Ambivalent Effects of Tumor Necrosis Factor Alpha on Apoptosis of Malignant and Normal Human Keratinocytes

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Keywords

Keratinocytes · Squamous cell carcinoma-13 · HaCaT · Tumour necrosis factor alpha · Apoptosis

Abstract

Introduction: Tumor necrosis factor alpha (TNFα) is a pro-inflammatory cytokine that may paradoxically induce either apoptosis or cell survival. It mediates its activity through binding of TNF-receptor (TNFR) 1 or 2. TNFR1 is mainly responsible for transmitting apoptotic signals. The activation of apoptotic mechanisms can either be intrinsic (mitochondrial) or extrinsic (death receptors). Death ligands such as TNF-related apoptosis-inducing ligand (TRAIL) specifically induce extrinsic apoptosis, while cytostatic drugs such as 5-fluorouracil (5FU) induce intrinsic apoptosis. **Objectives:** To investigate the effects of TNFα on apoptosis in malignant and normal human keratinocytes. **Methods:** Human cutaneous squamous cell carcinoma (SCC) cell line SCC-13 and immortalized human keratinocytes HaCaT as well as primary normal human keratinocytes (PNHK) were stimulated with

TNFα and then treated either with TRAIL or 5FU. Cell viability and cell proliferation, DNA fragmentation, apoptosis, and cytotoxicity were determined by WST-1 proliferation assay, ELISA, flow cytometry, and colorimetric analysis of lactate dehydrogenase, respectively. In addition, Western blotting was performed for analysis of caspase-3. **Results:** TNFα affected viability of SCC-13 and HaCaT cells in combination with 5FU or TRAIL. In contrast, TNFα did not influence cell viability of PNHK. It enhanced the apoptotic effects of both extrinsic and intrinsic stimuli in SCC-13 and HaCaT. In clear contrast, TNFα protected PNHK against TRAIL- and 5FU-induced apoptosis. The effects were dose-dependent and TNFα-specific; furthermore, the apoptosis pathway was caspase-dependent. **Conclusions:** In summary, opposing effects of TNFα in malignant versus normal human keratinocytes were observed with possibly relevant clinical implications, when patients are treated with TNFα inhibitors.

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Introduction

Tumor necrosis factor alpha (TNF α) is a pro-inflammatory cytokine mainly produced by macrophages and monocytes during acute inflammation but also by T cells in the later phase of immune response [1, 2]. It is responsible for several intracellular signaling events, which are exerted through its binding to cell membrane-bound TNF-receptor 1 (TNFR1) and TNFR2 [1]. Both receptors belong to the TNFR superfamily and are associated with adapter proteins that can rescue cells through the activation of nuclear factor kappa beta (NF- κ B). However, in many other cases, these signals may result in either necrosis or apoptosis. Paradoxically, TNF α can also induce cell survival. The diversity of its signaling makes TNF α also important for resistance to infections and cancer [3]. Interestingly, TNF α itself can upregulate its own mRNA, leading to an autocrine effect through binding on TNFR1 in keratinocytes [4]. Besides, TNF α is involved in several immunological processes such as potentiating innate immunity or co-stimulating T-cell activation and differentiation [5–8]. The targeting of TNF α is a well-established and efficacious therapy for several autoimmune diseases like psoriasis, rheumatoid arthritis, or hidradenitis suppurativa [7, 9–12].

Activation of either the intrinsic (mitochondrial) or the extrinsic (death receptor) pathway of apoptosis may lead cells to programmed cell death. Apoptosis is characterized by cell membrane blebbing, cell shrinkage, chromatin condensation, and DNA fragmentation, resulting in phagocytosis by macrophages or neighboring cells, thus avoiding an inflammatory response. The extrinsic apoptotic pathway is activated through the binding of apoptotic ligands on cell membrane death receptors, such as TNF-related apoptosis-inducing ligand (TRAIL). TRAIL binds to the death receptors DR4 and DR5, leading to recruitment of caspase-8 and FADD to form a death-inducing signaling complex, which finally activates effector caspase 3 [13]. TRAIL has been suggested as a promising target in cancer therapy for various tumors, including squamous cell carcinomas (SCC), since it may induce apoptosis in malignant cells but not in normal cells [13, 14].

