patients were dosed according to the German S-3 guideline on systemic treatment of psoriasis (see Supporting Information Table S1). Lesional skin biopsies (3 mm) were taken at baseline from the edge of a well-defined psoriasis plaque on the legs and after 12 weeks, from the same plaque, near to the previous biopsy site at baseline. The interval of 12 weeks was chosen because subjects had by then reached the maximum daily dosage of FAEs and after 12 weeks of treatment a clinically meaningful improvement is expected in daily clinical practice. Responders were defined as having a PASI improvement ≥75% at week 12 compared with baseline, while nonresponders were defined as having a PASI improvement <50%, as defined in the European treatment goal consensus.

Patients were randomly selected for gene expression profiling based on clinical improvement. The intermediate responders (PASI 50–75; n = 19) and dropouts (n = 13) were not included in the analysis.

The clinical study protocol was approved by the local medical ethical committee (MEC 2005-105), and all patients gave written informed consent prior to study enrolment. The study was conducted according to the principles of the Declaration of Helsinki.

RNA processing and microarray hybridization

From the 10 patients showing a clinical PASI improvement of more than 75% at week 12 (PASI >75 response), four patients were randomly selected as clinical responders and from the eight patients with a PASI <50 response, five patients were randomly selected as clinical nonresponders for microarray analysis. RNA was extracted from whole-skin biopsies (epidermis and dermis) of these nine patients and after 12 weeks of treatment a clinically meaningful improvement is expected in daily clinical practice. Responders were defined as having a PASI improvement ≥75% at week 12 compared with baseline, while nonresponders were defined as having a PASI improvement <50%, as defined in the European treatment goal consensus.

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Results

Clinical response to oral fumaric acid ester treatment

The baseline characteristics of the selected patients with psoriasis (six male and three female patients) are summarized in Table S3 (see Supporting Information). The median PASI reduction after 12 weeks of FAE treatment in the total group of patients (n = 50) was 65.2% [interquartile range (IQR) 50.6–77.8%]. The median PASI at baseline was 13.6 (IQR 11.3–16.4), which decreased to 5.5 (IQR 3.3–7.6) following 12 weeks of FAE treatment (P < 0.001). The clinical responders, randomly included for array analysis, had a median decrease in PASI of 84.3% (IQR 77.5–89.4%) whereas the clinical nonresponders had a median PASI decrease of 40.4% (IQR 34.8–44.7%). The difference in PASI reduction was statistically significant (P = 0.02; see Supporting Information Figure S1).

Differentially expressed genes in the skin of patients with psoriasis treated with fumaric acid esters

After 12 weeks of FAE treatment, 24 genes were differentially expressed in the lesional skin of patients with psoriasis (responders and nonresponders). Seven of these were downregulated [including psoriasin (S100A7), calgranulin-B (S100A9), pentraxin 3 (PTX3), matrix metalloproteinase 1, lipocalin 2 (LCN2), desmocollin 2 (DSC2) and 5-hydroxytryptamine (serotonin) receptor 3A]. Seventeen molecules were upregulated including dermcidin, secretogulobulin, prolactin-induced protein (PIP), keratins and NADPH dehydrogenase (NQO1; Table 1).

Most of the genes that were differentially expressed in the skin of patients with psoriasis are known markers of psoriatic inflammation, such as psoriasin, calgranulin-B, LCN2, DSC2 and PTX3. PIP is an immunosuppressive molecule.17,18

Differentially expressed genes in responders to fumaric acid esters

We also analysed gene expression changes separately in the group of responders and in the group of nonresponders, in order to identify molecules and pathways that might be responsible for the clinical improvement of the psoriasis lesions. In responders, 458 genes were differentially expressed in lesional skin before treatment vs. 12 weeks after the start of the treatment (166 upregulated, 292 downregulated, ≥ 2-fold, P < 0.05; Supporting Information Table S4). In the FAE-treated responders several upregulated keratin genes were detected, including keratin (K)15, which is downregulated in activated keratinocytes and in psoriatic skin.19,20 Thus the upregulation of K15 in FAE-treated responding patients was illustrative for the induced transition to normal skin. Similarly, the expression of K16 and K17, markers of keratinocyte hyperproliferation that are upregulated in psoriatic skin, were significantly downregulated in FAE responders after 12 weeks (Supporting Information Table S4). Several molecules of the
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Table 1  Complete list of differentially expressed genes in patients treated with fumaric acid esters (responders and nonresponders) week 0 vs. week 12

