Growth Arrest and DNA Damage-Inducible Proteins (GADD45) in Psoriasis

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Abstract

The interplay between T cells, dendritic cells and keratinocytes is crucial in the development and maintenance of inflammation in psoriasis. GADD45 proteins mediate DNA repair in many cells including keratinocytes. In the immune system, GADD45a and GADD45b regulate the function and activation of both T lymphocytes and dendritic cells and GADD45a links DNA repair and epigenetic regulation by mediating demethylation. Here, we analyzed the expression of GADD45a and GADD45b in the skin, dendritic cells and circulating T cells from psoriasis patients and their regulation by inflammatory signals. Psoriasis patients exhibited a lower expression of GADD45a at the epidermis but a higher expression in dermal infiltrating T cells in lesional skin. Expression of GADD45a and GADD45b was also higher in peripheral T cells from psoriasis patients, although no differences were observed in p38 activation. The expression and methylation state of the GADD45a target UCHL1 were evaluated, revealing a hypermethylation of its promoter in lesional skin compared to non-lesional skin and controls. Furthermore, hypermethylation correlated with lower levels of both GADD45a and UCHL1 in lesional skin. The demethylase function of GADD45a may account for its pleiotropic effects, and the complex and heterogeneous pattern of expression observed in psoriatic disease.

Introduction

Growth Arrest and DNA Damage-inducible proteins, namely GADD45a, GADD45b and GADD45g are small proteins of 18-20 kDa. They exert their functions by protein-protein interactions in the nucleus and cytoplasm and are involved in DNA repair, cell proliferation, survival and differentiation among other biological processes [1]. GADD45 family members are associated with stress responses through the stimulation of p38-JNK mitogen-activated protein kinases (MAPK) that play important roles in both innate and adaptive immune cells. T cell receptor (TCR) engagement induces GADD45 expression. In T cells, both GADD45b and GADD45g favor a sustained p38 activation and subsequently, lead to IFN-γ production and Th1 differentiation [2,3]. In T cells, GADD45b is also induced in response to the pro-inflammatory cytokines IL-12 and IL-18 [4]. In contrast to GADD45b and GADD45g, GADD45a is expressed in resting T cells. GADD45a has been shown to inhibit the alternative p38 activation pathway [5], since GADD45a-deficient T cells displayed constitutive p38 activation. Regarding the innate immune system, dendritic cells (DCs) from GADD45a-deficient mice exhibited a reduced production of Th1 cytokines, IL-12 and a lower expression of co-stimulatory molecules, such as CD40 [6]. Moreover, GADD45b-/- DCs show reduced IL-12 and IL-6 production, and GADD45b-deficient mice exhibited an impaired Th1 response [3].

GADD45 proteins have been associated with autoimmune-related diseases. Hence, GADD45a-deficient mice spontaneously develop an autoimmune disease characterized by the presence of autoantibodies against dsDNA [5]. Moreover, GADD45b-deficient mice showed exacerbated experimental autoimmune encephalomyelitis, while GADD45b and GADD45g double-deficient mice develop a spontaneous lymphoproliferative disease [7].

On the other hand, GADD45 expression has been associated with DNA repair in keratinocytes [8]. It has been described the induction of GADD45 expression by UV light in human keratinocytes [9,10], which has been linked to the production of reactive oxygen species (ROS) and the activation of NADPH oxidase, likely as a mechanism to counter-regulate the oxidative damage. In this sense, GADD45a is implicated in the G2/M cell cycle checkpoint in UV-irradiated cells, and GADD45a-deficient transgenic mice show genomic instability [11]. Interestingly, GADD45a acts as a link between DNA repair and epigenetic gene regulation by mediating demethylation [12]. GADD45a has been demonstrated to target the promoter of MMP-9 and to recruit thymine-DNA glycolase (TDG), inducing the demethylation of the MMP-9 promotor in keratinocytes, thus controling its expression [13]. The loss of GADD45a also significantly reduced ubiquitin carboxyl-terminal hydrolase L1 (UCHL1) expression via UCHL1 promoter methylation [14]. Interestingly, decreased UCHL1 expression has been detected in skin lesions with itch of psoriasis patients, while an increased number of DCs expressing UCHL1 has been described in atopic dermatitis patients [15].

We therefore hypothesized that GADD45 could have a role in the development of psoriasis, a disease where the interaction of keratinocytes and immune cells is pivotal, and where epigenetic modifications cause aberrant increases in epidermal thickness, keratinocyte differentiation, proliferation and inflammation [16].

Materials And Methods

Human patients and samples collection

This study was approved by the Institutional Review Board (IRB)/Independent Ethics Committee of Hospital de la Princesa, according to the Declaration of Helsinki Principles. All methods were performed in accordance with the relevant guidelines and regulations. All participants provided written informed consent. Thereafter, 20 control individuals and 30 patients with untreated plaque psoriasis were enrolled. Patients were eligible for the study if they were adult candidates to systemic therapy. The following washout periods were established: 14 days for topical corticosteroids, 28 days for systemic treatment including corticosteroids, methotrexate, cyclosporine, acitretin or phototherapy and 3 months for biologic agents. From each psoriasis patient, two non-sun-exposed cutaneous biopsies (10 mm) were taken, one from lesional psoriatic skin and another from apparently healthy skin (non-lesional skin). At the same time, 20 ml of peripheral venous blood were extracted. Normal leftover skin samples and peripheral venous blood samples were obtained from 10 surgical patients. Each biopsy was cut in half; one piece was snap frozen for RNA isolation, and the other one included in OCT and stored at -80ºC until processing for immunofluorescence stainings [17].