On the other hand, intrinsic apoptotic pathways start with the permeabilization of the mitochondrial outer membrane, which induces the release of proapoptotic mitochondrial molecules or destroys mitochondrial functions essential for cell survival. Cytostatics and UV radiation are classical inducers of intrinsic apoptosis [15]. In fact, 5-fluorouracil (5FU), a nucleoside analogue, exerts

its cytotoxic effects through DNA damage and, consequently, through induction of intrinsic apoptotic pathways [16].

TNF α can antithetically induce cell survival and apoptosis. We hypothesize that these opposing effects may depend on a premalignant/malignant condition of the cells themselves. In this work, we aimed to investigate the effects of TNF α on induction of apoptosis in human normal and malignant keratinocytes after activation of extrinsic and intrinsic apoptotic pathways.

Materials and Methods

Cell Lines and Cell Culture Conditions

Here, the human cutaneous cell line SCC-13 [17] and the immortalized keratinocyte cell line HaCaT [18] were used. Cells were grown in RPMI 1640 medium (Life Technologies, Darmstadt, Germany) supplemented with 10% FCS, 2 mM glutamine, 19 nonessential amino acids (alanine, asparagine, aspartic acid, glutamic acid, glycine, proline, and serine; Biochrom, Berlin, Germany) and penicillin/streptomycin. Primary normal human keratinocytes (PNHK) were derived from human foreskins, as previously described [19], and were maintained in culture using keratinocyte growth medium (Keratinocyte-SFM KGM, Gibco, Berlin, Germany) supplemented with 50 μ g/mL bovine pituitary extract (Gibco), 5 ng/mL recombinant epidermal growth factor (rEGF, Gibco), and penicillin/streptomycin. All cells were cultured in monolayers at 37°C in a humidified atmosphere containing 5% CO₂. Only subconfluent, 4th to 6th passage keratinocytes were used for the experiments. A day after the passage, cells received fresh growth medium supplemented with/without TNF α (10 ng/mL; Sigma-Aldrich, Munich, Germany) or TNF α and infliximab (IFX) (200 μ g/mL; Centocor Biotech Inc., County Cork, Ireland). Dose of infliximab was determined in pilot experiments based on the dose range of already published data [20, 21]. The stimulation time with TNF α alone or TNF α /IFX was 24 h. Then, cells were further treated for 18 h with soluble, human, recombinant TRAIL (KillerTRAIL, Alexis, Gruenberg, Germany ALX-201-073-C020; 30 ng/mL or 30, 15, 7.5 ng/mL for dose-dependent experiment), or for 48 h with 5FU (40 μ mol; Sigma-Aldrich, Munich, Germany).

Quantification of Cell Proliferation, Apoptosis, and Cytotoxicity

Cell proliferation and viability were quantified using the colorimetric, nonradioactive WST-1 assay (Roche, Mannheim, Germany), based on the cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells, following the protocol of the manufacturer. Apoptosis was quantified by a Cell Death Detection ELISA kit (Roche Diagnostics, Mannheim, Germany), which detects cytoplasmic histone-associated DNA fragments in apoptotic cells. Cytotoxicity was determined in parallel by quantification of lactate dehydrogenase (LDH) release in culture supernatants using a detection kit of Roche Diagnostics, according to the protocols of the supplier. Relative apoptosis and cytotoxicity values were calculated as compared to the values of untreated controls.

Fig. 1. Cell proliferation and cell viability of SCC-13, HaCaT, and PNHK stimulated with TNFα and treated with TRAIL (a) or 5FU (b) applying WST-1 assay. Data are presented as percentage of the control. Values are means ± SD of triplicates. The differences between the marked groups were analyzed using the Student *t* test.

