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PSORIASIS AND MELANOGENESIS:

WHICH DIFFERENCES BETWEEN PSORIATIC AND HEALTHY SKIN?

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Introduction

Psoriasis is a common chronic inflammatory skin disease characterized by hyperproliferative epidermis and mixed cutaneous lymphocytic infiltrate[1] that occurs in genetically predisposed individuals. The disease affects 1-3% of the world's population[2]; its onset is typically between 15 and 35 years. Psoriasis etiopathogenesis is still unknown, but evidences suggest that it is a complex disorder caused by the interaction among multiple genes, immune system, and environmental factors[3]. The dermatosis is considered to be a genetically programmed disease of dysregulated inflammation, which is driven and maintained by multiple components of the immune system[3]. Until relatively recently, psoriasis has been considered to be a mainly T helper (Th)1-driven inflammatory condition; however, several findings have now assessed a major role for Th17 cells in its pathogenesis [4,5]. Many immune-derived cytokines, including interleukin (IL)-1, IL-6, IL-17, IL-19, IL-20, IL-22, tumour necrosis factor (TNF)-a and interferon (IFN)s, are over-expressed in psoriasis skin which may contribute to psoriatic skin inflammation and can also regulate keratinocyte proliferation. Moreover, the inflammatory cytokine milieu influences the immune functions of fibroblasts and endothelium, with the latter being critical for leucocyte trafficking and extravasation[4]. It has been demonstrated that keratinocytes synthesize and secrete many cytokines; some of these interact with many other skin cells[3,6], particularly with melanocytes. Keratinocytes, melanocytes and dermal fibroblasts communicate with each other by secreted factors and by cell-cell contacts[7]. Melanocytes are specialized epidermal cells, originated from the neural crest, representing 2–5% of the cells in the epidermis[5]. These cells reside along the basal layer of epidermis, closely associated with both keratinocytes and Langerhans cells. They form the epidermal melanin units as a result of the relationship between one melanocyte and 30-40 associated keratinocytes.

The ratio of melanocytes to keratinocytes is 1:10 in the epidermal basal layer[6] . They produce melanin by melanogenesis complex biochemical pathway. Synthesis and distribution of melanin in the epidermis depend on many phases: transcription of melanogenic proteins, melanosome biogenesis, sorting of melanogenic proteins into the melanosomes, transport of melanosomes to the tips of melanocyte dendrites and finally transfer into keratinocytes, where they form a melanin cap over the nuclei to protect DNA from ultraviolet (UV) damage [3,7-9]. Two types of melanin are produced – pheomelanin and eumelanin [6]. They differ in color and the way of synthesis; both are derived products of 3,4-dihydroxyphenylalanine (DOPA) and both are formed in melanosomes from tyrosine through a series of oxidative steps. The melanin synthesis requires the enzyme tyrosinase and tyrosinase-related proteins (TRP-1 and TRP-2) that catalyse the oxidation of tyrosine to L-DOPA[7-9]. These events are closely regulated by a variety of paracrine and autocrine factors in response to endogenous and exogenous influences, principally UV irradiation [3,6]. Keratinocytes control melanocyte growth and activity through a system of paracrine growth factors and cell adhesion molecules[10]. The cross-talking of different signaling pathways between keratinocytes and melanocytes is a part of an epidermal complex network involved in the maintenance of skin homeostasis [6]. Potent mitogens are growth factors and hormones as stem cell factor (SCF), hepatocyte growth factor (HGF), endothelins (ETs), α-melanocyte stimulating hormone (MSH), adrenocorticotropic hormone (ACTH) and many other inflammatory and growth mediators[3]. α-MSH and ACTH are potent stimulators of melanogenesis through proopiomelanocortin (POMC) precursor and derived peptides; ET-1 is also involved in the regulation of melanogenesis, through activation of tyrosinase, increasing TRP-1 levels and stimulatiing melanocyte proliferation and dendrite formation[3,11,12]. A major role is attributed to microphthalmia-associated transcription factor (MITF) as the main melanocyte transcription factor influencing proliferation, dendrite formation,

melanin synthesis and inducing the expression of antiapoptotic genes; the MITF activates genes are responsible for the migration to the skin, preventing apoptosis in migrating cells, melanin production[3,13]. In addition, several keratinocyte-derived cytokines known to inhibit human melanogenesis have been identified; these include transforming growth factor-β (TGF-β), INF-β, IL-1, IL-6, TNF-α. Swope et al. first hypothesized and demonstrated that IL-1a, IL-6 and TNF-α epidermal cytokines may provide autocrine and paracrine regulatory inhibition signals for melanocytes. In fact, these cytokines could downregulate melanization and replication of melanocytes by a greater inhibitory effect on tyrosinase activity [3,14]. They also decrease melanocyte proliferation, but are not toxic to the cells. However, the effect of these cytokines on tyrosinase expression, protein level, and stability is not well-known [15]. Moreover, a hypopigmenting effect has been demonstrated for TGF-β using B16 ⁄F10 mouse melanoma cells as a model[16]. Members of the TGF-β family are also Bone Morphogenetic Proteins (BMPs)[15]. While originally identified as molecules that stimulate bone formation, it is now well recognized that BMPs signalling plays essential roles in the control of organ development, postnatal remodelling and regeneration by regulating proliferation, differentiation and apoptosis in many cell types [15-19]. During skin development, BMPs control the initiation phase of hair follicle morphogenesis, as well as being required for a proper control of keratinocyte differentiation in epidermis [15-19]. Currently, BMPs family consists of more than 20 secreted proteins that share structural homology exerting their biologic activity after interaction with specific BMP receptors [15,20]. Particularly, it was discovered that BMP-2 and BMP-4 have a prominent function in melanogenesis development. Bilodeau et al demonstrated that BMP-2 treatment of neural crest cells increases melanogenesis by promoting the synthesis of melanin rising the expression of tyrosinase gene; moreover, BMP-2 exerts no effect on the expression of the TRP-1 and TRP-2 or MITF, supporting the fact that BMP-2 does

not affect melanocyte differentiation [21]. Normal neonatal human melanocytes and keratinocytes express BMP-4. BMP-4 leads to decreased tyrosinase mRNA, decreasing message stability, repressing tyrosinase promoter activity, reducing tyrosinase protein and tyrosinase activity and melanin levels [17].

