Keratinocyte growth factor can increase the expression of the EDA⁺ fibronectin

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Introduction:

Psoriasis is considered to be a multigenic inflammatory skin disease with hyperproliferation and abnormal differentiation of the epidermal keratinocytes. In our previous work we showed that the fibronectin splice variant EDA⁺FN (oncofetal fibronectin), its receptor the α5-integrin, the keratinocyte growth factor (KGF), and its receptor (KGFR) are overexpressed in psoriatic uninvolved skin, compared to normal skin. EDA⁺FN and KGF both stimulate keratinocyte proliferation, moreover KGF is also known to induce α5-integrin expression.

Aim:

To measure the effect of exogenous KGF on the EDA⁺FN production of fibroblasts, keratinocytes and HaCaT cells.

Materials and Methods:

▪**Cell cultures:** Fibroblasts (5th passage) Keratinocytes (3rd passage) and HaCaT cells were seeded into 6 well plates at density of 0.3 x 10^6 cells per cm^2 in the appropriate media and were maintained in humidified atmosphere containing 5% CO_2. These cells were incubated with different concentrations of human recombinant KGF (10 ng/ml, 25 ng/ml, 50 ng/ml, 100 ng/ml). We measured the EDA⁺FN gene and protein levels 24 hours after exogenous KGF treatment.

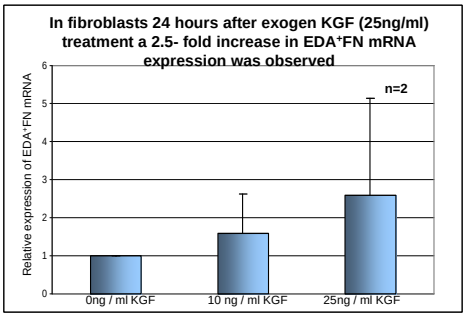
▪**Real-time RT-PCR**:
18S rRNA expression served as internal control. Results are expressed as mean ± SEM. Relative expressions were calculated using the ΔΔCT method.

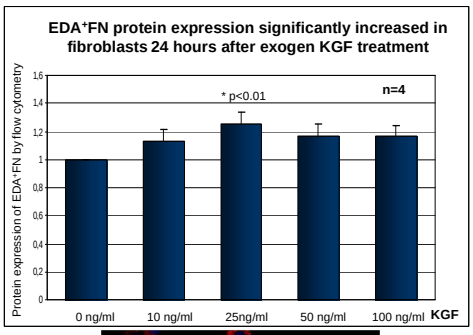
▪ **Immunocytochemistry on cultured human fibroblasts, keratinocytes and HaCaT cells:** primary antibody: mouse MoAb for EDA⁺FN (IST-9), DAPI was used for nuclear staining.
secondary antibody: goat anti-mouse IgG-Alexa 546

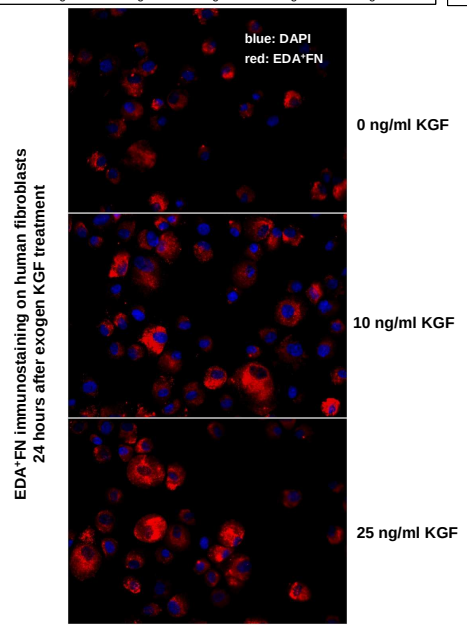
▪**Flow cytometry:**
primary antibodies: mouse MoAb for EDA⁺FN (IST-9), mouse MoAB IgG_1 (isotype control), secondary antibody: goat anti-mouse IgG-Alexa 647

Results:

