Identification of negative regulatory elements counteracting the *Propionibacterium acnes*-induced signaling pathways in *in vitro* cultured immortalized keratinocytes

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BACKGROUND

The human skin harbors a specialized microbial flora that is crucial for the maintenance of the epidermal homeostasis, but under special circumstances it can also contribute to the pathogenesis of skin diseases. One of the best examples for this is that the colonization of *Propionibacterium acnes* (*P. acnes*) grossly contributes to the pathogenesis of acne. Apart from the signaling events leading to the activation of immune and inflammatory events in response to the bacterium, negative regulatory signals must counteract the positive ones to maintain the epidermal homeostasis.

AIMS

To investigate the interplay of positive (TNFα) and negative regulatory factors (SIGIRR, TOLLIP, TNIP and TNFAIP3) of the Toll-like receptor (TLR)-induced signaling cascades, we analyzed their mRNA expression changes in response to *P. acnes 889* treatment in an *in vitro* cultured immortalized keratinocyte cell line; the HPV-KER.

MATERIALS AND METHODS:

*Human papilloma virus (HPV) E6/E16 immortalized human keratinocytes (HPV-KER; Polyánka 2011; Polyánka, 2013)

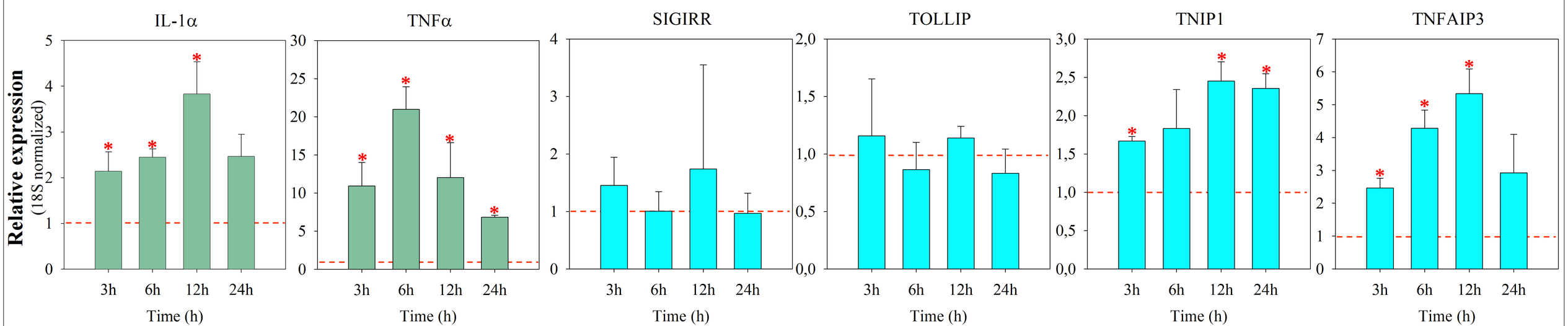
*RNA isolation, cDNA synthesis

*Real-time RT-PCR (UPL, Universal Probe Library System, Roche). Results represent the average changes of at least 2 independent induction experiments. All treatments were performed in triplicates. Error bars depict SEM. All data were normalized (18S rRNA) and relative gene expression was calculated using the ΔΔCt method.