Several evidences suggest that BMPs play a part in psoriasis pathophysiology. Long-term treatment of C57BL/6 mice with BMP-2 or BMP-4 result in thickening of the epidermis with development of psoriasis-like lesions. Moreover, elevated BMP signaling may stimulate angiogenesis because of up-regulation of vascular endothelial growth factor synthesis. Although the BMP role in psoriasis development remains to be determined [15].

The possible immunological interaction between psoriasis and melanogenesis is particularly interesting for clinical and therapeutic implications. There is some evidence that immunosuppressive therapy is associated with the development of eruptive benign melanocytic naevi and acquired dermal melanocytosis [22–25]. Moreover, Balato et al. showed that psoriatic subjects have a smaller number of melanocytic naevi, including atypical ones probably because the proinflammatory cytokine network in psoriasis skin might inhibit melanogenesis, melanocyte growth and ⁄or progression to naevi [3].

Aim of our study was to investigate possible differences in melanogenesis markers between psoriatic and healthy skin.

Materials and Methods

Study population

The overall study enrolment comprised 20 caucasian patients with moderate to severe psoriasis, without arthritis (mean age 41.2; range 18-50 years) as well as 20 aged-matched healthy controls (mean age 39.7 range 18-47 years). The study was conducted according to the Declaration of Helsinki principles. The local ethical committee approved the study, and each participant gave written informed consent before entering the study. Controls comprised healthy donors who had undergone to plastic surgery. All subjects undergoing treatment with immunosuppressant therapy including systemic corticosteroids, ciclosporin, methotrexate, psoralen plus UVA (PUVA) therapy and biologic drugs, patients affected by other inflammatory skin diseases (including obesity), or patients who had already received a diagnosis of atypical melanocytic naevi, were excluded. In both study groups, the number of melanocytic naevi, defined as all well-circumscribed brown flat or raised lesions with a distinct border and a diameter of 2 mm or larger (measured by over-laying a 2-mm stencil of a circle), was registered using two ranges (< 5 naevi; > 50 naevi), for each range 10 patients and 10 healthy controls were enrolled.

In vivo **expression of Tyrosinase, MITF and BMPs members family in Psoriatic Skin and Healthy Skin**

Skin punch biopsies (3mm diameter, location: trunk) were obtained from control group as well as psoriatic group (lesional and non lesional skin) and used for in vivo mRNA examination through real-time polymerase chain reaction (qRT-PCR).

Immunoistochemical detection of Tyrosinase and BMP-4 in Psoriatic Skin and Healthy Skin

Immunoistochemical detection of Tyrosinase and BMP-4 was carried out on the skin biopsies from the psoriatic skin before and after 16 weeks treatment with adalimumab (administered in according to drug bulletin) and healthy skin. Skin samples were immediately placed in tissue freezing medium (Jung, Leica, Wetzlar, Germany) and stored at −80°C. Five micrometer sections were cut with a cryostat and Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA) was used as follows: sections were incubated with blocking solution (horse serum diluted in buffer: phosphate buffered saline PBS + bovine serum albumin 1%) for 20 min at 22°C. Biopsies were stained with anti-tyrosinase(1 µg/ml, GIBCO, Grand Island, NY), BMP-4 (10 µg/ml Fitzgerald, Sudbury, USA) and incubated overnight at 4° C. In parallel, skin specimens were incubated with specific isotype control antibodies (Mouse IgG1 Isotype Control, Mouse IgG2B Isotype Control, Goat IgG Control, R&D System Inc, Minneapolis, MN) used at the same concentration as the corresponding primary antibody. The sections were then washed in buffer and incubated with biotinylated secondary antibody for 30 min at room temperature. Peroxydase activity was revealed using DAB substrate (ImmPACT DAB, Burlingame, CA). Counterstaining was performed with hematoxylin. Staining was observed using Nikon Eclipse E600 epifluorescence microscope (Nikon, Melville, NY).

Melanin Content Assay in Psoriatic Skin and Healthy Skin

Total cellular melanin content was performed in healthy skin and in psoriatic skin biopsies before and after 16 weeks treatment with adalimumab using Fontana-Masson staining (AMTS Inc., Lodi, CA, USA), according to the manufacturer’s instructions.

***Ex vivo* full-thickness skin organ cultures**

Skin specimens from healthy donors were cultured as follows: a hole was punched in a transwell filter (pore size 1 μm; Beckton Dickinson Labware, Franklin Lakes, NJ). The biopsy was inserted into the hole, and the filter containing the biopsy was placed in a 12-well culture plate (Beckton Dickinson Labware) with Dulbecco's modified Eagle's medium (DMEM, GIBCO, Grand Island, NY) containing 10% FBS (GIBCO), 2 mM L-glutamine (GIBCO) and antibiotics (100 IU/ml penicillin G, 100 μg/ml streptomycin, GIBCO). In this system the epidermis faces upwards at the liquid-air interface whereas the dermis is suspended in the culture medium. The tissue was incubated at 37° C in a humidified atmosphere containing 5% CO_2. Healthy skin biopsies were stimulated with TNF-α (GIBCO), at 20 ng/ml for 24 hrs.