Quantitative RT-PCR

GADD45a, GADD45b, UCHL1 and IFN-γ mRNA expression levels were determined by quantitative reverse transcription polymerase chain reaction (RT-PCR). Total RNA was isolated from skin samples, peripheral blood $CD4^+$ T cells and moDCs using the TRIzol reagent (Invitrogen) following the manufacturer's instructions. One microgram of RNA was reverse-transcribed to cDNA and amplified with the specific primers pairs using GoTaq qPCR Master Mix (Promega, WI USA). Real-time (RT)–PCR was performed in a CFX384 Real-time System (Bio-Rad) using SYBR Green PCR Master Mix (Applied Biosystems). The data were analysed using StepOne Plus Software (Applied Biosystems®, Carlsbad, CA). GADD45a, GADD45b, UCHL1 and IFN-γ mRNA levels were normalized to GAPDH levels and expressed as relative levels.

Immunofluorescence staining

Skin OCT sections of 5 µm were fixed (formaldehyde 4%), permeabilized (Triton X-100 0,2%) and blocked with 100 µg/ml human γ-globulin (Sigma-Aldrich, St Louis MO, USA) and a 1:100 dilution of donkey serum (Sigma-Aldrich) in phosphate buffer solution (PBS). Skin sections were then incubated over-night with 5µg/ml goat anti-human GADD45a and mouse anti-human CD45 antibodies (Abcam), followed by donkey anti-goat (DAG) Alexa Fluor 488 and DAM Alexa Fluor 555. Finally, cell nuclei were counterstained with DAPI. Negative controls were performed with omission of the primary antibody. Sections were examined with a Leica DMR immunofluorescence microscopy under the same acquisition conditions. Images were analysed using the ImageJ sowftware (http://imagej.softonic.com) GAD45a levels were analysed on regions of interest (ROIs) drawn for $CD3^+$ cells.

Peripheral blood $CD4^+$ T cells and monocyte derived DCs (moDCs) isolation and culture

Peripheral blood mononuclear cells (PBMCs) were obtained by density gradient and $CD4^+$ T cells were isolated by negative selection using magnetic microbeads (Miltenyi Biotec Bergisch Gladbach, Germany). Where indicated, $CD4^+$ T cells were incubated for 24 h in the presence of IL-12 (10ng/ml) plus IL-18 (10ng/ml). For moDCs, PBMCs were allowed to adhere for 30 min at 37ºC, and plastic adhered cells were cultured for 5 days in complete RPMI medium supplemented with 500 U/ml GM-CSF (Peprotech) and 10 ng/ml IL-4 (R&D systems). On day 6, 10ng/ml LPS were added and after 24 h cells were harvested for analysis.

Expression of p38 (pTryr180/pThr183) in $CD4^+$ T cells analysis by flow cytometry

Isolated $CD4^+$ T cells were incubated with anti-CD3/CD28 (10 µg/ml and 5 µg/ml respectively) during 30 min at 4ºC and then with anti-mouse Fc for additional 30 min at 4º and then immediately incubated at 37ºC. After 15min, cells were fixed, permeabilized and stained with mouse anti-human p38 (Becton-Dickinson®) following manufacturer's instructions and analyzed in a FACScanto flow cytometer (BD Bioscience).

Methylation datasets

Datasets from DNA methylation arrays were obtained from the repository Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/). We searched for available methylation datasets performed in psoriasis patients. Three datasets of psoriasis skin samples fulfilled these criteria: GSE63315[18], GSE73894[19] and GSE115797[20]. Skin punch biopsies of 4 mm diameter were collected. Methylation was analysed with an Illumina Infinium Human Methylation 450k BeadChip array following manufacturing protocol.

Although the three selected studies fulfil the inclusion criteria, and were performed with the same sampling and technology, we would like to point out the heterogeneity regarding the objectives of the three studies, and their differences: (1) GSE63315 dataset 1, data comes from 12 pre-UV irradiation moderate-to-severe psoriasis patients and 12 healthy controls; (2) GSE115797 dataset 3 contained lesional (L) and non-lesional (NL) samples from 24 moderate-to-severe plaque psoriasis patients; (3) GSE73894 dataset 2 combines both types of data including 114 samples of lesional skin, 41 of non-lesional skin and 62 of healthy subjects [18-22].

Statistical analyses

Data were analysed with GraphPad Prism (GraphPad Software, San Diego, CA, USA). The Kruskal-Wallis and Mann-Whitney U-tests were used, as appropriate. Where indicated, Wilcoxon signed rank test was used to analyze paired data. The Spearman test was used for correlation analysis. Significance was set at *p < 0.05, **p < 0.01, ***p < 0.001.