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Description</th>
<th>Fold change</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCD</td>
<td>Democidin</td>
<td>7.521</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SCGR2A2</td>
<td>Secretoglobin, family 2A, member 2</td>
<td>6.227</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SCGB1D2</td>
<td>Secretoglobin, family 1D, member 2</td>
<td>4.055</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PIP</td>
<td>Prolactin-induced protein</td>
<td>3.797</td>
<td>0.034</td>
</tr>
<tr>
<td>KRT19</td>
<td>Keratin 19</td>
<td>3.153</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CA6</td>
<td>Carbonic anhydrase V1</td>
<td>2.783</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>THRB</td>
<td>Thyroid hormone responsive</td>
<td>2.695</td>
<td>0.043</td>
</tr>
<tr>
<td>ATP6V0A4</td>
<td>ATPase, H+ transporting, lysosomal V0 subunit a4</td>
<td>2.464</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LGR5</td>
<td>Leucine-rich repeat containing G protein-coupled receptor 5</td>
<td>2.399</td>
<td>0.011</td>
</tr>
<tr>
<td>CLDN10</td>
<td>Claudin 10</td>
<td>2.342</td>
<td>0.008</td>
</tr>
<tr>
<td>DNER</td>
<td>Delta/notch-like EGF repeat containing</td>
<td>2.331</td>
<td>0.005</td>
</tr>
<tr>
<td>NQO1</td>
<td>NAD(P)H dehydrogenasequinone 1</td>
<td>2.244</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PFABG1A</td>
<td>Peroxisome proliferator-activated receptor gamma</td>
<td>2.157</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>REHNP2</td>
<td>Rhophilin, Rho GTPase binding protein 2</td>
<td>2.116</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>KRT7</td>
<td>Keratin 7</td>
<td>2.083</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>BTC</td>
<td>Betacellulin</td>
<td>2.04</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SLC12A2</td>
<td>Solute carrier family 12, member 2</td>
<td>2.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S100A9</td>
<td>S100 calcium binding protein A9 (calgranulin-A)</td>
<td>−2.065</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HTR3A</td>
<td>5-Hydroxytryptamine (serotonin) receptor 3A, ionotropic</td>
<td>−2.138</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DSC2</td>
<td>Desmocollin 2</td>
<td>−2.227</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LCN2</td>
<td>Lipocalin 2</td>
<td>−2.414</td>
<td>0.039</td>
</tr>
<tr>
<td>PTX3</td>
<td>Pentraxin 3, long</td>
<td>−2.48</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MMP1</td>
<td>Matrix metalloepitidase 1 (interstitial collagenase)</td>
<td>−2.589</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>S100A7A</td>
<td>S100 calcium binding protein A7A (psoriasin)</td>
<td>−4.443</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

epidermal differentiation complex were significantly downregulated after 12 weeks in responders to FAE treatment including LCE3D (late cornified envelope 3D), involucrin and several members of the small proline-rich (SPRR) family. The expression of inducible nitric oxide synthase and interleukin (IL)-20 was significantly reduced after 12 weeks of FAE treatment (Supporting Information Table S4).

The list of differentially expressed genes was analysed by IPA. In responders, the IL-17A pathway was the most significantly affected, with a downregulated expression of the chemokines CCL20, CXCL1 and CXCL6, the antimicrobial peptides β-defensin 2 (DEFB4), psoriasin (S100A7), calgranulin-A (S100A8) and calgranulin-B (S100A9) and the cytokines IL-8 and IL-17A (Table 2). When validating these findings with qRT-PCR, we confirmed a significant decrease in the expression of these genes after 12 weeks of FAE treatment (Supporting Information Figure S2). Expression of the signal transducer and activator of transcription (STAT)3 gene was significantly (−2.3-fold, P < 0.001) downregulated in biopsies of lesional psoriatic skin of responders after 12 weeks of FAE treatment (Supporting Information Table S4). To verify that STAT3 protein was also downregulated, immunofluorescent staining of phosphorylated STAT3 on these skin biopsies was performed. This showed a clear reduction of phosphorylated STAT3 at week 12 in responders, which is indicative of repression of the Th17 pathway (Fig. 1).

Furthermore, a downregulation of the expression of the proinflammatory cytokines IL-1β, IL-22, IL-36α (IL-1F6) and IL-36γ (IL-1F9) and an upregulation of the anti-inflammatory IL-37 (IL-1F7) was associated with successful FAE treatment (Supporting Information Table S4).