Normal human epidermal sheets

Normal human epidermal sheets were isolated from healthy donors as previously described [26]. Epidermal sheets were cultured as described for e*x vivo* full-thickness skin organ cultures and stimulated with TNF-α (GIBCO) at 20ng/ml for 24 hours.

RNA extraction, cDNA synthesis and qRT-PCR

RNA was extracted from every skin specimen (RNeasy Mini Protocol, Qiagen Valencia, CA) and cDNA was prepared (Transcriptor High Fidelity cDNA Synthesis, Roche, Indianapolis, IN) according to the manufacturer's instructions. PCR (LightCycler, Roche, Indianapolis, IN) was used to analyze the levels of expression of 18S, Tyrosinase, MITF, BMP-2, BMP-4, BMP-6, BMP-7. Each PCR was performed 2 times, each time in triplicate. PCR protocol and product quantification for 18S ribosomal RNA were performed as reported previously [27]. PCR primers for the selected genes were designed based on published sequences, and their specificity was verified with BLAST alignment search. To confirm amplification of the expected size fragment, amplification products were characterized by agarose gel electrophoresis.

Melting curve analysis was carried out after completion to confirm the presence of single amplified species. The amount of mRNA for a given gene in each sample was normalized to the amount of mRNA of 18S reference gene in the same sample. Fold induction of gene expression was calculated using the CT method as described previously [28]. Results obtained from each PCR were pooled and statistically analyzed. to the amount of mRNA of 18S reference gene in the same sample. Fold induction of gene expression was calculated using the ΔΔCT method as described previously [27]. Results obtained from each PCR were pooled and statistically analyzed.

Immunogold staining in Psoriatic Skin and Healthy Skin

Tissue samples were fixed in a mixture of 0.5% glutaraldehyde and 2% paraformaldehyde in PBS overnight at 4°C and washed in the same buffer. Following dehydration, samples were embedded in Epon resin, and ultrathin sections (60 nm) were collected on 200 mesh nickel grids. Samples were washed three times in distilled water for 5 min, equilibrated in PBS containing 1.5% goat serum and 1% BSA for 15 min and incubated overnight at 4 °C with anti-tyrosinase(1 µg/ml, GIBCO, Grand Island, NY), BMP-4 (10 µg/ml Fitzgerald, Sudbury, USA) diluted in PBS/BSA 1%. Following five 2-min washings in PBS and five washings in PBS/BSA 0.5%, sections were incubated in 1% PBS/BSA for 15 min and then for 1 h at room temperature with the goat anti-mouse secondary antibody (H & L) labeled with 20-nm gold particles (BBInternational, Cardiff, United Kingdom). Sequential washings were performed in 1% PBS/BSA for 15 min, in 0.5% PBS/BSA five times for 2 min, in PBS five times for 2 min, and in distilled water twice for 1 min. After staining with uranyl acetate, sections were analyzed using a Leo 912AB electron microscope (Carl Zeiss, Jena, Germany). Controls, based on the use of only secondary antibody, were run in parallel.

Detection of Serum Levels of BMP-4

Blood from patients and controls was obtained by venepuncture and collected in sterile heparinized glass vials and blood samples were centrifuged and the serum was collected and frozen at -80°C until analysis. Serum BMP-4 levels were determined using sandwich ELISA kits (Sigma, St Louis,. MO, USA), according to the manufacturer's instructions.

Statistical analyses

All statistical analyses were performed using GraphPad Prism 4.0 (GraphPad Software Inc, La Jolla, CA). Student's t test or Mann-Whitney test were used to calculate statistical differences. Analysis of biopsies from lesional *vs* non lesional psoriatic skin was performed with Wilcoxon matched pairs test. Values of $p < 0.05$ were considered significant and all data are displayed as means ± SD.

Results

In vivo expression of Tyrosinase and MITF in Psoriatic Skin and Healthy Skin

We examined the constitutive expression of Tyrosinase and MITF using qRT-PCR in lesional skin of psoriatic patients with respectively <5 naevi and >50 naevi; in non-lesional skin of these same patients and in healthy skin, matching <5 naevi and >50 naevi. The expression of Tyrosinase and MITF was significantly decreased in psoriatic skin respect to related non-lesional skin and healthy skin, without differences between skin from people with <5 naevi and > 50 naevi. Moreover, the relative expression of these melanogenesis factors did not differ in non-lesional skin from control skin (Figure 1).

In vivo expression of BMP family members in Psoriatic Skin and Healthy Skin

The recent identification of BMP members family in melanogenesis prompted us to investigate their involvement in psoriasis. Thus, we examined the constitutive expression of BMP-2, BMP-4, BMP-6 and BMP-7 using qRT-PCR in lesional skin of psoriatic patients with respectively <5 naevi and >50 naevi; in non-lesional skin of these same patients and in skin from normal donors, matching <5 naevi and >50 naevi. The expression of BMP-2, BMP-6 and BMP-7 did not show differences between examined groups; only BMP-4 presented a significant reduction in psoriatic skin versus corresponding non-lesional skin and healthy skin, without differences relative to <5 naevi and > 50 naevi skin and non-lesional skin from control skin (Figure 2).