Differentially methylated CpG sites between psoriasis and controls were detected by GEO2R analysis tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/) a web-based program that employs the Bioconductor packages GEOQuery [23] and limma[21] in R, with the Benjamini-Hochberg false-discovery rate (FDR). Log2Fold-change of methylation was calculated in psoriasis lesional skin referred to control (psoriasis non-lesional skin or healthy controls' skin). Thus, positive values mean that *UCHL1* is hypermethylated in psoriasis skin with respect to controls and negative values, hypomethylated. Although we have analysed epigenetic differences in all the methylation sites included in this array (485,000), we have focused on those sites located on CpG islands of *UCHL1* promoter that present a FDR adjusted p-value lower than 0.05. We have selected this region since CpG island hypermethylation is commonly associated with gene repression.

Results

Expression of GADD45a and GADD45b genes was analyzed in skin samples from lesional and non-lesional skin of psoriasis patients as well as in skin biopsies from control subjects. Lesional skin from psoriatic patients express lower levels of both GADD45a and GADD45b compared with non-lesional skin or skin from controls (Fig. 1A). Immunofluorescence assays showed the expression of GADD45a in keratinocytes and dermal T lymphocytes as indicated by the co-staining with CD3 (Fig. 1B). Unfortunately, antibody against GADD45b was not suitable for immunofluorescence techniques. We observed a complex pattern of expression of GADD45a in the skin samples, while the expression of this protein in keratinocytes was diminished in psoriasis its expression in dermal infiltrating lymphocytes increased (Fig. 1B).

As stated above, several inflammatory stimuli have been associated with the induction of GADD45 molecules. Thus, we postulated the existence of a possible correlation of GADD45 expression with IFN-γ or TNF-α levels. Our data showed a clear positive correlation between GADD45a and IFN-γ or TNF-α expression, both quantified by qRT-PCR (Fig. 2A). A positive correlation was also observed with the expression of GADD45b and TNF-α, however the association between the levels of GADD45b and IFN- γ was not significant (Fig. 2B).

Expression of GADD45 molecules was also quantified in peripheral blood $CD4^+$ T cells using qRT-PCR. Our data showed higher GADD45a and GADD45b mRNA levels in unstimulated $CD4^+$ T cells from psoriasis patients compared to controls (Fig. 3A). Expression of these molecules was also studied following stimulation with pro-inflammmatory cytokines, showing an induction of GADD45b but not of GADD45a (Supplementary figure 1). Interestingly, T cells from psoriasis patients expressed higher levels of GADD45b than controls after stimulation with a mixture of IL-12 and IL-18 (Fig. 3B). Besides T cells, DCs play a key role during different phases of the psoriasis development. Although the expression of GADD45a and GADD45b molecules was diminished in non-stimulated moDCs from psoriasis patients compared to controls (Fig. 3C), after LPS stimulation both patients and controls were able to upregulate the expression of GADD45a and GADD45b at similar levels (Fig. 3D).

Next, we wonder whether the differential expression of GADD45 proteins in T cells could be affecting the signaling pathway of p38 in psoriasis patients. Analysis of the activation of p38 in basal conditions and following TCR stimulation in patients and controls showed no statistical differences either in resting (Fig. 4A) or in anti-CD3/CD28 stimulated cells (Fig. 4B). These data indicate that the increased expression of GADD45a and GADD45b does not influence the p38 activation pathways in $CD4^+$ T cells from psoriasis patients.

It is known that GADD45a has a demethylase activity [24] and its depletion results in a hypermethylation of the promoters of several genes such as UCHL1 [14]. To determine the *UCHL1* methylation state we used publicly available datasets, containing methylation microarray information from psoriasis skin samples. Upon analyses of the differential methylation sites (DMS) between psoriasis skin and controls we have focused on those significant DMS located on CpG islands on *UCHL1* promoter (Table 1). As expected, most of these sites are hypermethylated in psoriasis skin samples with respect to controls. Moreover, expression of UCHL-1 was significantly lower in lesional skin compared to both non-lesional skin of psoriasis patients and skin of controls (Fig. 5A). Moreover, a positive correlation between the levels of GADD45a expression and its target UCHL-1 was observed (Fig. 5B).

Discussion

Members of the GADD45 family of proteins are involved in numerous biological processes, many of which seem to be closely related to the pathogenesis of psoriasis. The data provided in this work show a complex dysregulation in the patterns of expression of these molecules among cellular players with essential functions in psoriasis, such as keratinocytes, DCs and T lymphocytes. While epidermal cells from these patients express low levels of GADD45a, infiltrating lymphocytes and peripheral $CD4^+$ T cells exhibit a higher expression of these molecules compared to controls. A similar pattern seems to occur for GADD45b, revealing the complexity of GADD45 molecules, where different stimuli have been associated with cell-specific regulation of their expression. We detected a positive correlation between the expression of GADD45 molecules and the levels of IFN-γ. The pro-inflammatory environment in psoriatic skin could account for the increased expression of GADD45a and GADD45b. However, this statement seems to apply to leukocytes but not to keratinocytes.