**Differentially expressed genes in nonresponders to fumaric acid esters**

In the nonresponding patients, a differential expression of 35 genes was found: two of these were downregulated and 33 were upregulated. IPA showed activation of the glutathione signalling pathway [microsomal glutathione S-transferase (MGST)1, glutathione peroxidase 2 (GPX2)], the Nrf2 pathway (MGST1, NQO1 and GPX2), superoxide radical degeneration (NQO1) and that of xenobiotic mechanism signalling [MGST1, NQO1, peroxisome proliferator-activated receptor gamma]. These pathways were enriched in both nonresponders and responding patients (Table 2).

**Comparison of gene expression profiles between fumaric acid esters and etanercept**

The differentially expressed genes in the skin of FAE responders before and after treatment were compared with the differentially expressed genes before and after successful treatment with etanercept.10 When using the same cut-off values (> 2-fold change, P < 0.05), we found 112 upregulated and 208 downregulated genes (Fig. 2a). We compared the change in gene expression of psoriasis-related molecules during FAE and
etanercept treatment and found an overlap of 122 significantly downregulated genes and of 35 significantly upregulated genes. Overlapping downregulated genes included CCL20, CXCL1, DEFB4A and several S100 family genes (Fig. 2a). All overlapping genes are known markers of the psoriatic transcriptome and are likely important for lesion improvement as they were downregulated during successful treatment with both FAEs and etanercept.

Several molecules and pathways that were differentially expressed in FAE-treated patients did not alter during etanercept treatment. FAE downregulated 170 genes and upregulated 131 genes that were not differentially affected by etanercept treatment (Tables 3 and 4). FAE-specific pathways included the Nrf2, superoxide radicals degradation, eicosanoid signalling and IL-17 signalling in fibroblast pathways (Fig. 2). NQO1 is part of the Nrf2 pathway. The aryl hydrocarbon receptor (AHR) can regulate the Nrf2 pathway and AHR knockdown in keratinocytes partly inhibits NQO1 induction by coal tar. During FAE treatment the AHR was not differentially expressed; however, the AHR signalling pathway was differentially expressed in FAE responders, but not in etanercept responders.

### Table 2

<table>
<thead>
<tr>
<th>Canonical pathways</th>
<th>P-value</th>
<th>Upregulated</th>
<th>Downregulated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FAE responders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Role of IL-17A in psoriasis</td>
<td>&lt; 0.001</td>
<td>IL17</td>
<td>CCL20, CXCL1, CXCL6, DEFB4A/DEFB4B, IL8, IL17A, S100A8, S100A9</td>
</tr>
<tr>
<td>Role of cytokines in mediating communication between cells</td>
<td>&lt; 0.001</td>
<td>IL37</td>
<td>IL8, IL20, IL24, IL12B, IL17A, IL1A, IL1B, IL1RN, IL56A, IL56G, IL56N</td>
</tr>
<tr>
<td>Atherosclerosis signalling</td>
<td>&lt; 0.001</td>
<td>IL37, PLA2R1</td>
<td>ALOX12B, ALOX15B, IL8, IL1A, IL1B, IL1RN, IL56A, IL56G, IL56N, MMP1, PLA2G3, PLA2G2A, PLA2G4D, S100A8, SERPINA1</td>
</tr>
<tr>
<td>Dendritic cell maturation</td>
<td>&lt; 0.001</td>
<td>IL37, LEPR, PIK3C2G, PLCB4</td>
<td>CCR7, FCGR1A, FCGR1B, FCGR3B, IL12B, IL1A, IL1RN, IL56A, IL56G, IL56N, LTBR, STAT1</td>
</tr>
<tr>
<td>LXR/RXR activation</td>
<td>&lt; 0.001</td>
<td>IL37</td>
<td>ARG2, CCL7, IL1A, IL1B, IL1RN, IL56A, IL56G, IL56N, LDR, NOS2, S100A8, SAA1, SERPINA1</td>
</tr>
<tr>
<td>IL-10 signalling</td>
<td>&lt; 0.001</td>
<td>IL37</td>
<td>ARG2, IL1A, IL1B, IL1RN, IL56A, IL56G, IL56N, IL4R, STAT3</td>
</tr>
<tr>
<td>p38 MAPK signalling</td>
<td>&lt; 0.001</td>
<td>EEF2K, HSPB3, IL37</td>
<td>IL1A, IL1B, IL1RN, IL56A, IL56G, IL56N, PLA2G3, PLA2G2A, PLA2G4D, STAT1</td>
</tr>
<tr>
<td>Eicosanoid signalling</td>
<td>&lt; 0.001</td>
<td>ABR1C3, PLA2R1</td>
<td>ALOX12B, ALOX15B, FPR2, LTB4R, PLA2G3, PLA2G2A, PLA2G4D</td>
</tr>
<tr>
<td>Communication between innate and adaptive immune cells</td>
<td>&lt; 0.001</td>
<td>IL37</td>
<td>CCR7, IL8, IL12B, IL1A, IL1B, IL1RN, IL56A, IL56G, IL56N</td>
</tr>
<tr>
<td>LPS/IL-1 mediated inhibition of RXR function</td>
<td>&lt; 0.001</td>
<td>ABCC3, ALDH3A2, ALDH6A1, GSTM3, HSST6, IL37, SULT1E1, UST</td>
<td>ALAS1, ALDH1A3, HMGC1, HSST3A1, IL1A, IL1B, IL1RN, IL56A, IL56G, IL56N</td>
</tr>
<tr>
<td><strong>FAE nonresponders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione redox reactions</td>
<td>&lt; 0.001</td>
<td>GPX2, MGST1</td>
<td>GPX2, MGST1</td>
</tr>
<tr>
<td>Putrescine biosynthesis III</td>
<td>0.006</td>
<td>ODC1</td>
<td>ODC1</td>
</tr>
<tr>
<td>Nrf2-mediated oxidative stress response</td>
<td>0.013</td>
<td>GPX2, MGST1, NQO1</td>
<td>GPX2, MGST1, NQO1</td>
</tr>
<tr>
<td>Superoxide radicals degradation</td>
<td>0.017</td>
<td>NQO1</td>
<td>NQO1</td>
</tr>
<tr>
<td>Xenobiotic metabolism signalling</td>
<td>&lt; 0.001</td>
<td>MGST1, NQO1, PPARGC1A</td>
<td>MGST1, NQO1, PPARGC1A</td>
</tr>
</tbody>
</table>