Immunohistochemical detection of Tyrosinase and BMP-4 in Psoriatic Skin and Healthy Skin

To confirm qRT-PCR data, we also evaluated the immunohistochemical expression of Tyrosinase and BMP-4 in healthy skin, in psoriatic lesional and non-lesional skin. Moreover, in order to evaluate the relationship between anti-TNF-α treatment and melanogenesis factors, immunohistochemical detection of Tyrosinase and BMP-4 was

conducted in lesional and non-lesional psoriatic skin after 16 weeks of treatment with adalimumab.

Staining for Tyrosinase and BMP-4 showed a substantially lower intensity in psoriatic skin compared with normal skin. Non-lesional skin of psoriatic patients and normal skin presented similar intensity of staining (data not shown). Immunohistochemical detection of both Tyrosinase and BMP-4 in lesional and non-lesional (data not shown) psoriatic skin after adalimumab treatment registered analogous staining strength respect to normal skin. Immunohistochemical staining intensities of analyzed melanogenesis markers confirmed the results above reported (Figures 3, 4).

Melanin content assay in Psoriatic Skin and Healthy Skin

To corroborate immunohistochemical detections, we performed a histological visualization of melanin content, using the Fontana-Masson Stain Kit in healthy skin, in lesional and non-lesional psoriatic skin before and after anti-TNF-α treatment. Lesional skin contained dramatically a poor content of melanin compared to healthy skin and non-lesional skin (data not shown). Whereas, normal skin and non-lesional psoriatic (data not shown) skin presented the same staining levels like as psoriatic skin after adalimumab treatment (Figures 5).

Expression of Tyrosinase and BMP-4 in *ex vivo* full-thickness skin organ cultures

Given the down-regulation of Tyrosinase and BMP-4 in psoriatic skin, and the preminent role of anti-TNF-α treatment as condition that can be assimilated to non lesional skin and healthy skin, we sought to examine whether TNF-α regulate melanogenesis markers in *ex vivo* skin organ culture. qRT-PCR revealed that TNF-α significantly decreased Tyrosinase and BMP-4 mRNA expression, compared to untreated skin (Figure 6).

Expression of Tyrosinase and BMP-4 in *ex vivo* human epidermal sheet

In order to evaluate if the epidermis **was** involved in this decrement, we treated normal human epidermal sheet with TNF-α and then analyzed Tyrosinase and BMP-4 mRNA levels. qRT-PCR revealed that TNF-α significantly reduced tyrosinase and BMP-4 mRNA expression compared to untreated normal human epidermal sheet (Figure 7).

Detection of Serum Levels of BMP-4

Serum levels of BMP-4 were measured using ELISA assay in psoriatic subjects as well as healthy controls. Our findings showed that BMP-4 was increased in psoriatic patients respect to healthy individuals (Figure 8).

Discussion

The present work intended to evaluate the potential differences relative to melanogenesis markers between healthy and psoriatic skin. Immunological interactions among psoriasis and melanogenesis may probably be related to the reflection that some cytokines, involved in psoriasis pathogenesis, have a role in melanogenesis pathway [3]. In particular, IL-1a, IL-6, IL-17,TNF-A and TGF-β may inhibit melanogenesis and melanocytic growth because of their inhibitory effects on tyrosinase activity; the identical cytokines are implicated in the up-regulation of keratinocyte proliferation in the pathogenesis of psoriasis [1,3, 14, 29-33]. On these basis, we sought and demonstrated previously that psoriatic subjects showed a smaller number of melanocytic naevi, including atypical ones [3] and, after us, Di Cesare at al recorded a fewer number of melanocitic lesions in psoriatic subjects [34].

In the actual study, we examined in depth some of more relevant pro-melanogenesis and anti-melanogenesis factors in healthy and psoriatic skin.

First, we analyzed skin biopsy specimens obtained from psoriasis lesional and non-lesional skin and found that expression of Tyrosinase and MITF was significantly decreased in psoriatic skin respect to related non-lesional skin and healthy skin. Moreover, the relative expression of these melanogenesis factors was similar in non-lesional skin versus control skin. These results were extended and confirmed at proteic level by immunohistochemical examination of biopsies. In addition, our results were reinforced by histological visualization of a dramatically poor content of melanin in psoriatic lesional skin compared to healthy skin and non-lesional psoriatic skin. Moreover, since it is described outbreaks of eruptive benign melanocytic naevi during immunosuppressive therapy (including anti-TNF-α treatment for psoriasis) [23], we required to evaluate the relationship between an anti-TNF-α drug (adalimumab)

treatment and these melanogenesis markers, using immunohistochemical and melanin staining detection. We registered that adalimumab treatment, in both lesional and non-lesional skin, presented similar immunohistochemical staining and similar melanin content of normal skin. The most plausible speculation to give an explication for this observation may be that adalimumab therapy permit a recovery to an intact immunological status and it prompt to reduce cytokines that normally inhibits the proliferation of melanocytic lesions in psoriatic skin. After all, our data partially agree with a recent work of Wang et al.[33] ; they revealed a significant decrease in Tyrosinase and MITF levels in melanocytes after 48h exposure to IL-17 and TNF-α synergistically, but contrary to us they detected unexpected reduction of cellular melanin content in psoriasis skin. As well as us, they recognized an increased number of melanocytes, combined with a rapid recovery of pigmentation function during psoriasis resolution, in the majority of patients treated with etanercept. They ascribed this observation to an abundant production of melanin during treatment due to post-inflammatory hyper-pigmentation after psoriasis resolution [33]. We speculate that the reduction of Tyrosinase in psoriatic skin respect to non-lesional skin and healthy skin and the upturn of Tyrosinase in lesional and non-lesional skin after adalimumab treatment could reveal the fact that the local citokines re-balance pile out also in the keratinocyte-melanocyte cross talking. These data were also emphasized using *ex vivo* full-thickness skin organ cultures and in *ex vivo* human epidermal sheet stimulated with TNF-α.