The study of GADD45 proteins in human autoimmune diseases is barely explored. Polymorphisms in GADD45a and GADD45b genes and protein expression have been investigated in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) patients. GADD45b but not GADD45a mRNA levels were significantly lower in RA patients compared to control cases. Regarding SLE, no differences in the expression of either molecule was detected [25]. Similarly to the findings of RA patients, our data show that lesional skin from psoriasis patients express low levels of GADD45a and GADD45b. However, surprisingly a higher expression of IFN-γ was found in psoriasis patients. This unexpected inverse correlation has also been found in synovial fibroblasts of RA patients where a diminished expression of GADD45b was detected despite the higher activation of NF-kβ [26]. The absence of GADD45b has been associated with a higher expression of pro-inflammatory molecules in myeloid cells [3], but the opposite has been described for GADD45a [6]. These findings support a complex counter-regulation of these molecules, where the outcome of the inflammation results from the balance between positive and negative signals. In psoriasis, the function of GADD45b and other anti-inflammatory signals seems to be overcomes by pro-inflammatory triggers. Unfortunately, we were unable to evaluate the expression of GADD45b by immunofluorescence and cannot rule out a differential expression of this molecule in the different cells of the skin.

T cells are key players for the psoriasis development. In this sense, our data demonstrate that GADD45a and GADD45b are upregulated in peripheral $CD4^+$ T cells from psoriasis patients at basal conditions. GADD45a and GADD45b show differences and similarities regarding the activation of T cells, while GADD45a acts as a negative regulator after TCR activation [5], GADD45b seems to play a dual role favoring the activation of T cells at early time points but self-limiting cell activation during the chronic phase of inflammation [27]. Although the increment of both GADD45a/b molecules at basal conditions in psoriasis may be a consequence of the pro-inflammatory environment, we did not observe any difference in the activation of the p38 pathway compared to controls. The opposing effects of GADD45a and GADD45b molecules on T cell activation may counterbalance and account for the observed phenotype; the inhibitory effect of GADD45a on p38 activation seems to be overcome by the upregulated expression of GADD45b in psoriasis patients not only in unstimulated T cells but also following activation with IL-12 plus IL-18 cytokines. Further studies will be necessary to elucidate whether the increased expression of these molecules could be affecting the survival of activated $CD4^+$ T cells.

GADD45 proteins act as stress sensors and are rapidly induced by genotoxic agents such as UV radiation and oxidative stress. In keratinocytes, GADD45a promotes cell cycle arrest, favoring genomic DNA repair and inhibiting cell death [11]. Recently, it has been reported that GADD45a silencing promotes cell proliferation and inhibits apoptosis in skin squamous cell carcinoma [28]. Moreover, the silencing of GADD45a induced a local increased expression of cytokines such as IL-1, IL-6, TNF-α and VEGF. It is conceivable that the reduction of GADD45a expression in keratinocytes from psoriasis patients could promote keratinocyte hyper-proliferation and the production of pro-inflammatory mediators.

Besides the above described functions, GADD45 proteins are involved in DNA demethylation. GADD45a interacts with components of DNA repair complexes, promoting their recruitment to specific sites and resulting in the replacement of methylated by unmethylated cytosines [24,29,30]. GADD45a depletion results in hypermethylation of UCHL1 promoter [14]. Accordingly, we have observed that most of the analyzed CpG sites located on *UCHL1* promoter were hypermethylated in psoriasis skin samples with respect to controls. UCHL1, also known as PGP 9.5, is an enzyme with ligase and hydrolase activities, mainly expressed in neuroendocrine cells and the central nervous system. Our data demonstrate not only that the skin of psoriasis patients expresses lower levels of UCHL1 compared to controls in agreement with previously published data [31], but also a clear positive correlation with GADD45a expression. UCHL1 determines cellular levels of ubiquitins and glutathione and regulates cell cycle [32]. Interestingly, it is known that, in keratinocytes, UCHL1 inhibits the secretion of IL-8, IFN-I and MIP3, and suppresses the NF-kβ activity induced by TNF-α [33]. Its expression has also been associated to the suppression of iNOS induced by TNF-α and NFκ-β activation [34,35] (Supplementary Figure 2).

Psoriasis is a very complex disease with numerous deregulated molecules that participate in triggering a sustained activation of the immune system and epidermal cells, leading to the characteristic clinical manifestations of this disease. GADD45 proteins have been implicated in numerous biological processes and the finding that they relieve epigenetic gene silencing may account for some of the pleiotropic effects observed in the psoriatic pathogenesis.

Declarations

CONFLICTS OF INTEREST: Prof F Abad-Santos has been a consultant or investigator in clinical trials sponsored by the following pharmaceutical companies: Abbott, Alter, Chemo, Farmalíder, Ferrer, Galenicum, GlaxoSmithKline, Gilead, Janssen-Cilag, Kern, Normon, Novartis, Servier, Teva and Zambon. MC Ovejero-Benito has potential conflicts of interest (honoraria for speaking and research support) with Janssen-Cilag and Leo Pharma, respectively.