*western blot analysis, and a subsequent semiquantitative image analysis

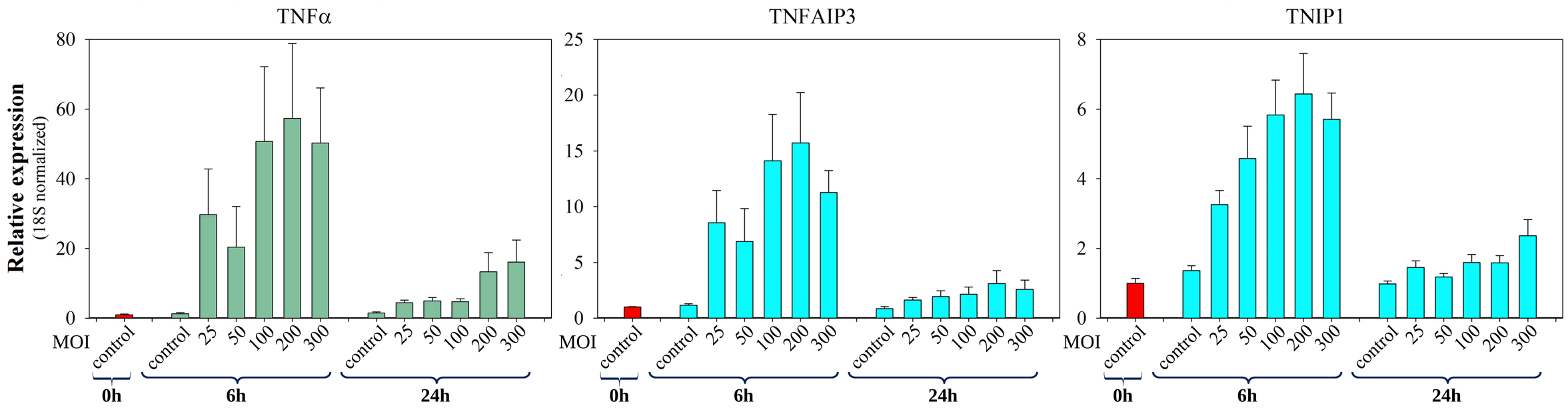
*Luciferase reporter assay

Basal expression of the pro- and anti inflammatory molecules regulating TLR signaling in HPV-KER cells and in response to *P. acnes 889* treatment



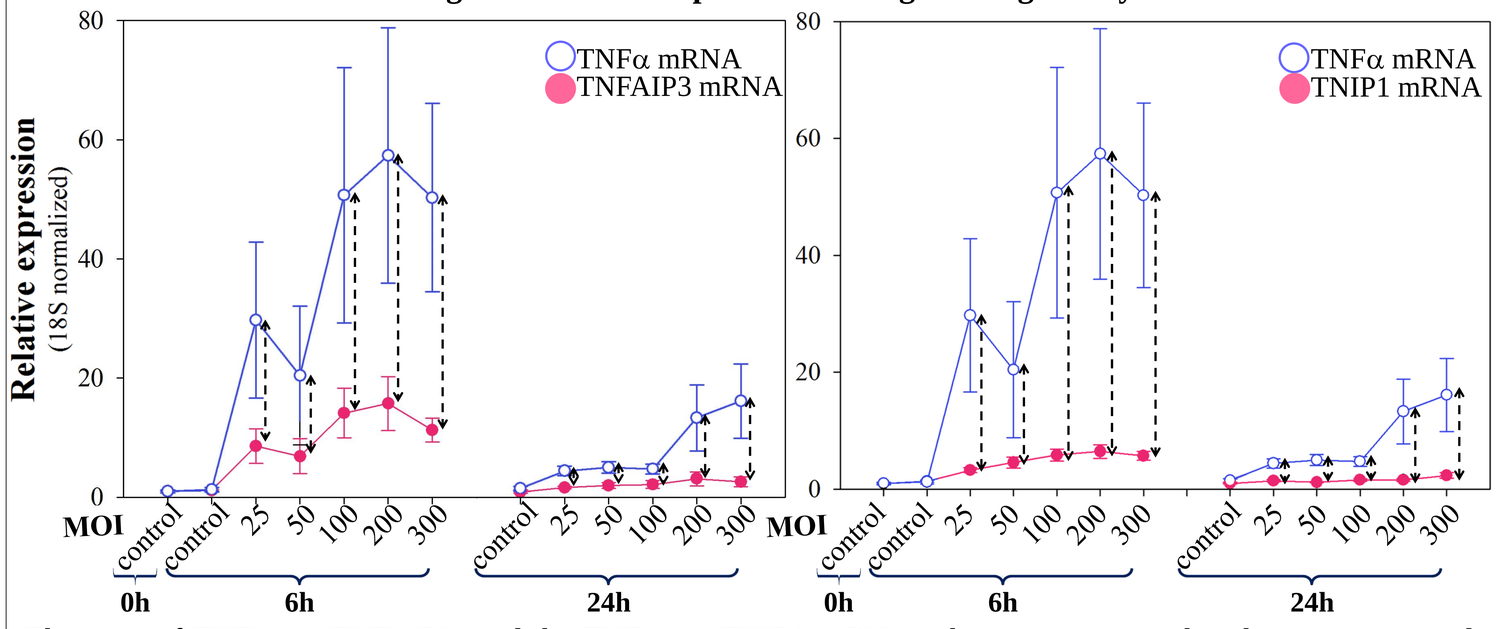
Apart from the expression of the pro-inflammatory cytokines TNFα and IL-1α, the studied negative regulators of TLR signaling are also expressed in HPV-KER cells. The mRNA expression of TNIP1 and TNFAIP3 increases in response to *P. acnes 889* treatment (MOI=100). All data were compared to time-matched untreated control values. (*: one-way ANOVA, post hoc Dunnet's test, $p<0.05$)