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. TNFα, tumor necrosis factor alpha; TRAIL, TNF-related apoptosis-inducing ligand; 5FU, 5-fluorouracil; SCC, squamous cell carcinoma; PNHK, primary normal human keratinocytes.

Cell cycle analyses and quantification of apoptosis were additionally performed by flow cytometry, as described before [22]. In brief, cells were stained with propidium iodide (Sigma-Aldrich, Taufkirchen, Germany; 200 mg/mL, 1 h), and cell fractions in G1, G2, S-phase, and sub-G1 were quantified by flow cytometry (FAC-SCalibur, BD Bioscience, Bedford, MA, USA). Sub-G1 cell fraction corresponds to apoptotic cells due to washing out of fragmented DNA.

Western Blot Analysis

For protein extraction, cells were seeded in 75-cm² culture flasks and harvested at a confluence of 60–80%. Cells were washed with PBS before lysis buffer (10 mM Tris-HCl, pH 7.5; 150 mM NaCl; 1 mM EDTA; 2 mM PMSF; 1 mM leupeptin; 1 mM pepstatin;

0.5% SDS; and 0.5% Nonidet P-40) was added. Protein extraction and Western blot analysis were performed, as previously described [23]. For protein detection, the following antibodies were used: GAPDH (6C5, sc-32233, mouse, 1:200, Santa Cruz, CA, USA), β-actin (C4, sc-47778, mouse, 1:1,000, Santa Cruz, CA, USA), and caspase-3 (sc-9661, rabbit, 1:1,000, Santa Cruz, CA, USA).

Statistical Analysis

Experiments consisted of triplicate values and were at least twice independently repeated. Mean values, standard deviations, and statistical significance of representative experiments are shown. The significance of differences between experimental variables was determined by the paired *t* test, and a *p* value <0.05 was considered statistically significant.

Fig. 2. Apoptosis of SCC-13, HaCaT, and PNHK stimulated with TNFα and treated with TRAIL (a) or 5FU (b) quantified by the Cell Death Detection ELISA kit detecting DNA fragments in apoptotic cells. The relative values were normalized to untreated controls. Values are means ± SD of triplicates. The differences between the

marked groups were analyzed using the Student *t* test. **p* ≤ 0.05, ***p* ≤ 0.01, ****p* ≤ 0.001. TNFα, tumor necrosis factor alpha; TRAIL, TNF-related apoptosis-inducing ligand; 5FU, 5-fluorouracil; SCC, squamous cell carcinoma; PNHK, primary normal human keratinocytes.

Results

Loss of Cell Viability and Cell Proliferation

For investigating cell proliferation and cell viability in response to apoptosis induction in combination with TNFα, the WST-1 assay was applied. Treating cells with TNFα for 42 h did not show any significant effects in SCC-13, HaCaT, or PNHK. Also, treatment with TRAIL for 18 h remained without significant effect on cell proliferation in SCC-13 and PNHK, while TRAIL signifi-

cantly reduced cell proliferation in HaCaT (*p* < 0.01, Fig. 1). The combination of TRAIL with TNFα decreased cell proliferation in all cell types as compared to TRAIL alone, which was significant in SCC-13 and PNHK (*p* < 0.05) and showed a tendency in HaCaT (*p* = 0.06; Fig. 1a). Furthermore, cell proliferation of SCC-13, HaCaT, and PNHK was significantly suppressed by 5FU alone at 48 h. The combination with TNFα (72 h) further significantly decreased cell proliferation in SCC-13 and HaCaT, while the effect was not significant in PNHK (*p* = 0.482; Fig. 1b).