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Tables

Table 1. Differentially methylated CpG sites in *UCHL1* promotor in skin samples from psoriasis patients

Dataset	Reference	DMS	**Location**	**CpG-site neighborhood**	Adj p-value	Gene context	log2 FC	Methylation state
GSE63315 (L vs HC)	1	cg16026922	**41259044**	**Island**	1,38E-03	Body	5,68E-02	Hypermethylated
		cg16142306	**41258935**	**Island**	1,94E-03	1stExon;5'UTR	3,70E-02	Hypermethylated
GSE73894 L vs HC	2	cg24715245	**41258794**	**Island**	1,49E-03	TSS200	-3,22E-02	Hypomethylated
		cg09921610	**41259866**	**Island**	6,56E-03	Body	2,95E-02	Hypermethylated
GSE73894 L vs NL		cg09921610	**41259866**	**Island**	6,06E-03	Body	-3,50E-02	Hypomethylated
GSE115797	3	cg07068756	**41258910**	**Island**	1,05E-02	1stExon;5'UTR	7,52E-02	Hypermethylated

Location: genomic coordinate_37 of the CpG site interrogated by the probe. CpG-site neighborhood: location of the gene-associated CpG-site(s) within the CpG-site neighborhood. Gene context: location of the gene-associated CpG-site(s) with respect to the gene context. Abbreviations: Adj p-value: adjusted p value; CpG: Cytosine-Phosphate-Guanine sites; DMS: differentially methylated sites; HC: Healthy control skin; Log2FC: logarithm of the fold change of methylation values in psoriasis samples with respect to controls; L: psoriasis lesional skin; NL: psoriasis non-lesional skin; TSS200 Promoter regions 200 bp (base pairs) upstream of the transcription start site; 5'-UTR: 5'-untranslated region; vs: versus.

Figures

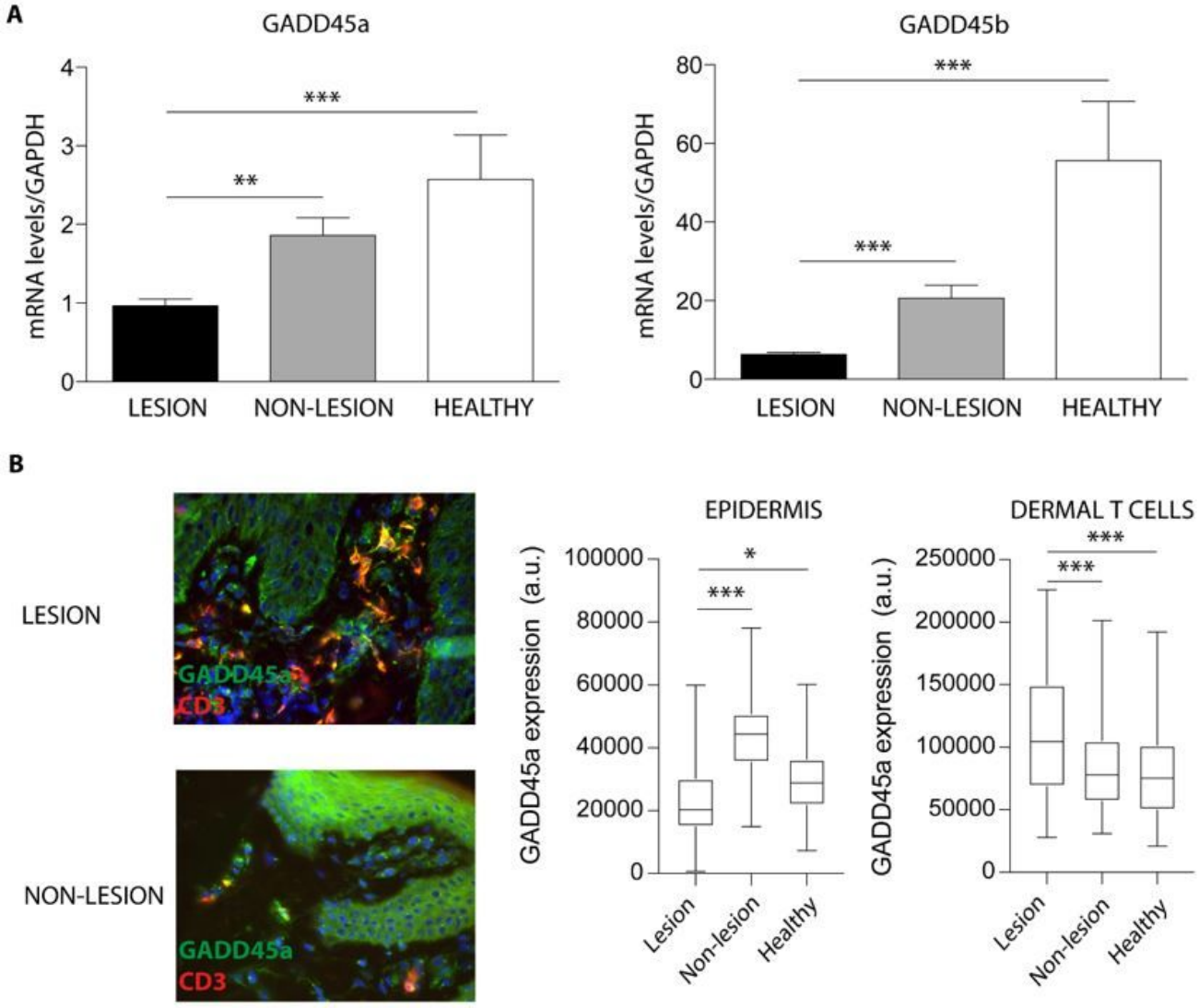

Figure 1

Lesional skin from psoriasis patients expresses low levels of GADD45a and GADD45b. A, mRNA levels of GADD45a (left) and GADD45b (right) were analyzed by qRT-PCR in skin samples from 30 patients with psoriasis and 20 controls. GAPDH was used to normalize gene expression. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparisons test, *** $p<0.001$, ** $p<0.01$. B, Representative staining of GADD45a (green) and CD3 (red) in skin samples from lesional skin and non-lesional skin. Quantification of immunofluorescence stainings, fluorescence intensity of GADD45 in epidermal (left) and dermal (right) infiltrating T cells was calculated using the Image J software. Differences between groups were determined by one-way ANOVA followed by Tukey's multiple comparisons test, *** $p<0.001$, **$p<0.01$, *$p<0.05$.

