



# The effect of etanercept on vascular endothelial growth factor production by cutaneous mesenchymal stem cells from patients with psoriasis

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## Abstract

**Objective:** To evaluate prospectively the effect of etanercept (a tumour necrosis factor [TNF]- $\alpha$  inhibitor) on vascular endothelial growth factor (VEGF) production by mesenchymal stem cells (MSC) from patients with psoriasis.

**Methods:** MSCs from lesional and perilesional skin were isolated, cultured and characterized. VEGF production was evaluated at baseline and after 12 weeks' etanercept treatment.

**Results:** Etanercept treatment resulted in significant reductions in VEGF production compared with baseline in both lesional MSCs ( $256.42 \pm 3.07$  pg/ml per  $10^6$  cells at baseline vs  $27.66 \pm 2.03$  pg/ml per  $10^6$  cells after treatment) and perilesional MSCs ( $235.03 \pm 2.52$  pg/ml per  $10^6$  cells vs  $41.65 \pm 4.72$  pg/ml per  $10^6$  cells).

**Conclusions:** Etanercept reduces the production of VEGF in MSCs, which may modulate angiogenesis and contributes towards preventing the start of the "psoriatic march".

## Keywords

Angiogenesis, cutaneous mesenchymal stem cells, etanercept, psoriasis, vascular endothelial growth factor

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## Introduction

Pathophysiological events leading to the immune-mediated inflammatory disease, psoriasis, begin at the stem-cell level; mesenchymal stem cells (MSCs) are implicated early in the hypothesized cascade of events linking psoriasis and cardiovascular comorbidities, collectively known as the “psoriatic march”.<sup>1–3</sup> MSCs may produce vascular endothelial growth factor (VEGF), a proangiogenic cytokine that is directly implicated in influencing both dermatologic and systemic involvement in patients with psoriasis.<sup>4,5</sup> Tumour necrosis factor (TNF)- $\alpha$  inhibitors have radically changed the management of psoriasis, a well known T-helper (Th)1–Th17 disease, and the effects of these agents on differentiated cutaneous cells are well described.<sup>6–9</sup>

The aim of the present study was to evaluate the effect of etanercept (a TNF- $\alpha$  inhibitor) on VEGF production by MSCs obtained from patients with psoriasis.

## Patients and methods

### Study population

This prospective clinical study recruited consecutive patients with stable, moderate-to-severe plaque psoriasis attending the Dermatological Clinic, Polytechnic Marche University, Ancona, Italy between November 2013 and January 2014. Inclusion criteria were: aged  $\geq 18$  years; plaque body surface area (BSA)  $>10\%$ ; Psoriasis Area Severity Index (PASI)  $>10$ ;<sup>10</sup> Dermatology Life Quality Index (DLQI)  $>10$ ;<sup>11</sup> absence of psoriatic arthritis; no adequate response to previous conventional treatments including topical corticosteroids, retinoids or vitamin D<sub>3</sub> derivatives, systemic cyclosporine or methotrexate and PUVA (psoralen combined with ultraviolet A), ultraviolet A or ultraviolet B narrowband therapy. Exclusion criteria were: nonplaque psoriasis (guttate, erythrodermic, generalized pustular psoriasis or palmoplantar pustulosis); systemic

administration of methotrexate or cyclosporine in the 4 weeks before enrolment; TNF- $\alpha$  inhibitor treatment in the 12 weeks before enrolment; acitretin treatment in the 2 years before enrolment (owing to its long half-life).<sup>12</sup>

The Polytechnic Marche University ethics committee approved the study, which was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent prior to enrolment.

### VEGF quantification

Patients were evaluated both at baseline and after treatment (50 mg etanercept subcutaneous injection, administered twice weekly for 12 weeks). Lesional and perilesional skin punch biopsies were performed, and MSCs were isolated, cultured and characterized as described.<sup>13,14</sup> MSCs at passage 4–6 were seeded at 4000 cells/cm<sup>2</sup> and incubated with the culture medium  $\alpha$ -MEM. After 3 days, culture media were collected and VEGF was quantified as described,<sup>15</sup> using a commercial enzyme immunoassay kit (human VEGF TiterZyme<sup>®</sup> EIA kit; Assay Designs, Ann Arbor, MI, USA). All assays were carried out in triplicate according to the manufacturers' instructions. Absorbance was read at 450 nm using a plate reader (Sunrise<sup>™</sup>; Tecan Group, Mannedorf, Switzerland). Free VEGF concentrations were extrapolated from the standard curve, and expressed as pg/ml per 10<sup>6</sup> cells. The same volume of nonconditioned medium was placed in the incubator under identical conditions and was used as negative control.

### Statistical analyses

Data were expressed as mean $\pm$ SD for continuous variables, and *n* (%) for categorical variables. Normal distribution of continuous variables was verified with

Kolmogorov–Smirnov test. Between-group differences were tested using one-way analysis of variance. Homogeneity of variance was tested using Cochran’s C test and post hoc comparison (Newmann–Keuls) was used to discriminate between means of values. When necessary, the nonparametric Mann–Whitney *U*-test was used. Data were analysed using Prism version 5.3, (GraphPad, San Diego, CA, USA). *P*-values < 0.05 were considered statistically significant.

## Results

The study included MSCs from three male and two female patients; mean age  $54.6 \pm 10.8$  years; age range 43–67 years). Treatment with etanercept for 12 weeks resulted in significant reductions in VEGF production compared with baseline in both lesional MSCs ( $256.42 \pm 3.07$  pg/ml per  $10^6$  cells at baseline vs  $27.66 \pm 2.03$  pg/ml per  $10^6$  cells after treatment; *P* < 0.05) and perilesional MSCs ( $235.03 \pm 2.52$  pg/ml per  $10^6$  cells at baseline vs  $41.65 \pm 4.72$  pg/ml per  $10^6$  cells after treatment; *P* < 0.05).

## Discussion

Studies of differentiated skin cells have demonstrated that TNF- $\alpha$  inhibitors are able to reduce angiogenesis in both lesional and perilesional skin from patients with psoriasis.<sup>4,16</sup> Our results demonstrate that etanercept reduces the production of VEGF in cutaneous MSCs from these patients.

Etanercept is known to modulate angiogenesis in psoriasis,<sup>4</sup> and the ability of systemic therapy to reduce both cutaneous involvement and comorbidities in psoriasis largely depends on its ability to limit inflammatory and angiogenic phenomena.<sup>4–18</sup>

It is thought that MSCs are involved in the early stages of the “psoriatic-march”.<sup>19</sup> Our findings suggest that anti-TNF- $\alpha$  therapies may prevent the initiation of this process via their actions on MSCs.

## Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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