Fig. 3. Apoptosis (DNA fragmentation) of HaCaT (**a**) and PNHK (**b**) stimulated with TNF α and treated with different doses of TRAIL; and apoptosis (DNA fragmentation) of SCC-13 (**c**) and PNHK (**d**) stimulated with TNF α , infliximab, or both and treated with TRAIL and 5FU, respectively, detected by using the Cell Death Detection ELISA kit. The relative values were normalized to

untreated controls. Values are means $\pm$ SD of triplicates. The differences between the marked groups were analyzed using the Student *t* test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. TNF α , tumor necrosis factor alpha; TRAIL, TNF-related apoptosis-inducing ligand; 5FU, 5-fluorouracil; SCC, squamous cell carcinoma; PNHK, primary normal human keratinocytes.

Induction of Apoptosis

To further investigate the effects of TNF α , TRAIL, and 5FU on apoptosis induction, we performed DNA fragmentation analysis by using the Cell Death Detection ELISA kit and quantification of cells with fragmented DNA (sub-G1 cells) by flow cytometry as well as quantification of cytotoxicity by LDH release assay. As shown in Figure 2, TNF α caused apoptosis in SCC-13 and HaCaT cells in

a limited but statistically significant degree, as compared to controls. The apoptotic effects of TRAIL on the cells were stronger, and TNF α stimulation followed by TRAIL treatment led to a significant enhancement of apoptosis, as compared to TRAIL alone (SCC-13, 1.6-fold; HaCaT, 1.9-fold, $p < 0.05$). In clear contrast, TNF α did not induce apoptosis in PNHK, and it strongly diminished the proapoptotic effects of TRAIL by 1.9-fold ($p < 0.05$, Fig. 2a).

Fig. 4. a Apoptosis quantified by flow cytometry using PI staining of SCC-13 and PNHK stimulated with TNFα and treated with 5FU. Gate M1 corresponds to sub-G1 phase of the cell cycle including apoptotic cells with hypodiploid nuclei. Data are presented as percentage of the gated cells in a representative experiment. **b** Western blot analysis of prodromal caspase-3 before cleavage in

SCC-13 and PNHK stimulated with TNFα and treated with 5FU or TRAIL, respectively. GAPDH or β-actin was used as loading controls. TNFα, tumor necrosis factor alpha; TRAIL, TNF-related apoptosis-inducing ligand; 5FU, 5-fluorouracil; SCC, squamous cell carcinoma; PNHK, primary normal human keratinocytes.

Comparable results were obtained from the combination of TNFα stimulation and intrinsic apoptosis induction with 5FU. Accordingly, treatment of SCC-13 and HaCaT cells with 5FU for 48 h led to slightly induced apoptosis. A significant boost of apoptosis was observed, when cells were stimulated with TNFα before 5FU treatment, resulting in 2.9-fold increased apoptosis in SCC-13 and 3.4-fold in HaCaT, both as compared to only 5FU. Again in clear contrast, the proapoptotic effects of 5FU in PNHK were significantly diminished to 1.52-fold ($p < 0.05$, Fig. 2b).

We further considered possible dose-dependent effects of TRAIL in this setting. Thus, HaCaT and PNHK were treated with different concentrations of TRAIL (30, 15, and 7.5 ng/mL) with or without TNFα stimulation. Apoptosis induction in these cells by TRAIL appeared as clearly dose-dependent (Fig. 3a, b). Highly comparable effects were seen for the 3 TRAIL concentrations in combination with TNFα, namely, apoptosis induction by TRAIL in HaCaT cells was enhanced by TNFα (1.8-, 1.4-, and 1.9-fold; $p < 0.05$; Fig. 3a), while it significantly decreased apoptosis in PNHK (1.5-, 1.2-, and 2.3-fold; $p < 0.05$; Fig. 3b).

To exclude possible autocrine influence of endogenous TNF α or any nonspecific effects of added TNF α , we blocked its activity by coculturing cells with IFX, a chimeric monoclonal antibody against human TNF α . Thus, SCC-13 cells were treated with TNF α , infliximab, and TRAIL, while PNHK received TNF α , infliximab, and 5FU, in order to investigate effects both on extrinsic and intrinsic apoptosis pathways and in malignant and normal cells. While infliximab alone remained without effect on apoptosis in SCC-13 and PNHK, in combination, it diminished apoptosis induction by TNF α /TRAIL in SCC-13 to a similar level as TRAIL alone, and it enhanced apoptosis induction by TNF α /5FU in PNHK to a similar level as 5FU alone (Fig. 3c, d).