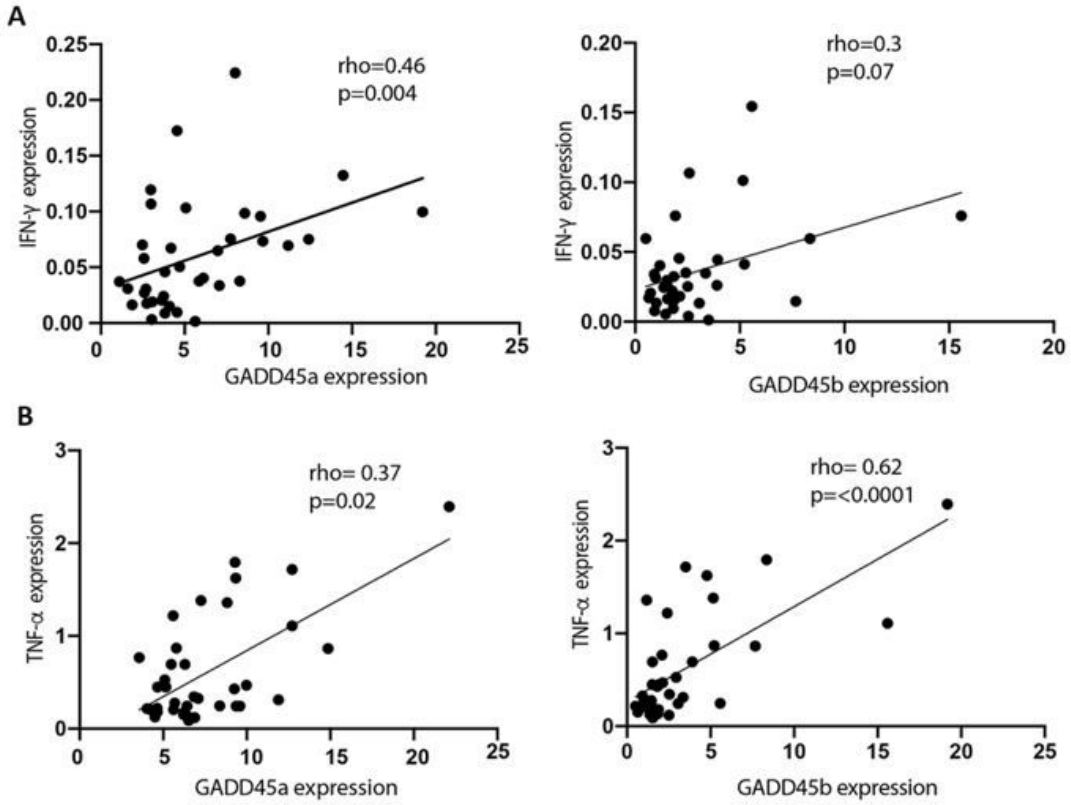

Figure 2

Expression of GADD45a correlates with IFN-γ and TNF-α in lesional skin of psoriasis patients. A. Scatter graphs showing the correlation between mRNA levels of GADD45a and GADD45b with IFN-γ in lesional skin of psoriasis patients. B. Scatter graphs showing the correlation between mRNA levels of GADD45a and GADD45b with TNF-α as in A. GADD45 expression was analyzed using qRT-PCR using GADPH expression as control. Data were analyzed using Spearman test, n=30.