In this study, we also analyzed the role of BMPs members family in psoriasis. The expression of BMP-2, BMP-6 and BMP-7 did not evidence differences between examined groups; only BMP-4 expression significantly was reduced in psoriatic skin *versus* corresponding non-lesional skin and healthy skin, without differences relative to <5 naevi and > 50 naevi skin and without discrepancies among non-lesional skin from control skin. This result was confirmed at proteic level, through immunhistochemistry

and histological visualization of melanin, and *in vitro*, using *ex vivo* full-thickness skin organ cultures and human epidermal sheet stimulated with TNF-α.

Given data previously reported in the literature [3, 33, 34], the observed tendency to reduction of Tyrosinase and MITF was expected; but the decreasing of BMP-4 in psoriatic skin was not estimated. This decrement was not confirmed at serum level, using ELISA, because BMP-4 serum level was increased in psoriatic patients respect to healthy individuals.

The activities of BMPs are precisely regulated by certain classes of molecules that are recently recognized as BMPs antagonists that prevent BMP binding to BMP receptors [35, 36]. BMPs activity in developing and in adult tissues is modulated and antagonized on different levels (extracellular, cytoplasmic, and nuclear). All antagonist proteins bind members of the BMP family with higher affinity than the BMPR complex, thus restricting BMP activity to the tissue compartments. Also, some BMP antagonists are able to bind simultaneously BMP and other growth factors and institute a balance of growth regulators in specific tissue, including skin during hyperproliferative disorders [15, 35]. The detection of higher BMP-4 serum level in psoriatic subjects respect to healthy controls let us to speculate that BMP-4 could sustain inflammation systemic status in psoriatic patients as well as atherosclerosis or metabolic syndrome [37-40].

Moreover, it is recognized that BMPs stimulate angiogenesis, key pathogenic feature of psoriasis [41], through VEGF-independent pathways via indirect or direct effects on endothelial cells, as is shown in osteoblasts [15,42-44].

Conclusions

Our study evidenced differences between Tyrosinase and MITF, master regulators taken part in activation of melanogensis pathway, among healthy and psoriasis skin. In particularly, these data showed a possible involvement of Tyrosinase in "hyperpigmentation" post anti-TNF-α therapy.

Figures and Legends

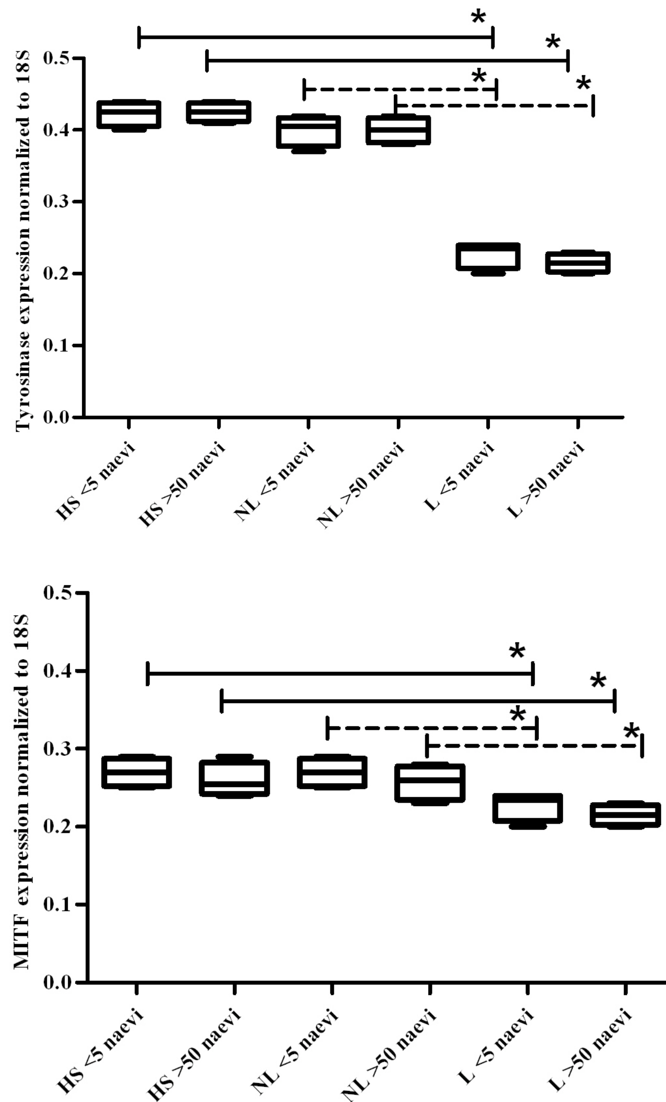

Figure 1: *In vivo* expression of Tyrosinase and MITF in Psoriatic Skin and Healthy Skin

The expression of Tyrosinase and MITF was significantly reduced in psoriatic skin respect to related non-lesional skin and healthy skin, without differences between skin from people with <5 naevi and > 50 naevi. Moreover, the relative expression of these melanogenesis factors did not differ in non-lesional skin from control skin. *(* p<0.1)*