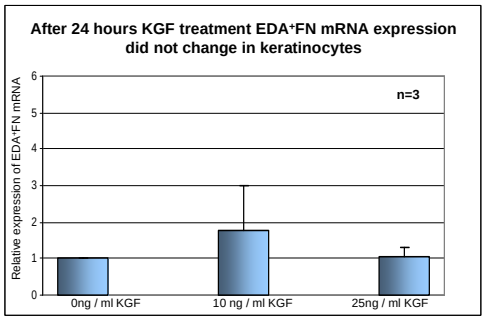
Normal human fibroblasts

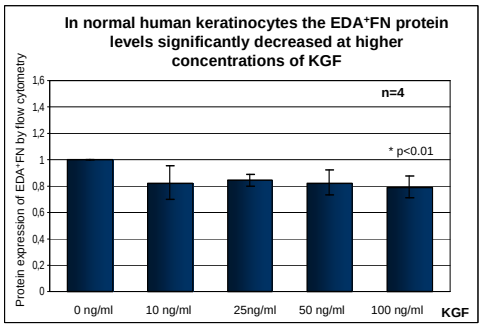


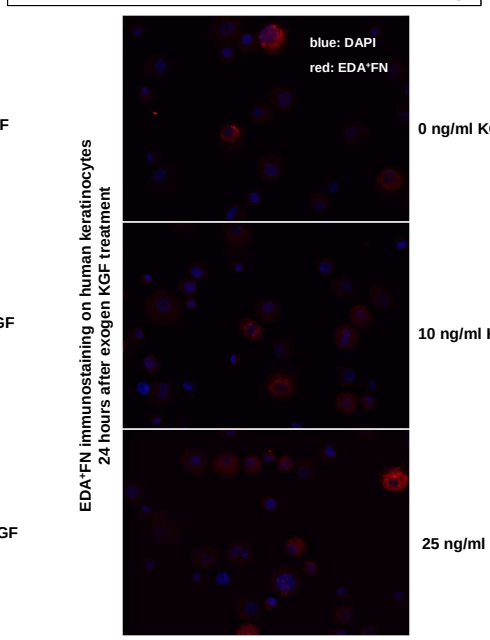




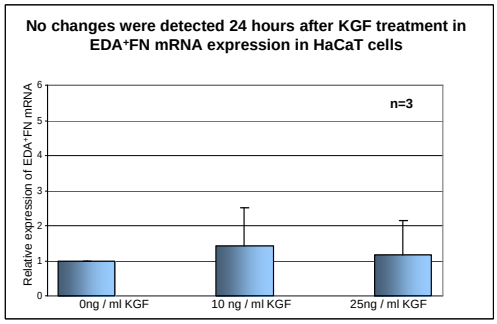
Normal human keratinocytes

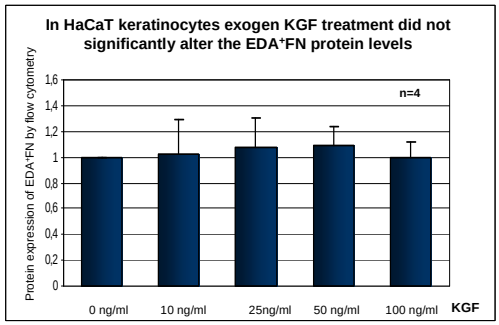


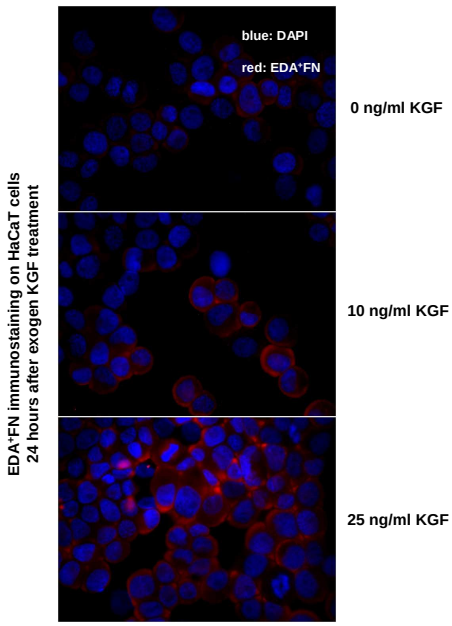




HaCaT cells







Conclusion:

Our results suggest that KGF can promote the production of EDA⁺FN, therefore it may contribute to the altered homeostasis in the uninvolved skin of psoriatic patients.

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