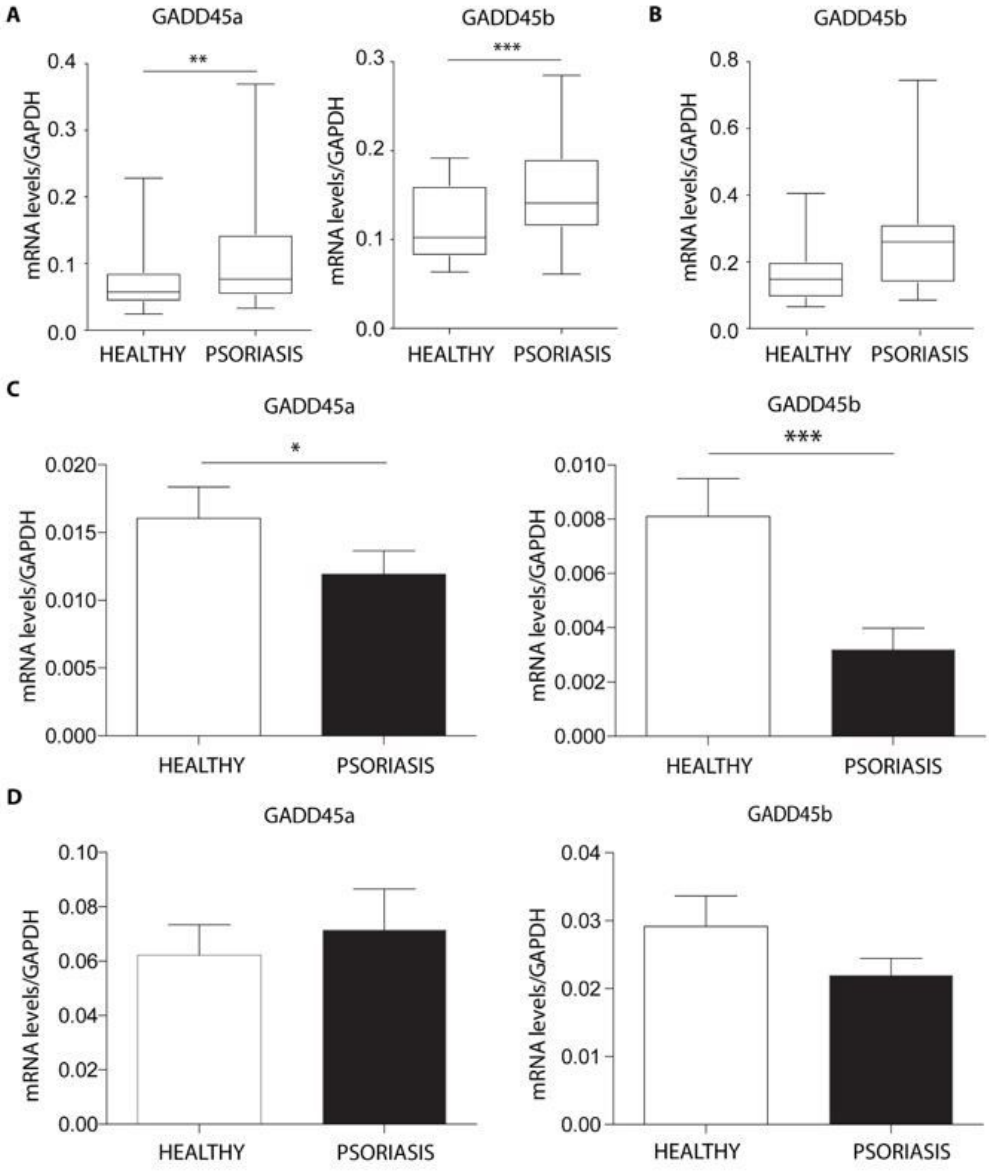

Figure 3

Psoriasis patients peripheral blood CD4+ T cells express high levels, while immature moDCs express low levels of GADD45. A. Box charts show the basal mRNA expression of GADD45a (left) and GADD45b (right) in CD4+ T cells. GADD45a and GADD45b expression was analyzed using qRT-PCR. GAPDH was used to normalize data. Differences between groups was determined using Mann-Whitney U test, $p<0.05$*, $p<0.01$ ** $p<0.001$ ***. B. Expression of GADD45b in isolated CD4+ T cells incubated in the presence of a mixture of IL-12 and IL18 (10ng/ml). GADD45b expression was detected and analyzed as in A. C, Basal expression of GADD45a (left) and GADD45b (right) in moDCs, that were differentiated from peripheral monocytes as described in Material and Methods. Bar charts show GADD45a and GADD45b expression levels, analyzed using qRT-PCR. GAPDH was used to normalize data. Differences between groups was determined using Mann-Whitney U test, $p<0.05$*, $p<0.01$ **, $p<0.001$ ***.D, Expression of GADD45a and GADD45b in moDCs after 24h of stimulation with LPS (10 ng/ml). GADD45 expression was detected and analyzed as in A.