HS: Healthy Skin; NL: Non Lesional psoriatic skin; L: Lesional psoriatic skin

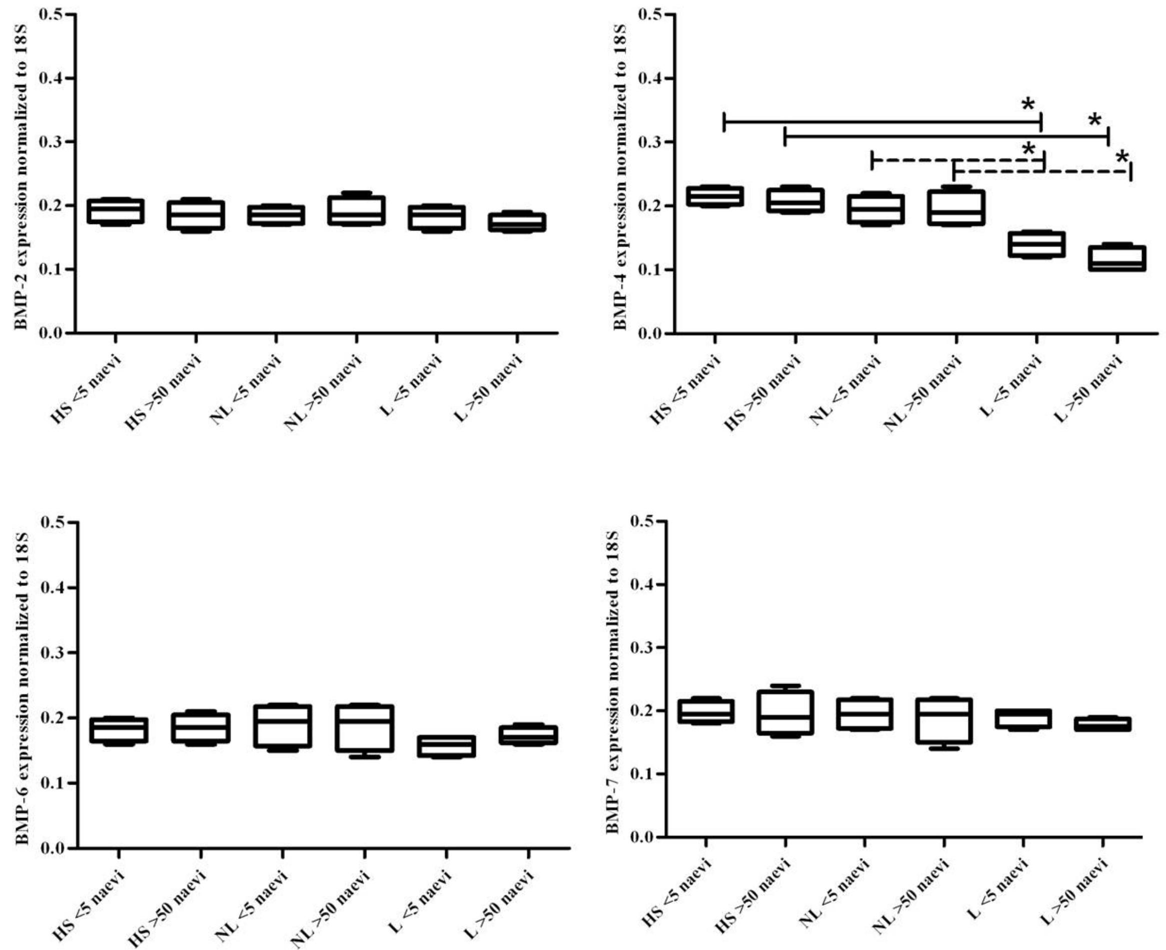

Figure 2: ***In vivo*** **expression of BMP family members in Psoriatic Skin and Healthy Skin**

The expression of BMP-2, BMP-6 and BMP-7 did not show differences between examined groups; only BMP-4 presented a significant reduction in psoriatic skin versus corresponding non-lesional skin and healthy skin, without differences relative to <5 naevi and > 50 naevi skin and non-lesional skin from control skin. *(* p<0.1)*

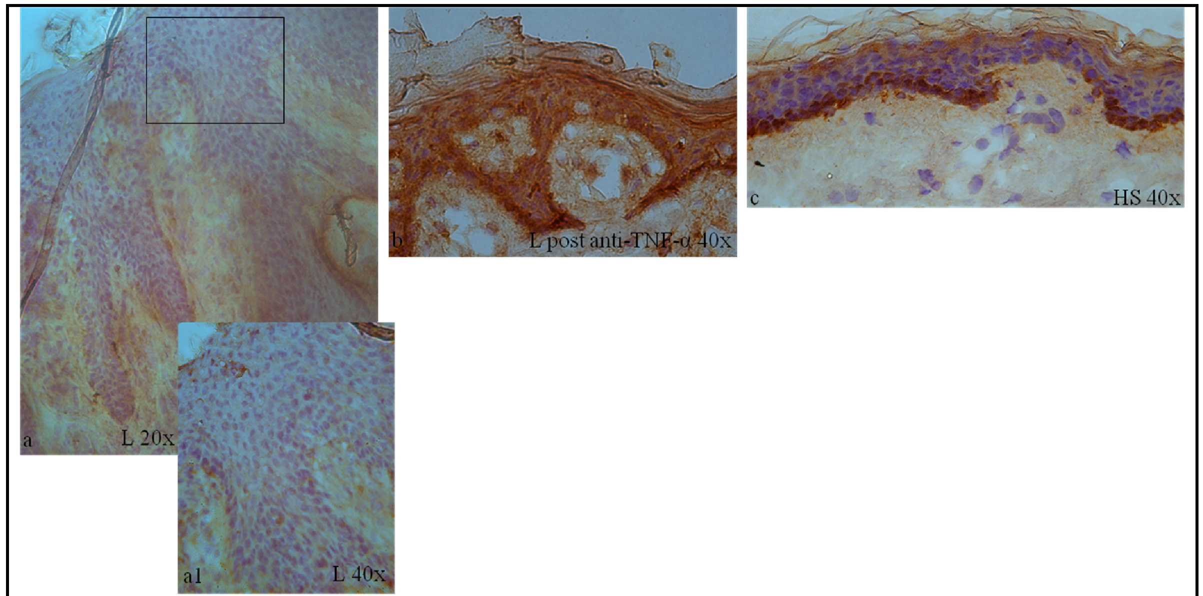

Figure 3: Immunohistochemical detection of Tyrosinase in Psoriatic Skin and Healthy Skin