Dose dependent expression of the pro- and anti inflammatory molecules regulating TLR signaling in HPV-KER cells and in response to *P. acnes 889* treatment



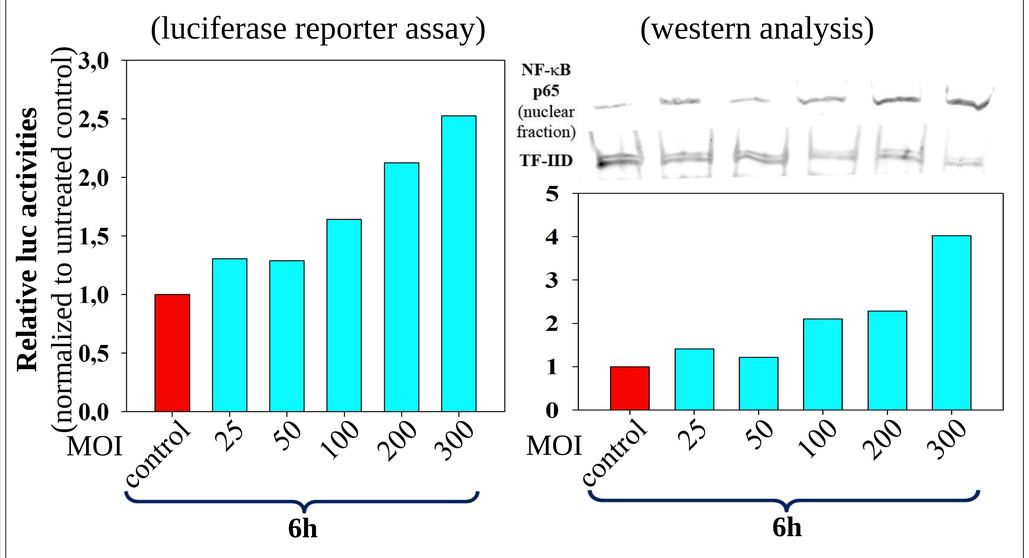
mRNA expressions of the positive regulatory TNFα and the negative regulatory TNFAIP3 and TNIP1 increase dose-dependently parallel to the increasing MOI (multiplicity of infection) of *P. acnes 889*. All data were compared to the 0h untreated control values.

Changes of the ratio of positive and negative regulatory mRNAs



The ratio of TNFα *vs.* TNFAIP3, and the TNFα *vs.* TNIP1 mRNAs changes in a way that the positive signals increasingly outgrow the negative ones. All data were compared to the 0h untreated control values.

Dose-dependent activation of the NF-κB signaling



Parallel to the increased *P. acnes* dose, a similar dose dependent activation of the NF-κB transcription factor can be measured by a reporter analysis, and also by western-blotting detecting the amount of nuclear p65 isoform.

CONCLUSION:

The induced positive and negative signaling changes are increasing parallel to the increase in the applied *P. acnes* dose. The net effect of these events may determine the keratinocytes' cellular behavior, and might contribute to acne severity.

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