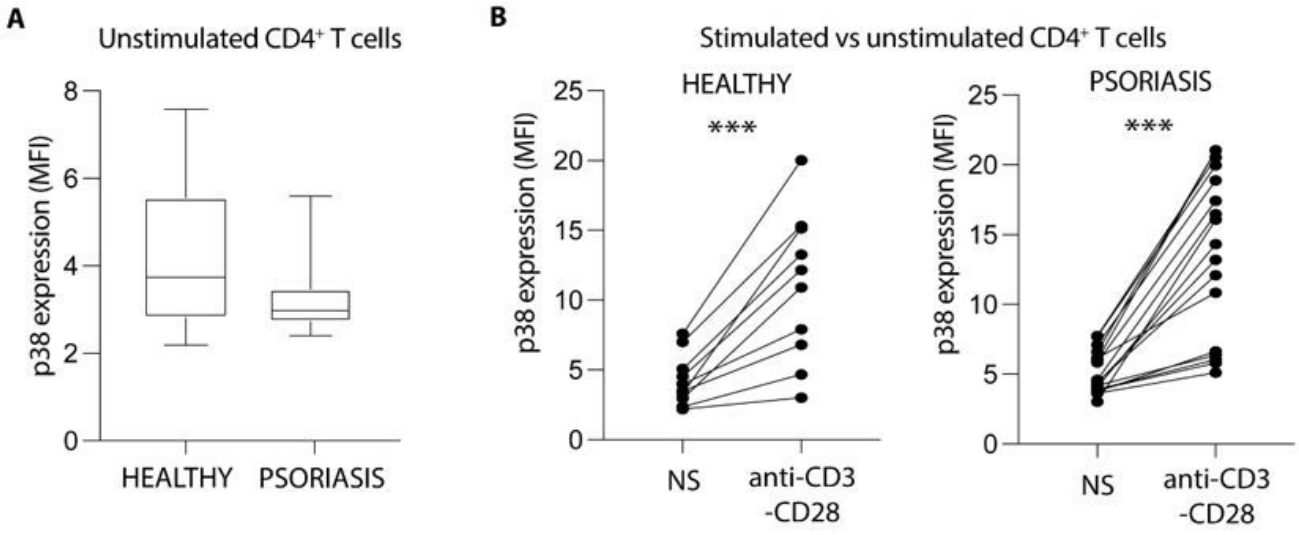

Figure 4

p38 activation is not affected in CD4+ T cells from psoriasis patients. A, Dual phosphorylation of p38 was evaluated by flow cytometry in peripheral blood CD4+ T cells from psoriasis patients (n=10) and healthy subjects (n=10), immediately after isolation. Data were analyzed using Mann-Whitney U test. B, Activation of p38 before and after TCR stimulation. T cells were either left untreated (not stimulated, NS) or activated in the presence of anti-CD3 (10ug/ml) and anti-CD28 (5ug/ml) antibodies during 15 min. Thereafter, dual phosphorylation of p38 was evaluated by flow cytometry as in A. Data were analyzed using Wilcoxon matched signed rank test.

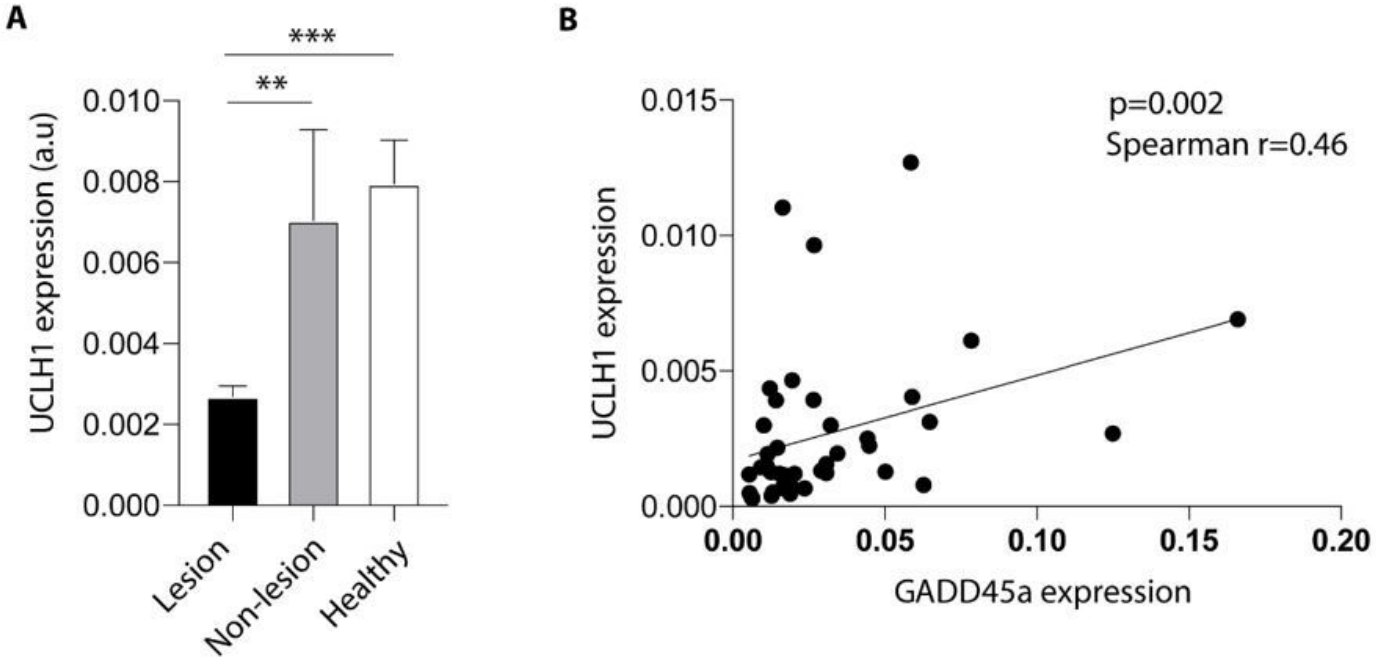

Figure 5

Lesional skin of psoriasis patients express low levels of UCHL1 that correlate with GADD45a levels. A, Expression of UCHL1 in skin samples from psoriasis patients (lesional and non-lesional) and control subjects. UCHL1 expression was analyzed using qRT-PCR and data were normalized using GADPH. Data were analyzed using One-way ANOVA and Tukey's multiple comparisons test, p<0.01 **,

p<0.001 ***. B, Scatter graphs showing the positive correlation correlation between the levels of GADD45a and UCHL1 in lesional skin of psoriasis patients. Data were analyzed using Spearman test.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure109.06.20.ai
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- GraphicalAbstract.png
- Graphicalabstract.docx