For further confirmation of the contrasting effects of TNF α on malignant and benign human keratinocytes, cell cycle analysis by flow cytometry and Western blot analysis was additionally performed. For cell cycle analysis, SCC-13 and PNHK were treated with TNF α and/or 5FU. The results largely confirmed the previous data of DNA fragmentation analysis. Thus, TNF α enhanced the 5FU-induced sub-G1 cell population (M1 = apoptotic cells) in SCC-13, while it slightly decreased the 5FU-induced sub-G1 cell population (M1) in PNHK (Fig. 4a).

Effects found at the level of procaspase-3, which is cleaved in caspase-dependent apoptosis, were largely in parallel with apoptosis assays. Western blotting showed stronger caspase-3 degradation in malignant SCC-13 cells in response to TNF α /5FU treatment than in cells treated with 5FU alone. Similarly, the TRAIL-induced degradation of procaspase-3 in PNHK was reversed by TNF α (Fig. 4b), suggesting caspase-3-mediated apoptosis regulation in this setting. Importantly, no significant cytotoxicity of the abovementioned treatments could be detected by LDH measurement in culture supernatants (data not shown).

Discussion/Conclusion

As shown in many scientific investigations, TNF α is a pleiotropic cytokine that can trigger opposing events in target cells [3]. In this study, we found opposing effects of TNF α in normal and malignant keratinocytes on extrinsic and intrinsic apoptosis controls. As models in this study, we used a well-established cell line of malignant keratinocytes, the cutaneous SCC cell line SCC-13; the immortalized keratinocyte cell line HaCaT; and cultures of PNHK. It has been controversially discussed whether HaCaT may be more representative for malignant or for

normal cells [18, 24–26]. As shown here, HaCaT revealed parallel to malignant keratinocytes in terms of apoptosis regulation through 5FU, TRAIL, and TNF α , rather than to normal keratinocytes. As shown in quantitative analysis, HaCaT cells responded 10 times stronger in terms of DNA fragmentation than PNHK. This may be related to faster cellular metabolism and proliferation rates of HaCaT cells, in contrast to normal keratinocytes.

Already published data had shown that stimulation with TNF α did not significantly affect apoptosis of PNHK [27], while HaCaT and SCC-13 were more susceptible to TNF α -induced apoptosis [22, 28]. In contrast, both TRAIL and 5FU significantly affected cell proliferation and cell viability in all 3 keratinocyte cell populations, which is in parallel with previous data [29–32]. Induction of apoptosis by TRAIL appeared as clearly dose-dependent, as shown for HaCaT and PNHK.

The opposing effects of TNF α in normal and malignant keratinocytes were evident by decrease or enhancement of apoptosis in combination with characteristic inducers of the extrinsic and intrinsic apoptosis pathway (TRAIL and 5FU). In fact, pretreatment of HaCaT and SCC-13 cells with TNF α before stimulation enhanced the apoptotic effects of 5FU and TRAIL, while TNF α protected normal keratinocytes against both proapoptotic stimuli. The specific dependence of these effects of TNF α was proven by simultaneous incubation with infliximab, a chimeric anti-TNF α monoclonal antibody. Hence, infliximab abolished the TNF α -mediated effects both in normal and malignant keratinocytes.