Staining for Tyrosinase showed a substantially lower intensity in psoriatic skin (a; a1) compared with normal skin (c). Immunohistochemical detection of Tyrosinase in lesional psoriatic skin after adalimumab treatment (b) registered analogous staining strength respect to normal skin (*20x magnification; 40x magnification).*

HS: Healthy Skin; L: Lesional psoriatic skin

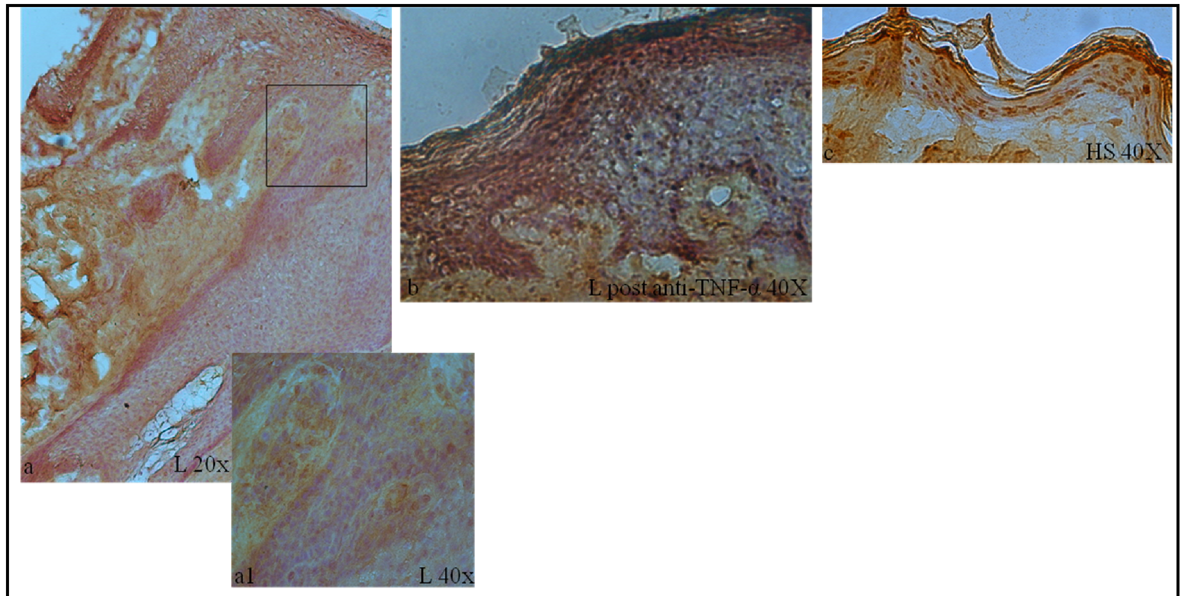

Figure 4: Immunohistochemical detection of BMP-4 in Psoriatic Skin and Healthy Skin

Staining for BMP-4 showed a lower intensity in psoriatic skin (a; a1) respect to normal skin (c). Immunohistochemical detection of BMP-4 in lesional psoriatic skin after adalimumab treatment (b) registered similar staining potency in comparison to normal skin (*20x magnification; 40x magnification)*.

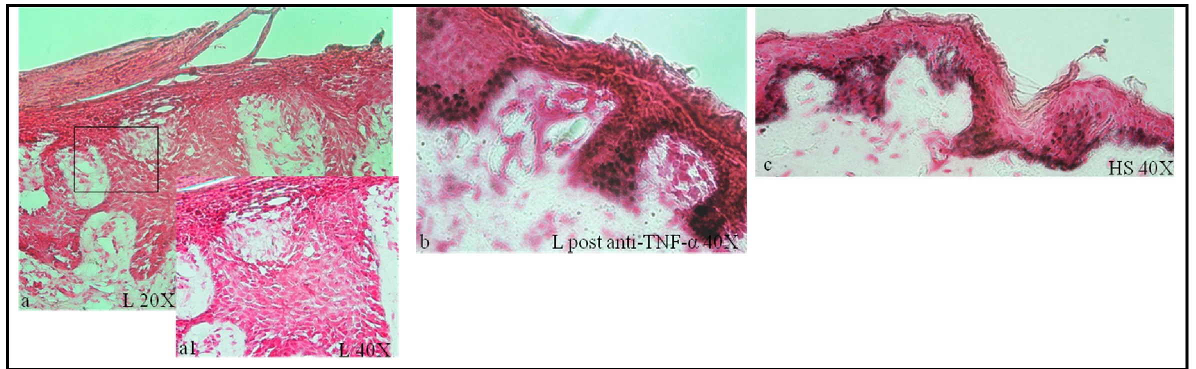

Figure 5: Melanin content assay in Psoriatic Skin and Healthy Skin

Histological visualization of melanin content showed that lesional skin (a, a1) contained dramatically a poor content of melanin compared to healthy skin (c) and psoriatic skin after adalimumab treatment (b). Moreover normal skin (c) and skin after adalimumab treatment (b) presented the same staining levels *(20x magnification; 40x magnification).*