IL, interleukin; LXR, liver X receptor; RXR, retinoid X receptor; PLS, lipopolysaccharide.
Transcription factors

The differential expression of transcription factors due to FAE treatment was compared with that for etanercept treatment. In etanercept responders five transcription factors were differentially expressed, whereas in the FAE responders nine transcription factors were differentially expressed (Table 5). FAE treatment specifically reduced the transcription regulator NFκBIZ (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta) and PTTG1 (pituitary tumour transforming gene 1) in lesional psoriatic skin (Table 5). In addition, FAE specifically upregulated the transcription factors NR3C1 (nuclear receptor subfamily 3, group c, member 1/glucocorticoid receptor) and GATA3, which is an important regulator in T-cell differentiation.26 These transcription factors were not differentially expressed during etanercept treatment.

Discussion

This study assessed the mode of action of FAEs by analysing their effects on the gene expression profile in lesional psoriatic skin before and after 12 weeks of FAE treatment. We compared our findings of FAE treatment with a previously published study that investigated gene expression during etanercept treatment.10 The comparison shows that FAE and etanercept have a considerable overlap in the affected pathways.
leading to psoriasis improvement, including the IL-17 pathway. Responders to FAE treatment showed a differential expression after 12 weeks of many antimicrobial peptide genes. Antimicrobial peptides are significantly upregulated in psoriatic skin representing a disturbance in innate immunity, an important aspect of the pathogenesis of the disease.\(^27\) Human \(\beta\)-defensin 2 even serves as a psoriasis disease severity biomarker.\(^28\) The differential expression during successful FAE treatment is similar to the molecular effects of other systemic treatments for psoriasis and may represent a fingerprint of a successful psoriasis treatment response.