Depending on the type of TNFR and the subsequent recruitment of the associated intracellular adapter proteins, TNF α can induce controversial signals such as apoptotic/anti-apoptotic or pro-inflammatory/anti-inflammatory. TNFR1 engagement can induce either apoptosis through the subsequent cleavage of caspase-8 or can inhibit apoptosis, when TRAF2 is recruited, leading to activation of NF- κ B [33–35]. Unlike TNFR1, TNFR2 lacks a death domain; therefore, the transduction of TNF α signaling through TNFR2 leads via NF- κ B activation to an anti-apoptotic effect and inflammation [36]. In the case of human head and neck SCC, it was shown that TNF α can induce survival through activation of TNFR-associated factor 2 pathway, whereas apoptosis was induced through FADD-caspase-8-caspase-3 and ASK-JNK-p53-Noxa pathways [37]. These unpredictable effects of TNF α may at least partly explain its limited value as an antitumor agent. Furthermore, in clinical trials, TNF α was found to be unsuitable for systemic administration at clinically relevant doses, since it caused toxic

side effects that were associated with its strong pro-inflammatory activity, including fever, lung or liver failure, increased blood clotting, and hypotension [38].

Interestingly, TNFR2 was shown not to be expressed by PNHK [39]. However, similarly to epithelioid and giant cells in the hyperplastic human lymphoid tissue [40], normal human keratinocytes might upregulate expression of TNFR2 in the presence of TNF α . This may partly explain the observed protective effects seen for TNF α in PNHK against apoptosis in response to TRAIL and 5FU. A further explanation of the different effects of TNF α on apoptosis of malignant and normal human keratinocytes may be the different expression of TRAF2 in these cells. In fact, TRAF2 expression and activation can be regulated in human keratinocytes [41]. However, it should be noted that no studies have been published focusing on TRAF2 expression in SCC-13 cells, so far. Publications about TRAF2 expression in PNHK and HaCaT are very rare, as well [41].

Although considering the limitations of these in vitro results and the clear gap between bench and bedside conditions, our findings may provide some insights concerning anti-TNF α therapies in patients with tumors of malignant keratinocytes. Indeed, an increased risk of NMSCs, associated with exposure to anti-TNF α , was observed in a meta-analysis of studies with patients on biological treatments [42]. Blocking the enhancing apoptotic effects of TNF α with monoclonal antibodies in malignant keratinocytes might reduce the response of cytostatics like 5FU or even shut down the protective effects of TNF α produced by normal keratinocytes. This hypothesis undoubtedly needs to be further investigated.

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References

- 1 Sedger LM, McDermott MF. TNF and TNF-receptors: from mediators of cell death and inflammation to therapeutic giants: past, present and future. *Cytokine Growth Factor Rev.* 2014 Aug;25(4):453–72.
- 2 Sabat R, Wolk K, Loyal L, Döcke WD, Ghoreschi K. T cell pathology in skin inflammation. *Semin Immunopathol.* 2019 May;41(3):359–77.
- 3 Idriss HT, Naismith JH. TNF alpha and the TNF receptor superfamily: structure-function relationship(s). *Microsc Res Tech.* 2000 Aug 1;50(3):184–95.
- 4 Lisby S, Faurschou A, Gniadecki R. The autocrine TNF α signalling loop in keratinocytes requires atypical PKC species and NF-kappaB activation but is independent of cholesterol-enriched membrane microdomains. *Biochem Pharmacol.* 2007 Feb 15;73(4):526–33.
- 5 Yi F, Frazzette N, Cruz AC, Klebanoff CA, Siegel RM. Beyond cell death: new functions for TNF family cytokines in autoimmunity and tumor immunotherapy. *Trends Mol Med.* 2018 Jul;24(7):642–53.
- 6 Krause K, Sabat R, Witte-Händel E, Schulze A, Puhl V, Maurer M, et al. Association of CCL2 with systemic inflammation in Schnitzler syndrome. *Br J Dermatol.* 2019 Apr;180(4):859–68.

Statement of Ethics

This was an experimental in vitro work with cell lines. An ethic approval was not required.