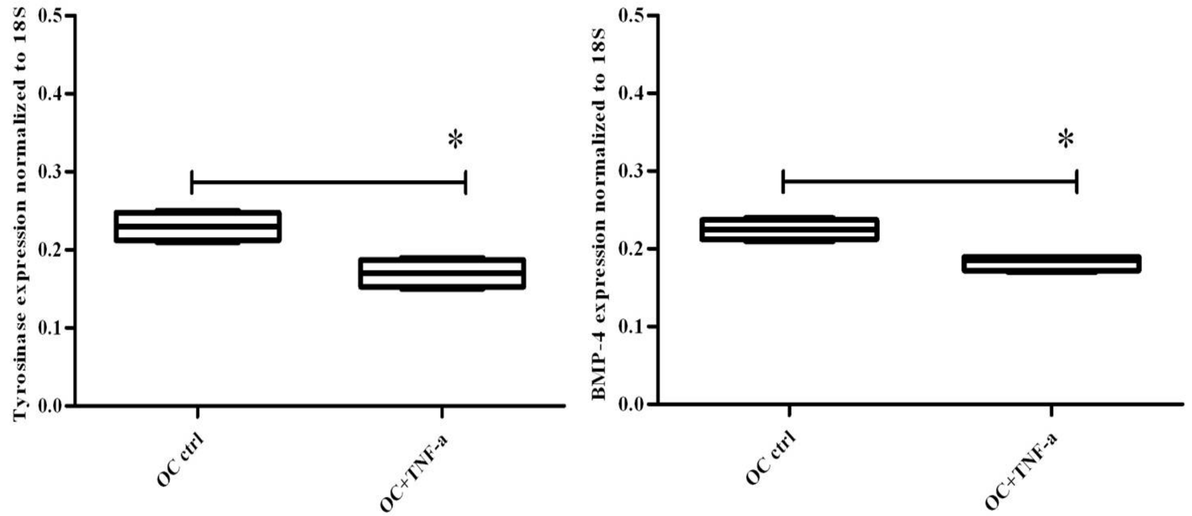

Figure 7: Expression of Tyrosinase and BMP-4 in *ex vivo* full-thickness skin organ cultures.

In *ex vivo* skin organ culture stimulated with TNF-α, Tyrosinase and BMP-4 mRNA expression significantly decreased, compared to untreated skin. *($p<0.1$)*

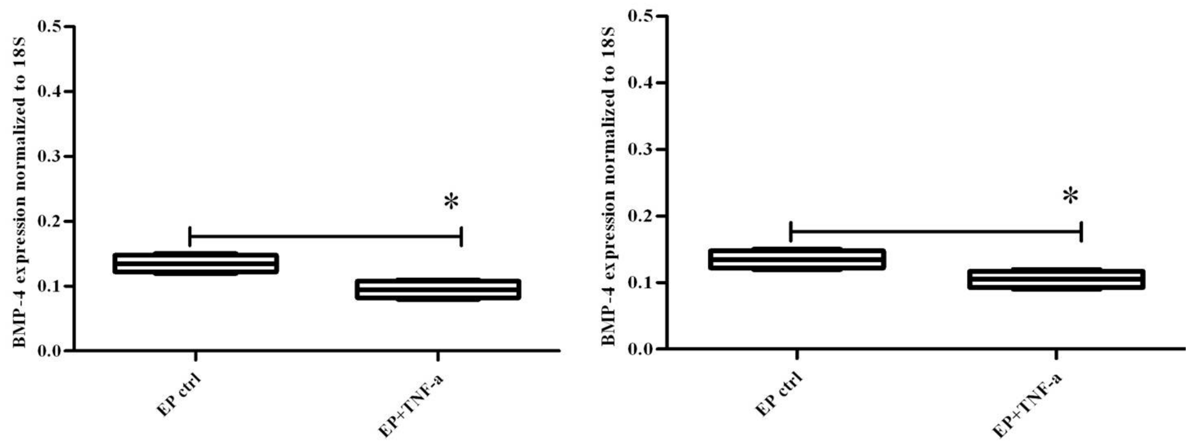

Figure 8: Expression of Tyrosinase and BMP-4 in *ex vivo* human epidermal sheet

In normal human epidermal sheet treated with TNF-α Tyrosinase and BMP-4 mRNA levels were reduced compared to untreated normal human epidermal sheet. *(* $p<0.1$)*

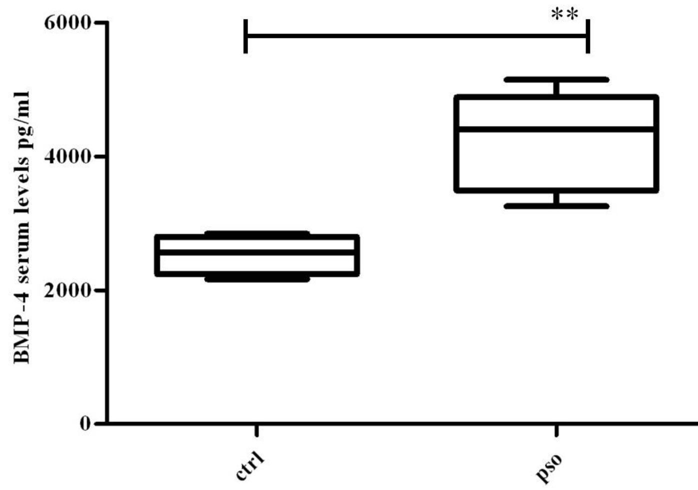

Figure 9: Detection of Serum Levels of BMP-4

BMP-4 serum level was increased in psoriatic patients respect to healthy individuals. *(** p<0.01)*

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