In vitro, dimethylfumarate and its primary metabolite monomethylfumarate induce the expression of the Nrf2/ NQO1 pathway in endothelial cells.\(^29\) The Nrf2 pathway can
be regulated by FAE in neurons, and the neuroprotective effects of fumarates are dependent on Nrf2-mediated antioxidative pathways. An FAE formulation containing dimethylfumarate (BG-12, Biogen Idec, Cambridge, MA, U.S.A.) was recently approved by the U.S. Food and Drug Administration for the treatment of multiple sclerosis. The nervous system and neuronal factors promote inflammation in psoriasis lesions, which are characterized by a high density of nerves and an increased expression of neurotrophins. The Nrf2 pathway has an important antioxidative function and is involved in epidermal barrier function. Our results show that in FAE-treated responding as well as nonresponding patients the Nrf2 pathway is activated and the expression of its major effector molecule NQO1 is induced. The AHR can regulate the Nrf2 pathway, and AHR knockdown in keratinocytes partly inhibits the induction of NQO1 by coal tar. However, during FAE treatment the AHR was not differentially expressed; therefore additional mechanisms might play a role in FAE-induced activation of the Nrf2 pathway. The Nrf2 pathway was differentially expressed in FAE responders as well as nonresponders, but not in the etanercept-treated responders. This suggests that Th2 cell development/skewing is an important target of FAE, which is also illustrated by the known induction of IgE by FAE. In addition, NR3C1 was significantly upregulated only in the FAE-treated responders, but not in the etanercept-treated responders. This was not differentially expressed in etanercept-treated patients, including GPX2, MGST1 and glutathione S-transferases and depletion enzymes in both responding and treated patients.

### Table 5 Differentially expressed transcription factors in patients treated with etanercept and fumaric acid esters (FAEs)

<table>
<thead>
<tr>
<th>FAE Upstream regulator</th>
<th>Fold change</th>
<th>P-value</th>
<th>Etanercept Upstream regulator</th>
<th>Fold change</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFKBIZ</td>
<td>-2.96</td>
<td>0.003</td>
<td>EHF</td>
<td>-5.33</td>
<td>0.005</td>
</tr>
<tr>
<td>ELF3</td>
<td>-2.22</td>
<td>0.046</td>
<td>TP63</td>
<td>-2.49</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ZEB1</td>
<td>-2.06</td>
<td>0.001</td>
<td>STAT3</td>
<td>-3.11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>GATA3</td>
<td>2.12</td>
<td>0.046</td>
<td>ST2</td>
<td>-3.18</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NR3C1</td>
<td>2.30</td>
<td>0.046</td>
<td>ZEB1</td>
<td>2.03</td>
<td>0.001</td>
</tr>
</tbody>
</table>

FAEs have shown in vitro effects on T cells and Th-cell differentiation. GATA3 is an important transcription factor and regulator in T-cell development, and regulation by GATA3 leads to Th2 cell differentiation. In addition, GATA3 is involved in normal epidermal development. GATA3 was significantly upregulated only in the FAE-treated responders, which suggests an FAE-specific effect.

In conclusion, FAE-specific induced pathways in the skin demonstrate that the expression of NFKBIZ, which is required for Th17 development in mice. NFKBIZ-deficient mice are not able to produce Th17 cells. The observed downregulation of NFkB in human psoriatic skin samples is likely important in the inhibition of the Th17 pathway, although this should be confirmed in further experiments. Interestingly, this transcription factor was not differentially expressed in etanercept-treated patients, but only in FAE-treatment responders.

In conclusion, FAE-specific induced pathways in the skin include activation of the Nrf2 and glutathione pathways. FAE-specific molecules that are related to response to treatment are the transcription factors TTG1 and NR3C1, which are important, respectively, in keratinocyte regulation and normal cutaneous...
development, and GATA3 and NFκBIZ, which are important in Th2 and Th17 cell development, respectively.

Acknowledgments

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References

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig S1. Clinical response during fumaric acid ester (FAE) treatment. (a) Change in median Psoriasis Area and Severity Index (PASI) in the total group of patients (n = 50) during FAE treatment. Bars represent median and interquartile range. Wilcoxon signed-rank test *P < 0.05. (b) PASI reduction in the selected responders (n = 4) and nonresponders (n = 5) after 12 weeks. Bars represent median and interquartile range. Mann–Whitney U-test *P < 0.05.

Fig S2. Selected molecules for confirmation by quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) in responding patients after 12 weeks of fumaric acid ester (FAE) treatment. The mRNA expression was measured by qRT-PCR in biopsies from psoriatic lesions at baseline and after 12 weeks of FAE treatment in the responders (n = 6–15). The y-axis shows expression relative to that of the housekeeping gene ABL1. Wilcoxon signed-rank test, *P < 0.05, error bars indicate SEM.

Table S1. Dosage schedule of fumaric acid ester treatment.

Table S2. Primers and probes used for quantitative reverse-transcriptase polymerase chain reaction.

Table S3. Demographic and clinical characteristics of the study population.

Table S4. Top 20 genes significantly downregulated in responders following 12 weeks of treatment with fumaric acid esters.