Conflict of Interest Statement

G.K. has received honoraria for participation in advisory boards, in clinical trials, and/or as a speaker from Abbott GmbH, AbbVie Deutschland GmbH & Co. KG, Actelion Pharmaceuticals Ltd., Basilea Pharmaceutica Ltd., Bayer AG, Biogen IDEC GmbH, Charité Research Organisation GmbH, Celgene GmbH, Janssen-Cilag GmbH, LEO Pharma GmbH, Lilly Deutschland GmbH, MSD Sharp & Dohme GmbH, Novartis Pharma GmbH, Parexel International GmbH, Pfizer Deutschland GmbH, and UCB Pharma GmbH. R.S. has received research grants, scientific awards, or honoraria for participation in advisory boards, clinical trials, or as a speaker for 1 or more of the following: AbbVie Inc., AbbVie Deutschland GmbH & Co. KG, AMGEN GmbH, Bayer Schering Pharma AG, Biogen IDEC GmbH, Boehringer Ingelheim Pharma GmbH & Co. KG, Celgene GmbH, Celgene International II Sàrl, Charité Research Organisation GmbH, CSL Behring GmbH, Dr. Willmar Schwabe GmbH & Co. KG, Flexopharm GmbH & Co. KG, Generon Corporation Ltd., Janssen-Cilag GmbH, La Roche-Posay Laboratoire Dermatologique Deutschland, Novartis Pharma GmbH, Parexel International GmbH, Pfizer Deutschland GmbH, Sanofi–Aventis Deutschland GmbH, TFS Trial Form Support GmbH, and UCB Pharma GmbH. S.K. has received honorary for her participation in an advisory board of UCB. S.A. W.S. and J.E. have no conflicts of interest to declare.

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Author Contributions

G.K., R.S., W.S., S.K., and J. E. conceived the experiments and designed the research. G.K. and J.E. performed the research. G.K. and R.S. performed data analysis and interpretation. All authors contributed to the development of the methodology and preparation of the manuscript.

- 7 Kokolakis G, Giannikaki E, Stathopoulos E, Avramidis G, Tosca AD, Krüger-Krasagakis S. Infliximab restores the balance between pro- and anti-apoptotic proteins in regressing psoriatic lesions. *Br J Dermatol*. 2012 Mar; 166(3):491–7.
- 8 Psarras Antonios, Antanaviciute Agne, Alase Adewonuola, Carr Ian, Wittmann Miriam, Emery Paul, et al. TNF- α Regulates Human Plasmacytoid Dendritic Cells by Suppressing IFN- α Production and Enhancing T Cell Activation. *J Immunol*. 2021.
- 9 Croft M, Benedict CA, Ware CF. Clinical targeting of the TNF and TNFR superfamilies. *Nat Rev Drug Discov*. 2013 Feb;12(2):147–68.
- 10 Kokolakis G, Bachmann F, Wolk K, Sabat R, Philipp S. Efficacy of adalimumab for nail psoriasis during 24 months of continuous therapy. *Acta Derm Venereol*. 2020 Jul 28; 100(14):adv00214.
- 11 Sabat Robert, Jemec Gregor B E, Matusiak Łukasz, Kimball Alexa B, Prens Errol, Wolk Kerstin. Hidradenitis suppurativa. *Nat Rev Dis Primers*. 2020;6(1):18.
- 12 Ghoreschi Kamran, Balato Anna, Enerbäck Charlotta, Sabat Robert. Therapeutics targeting the IL-23 and IL-17 pathway in psoriasis. *Lancet*. 2021.
- 13 Yuan X, Gajan A, Chu Q, Xiong H, Wu K, Wu GS. Developing TRAIL/TRAIL death receptor-based cancer therapies. *Cancer Metastasis Rev*. 2018 Dec;37(4):733–48.
- 14 Derakhshan A, Chen Z, Van Waes C. Therapeutic small molecules target inhibitor of apoptosis proteins in cancers with deregulation of extrinsic and intrinsic cell death pathways. *Clin Cancer Res*. 2017 Mar 15;23(6): 1379–87.
- 15 Jin Z, El-Deiry WS. Overview of cell death signaling pathways. *Cancer Biol Ther*. 2005 Feb; 4(2):139–63.
- 16 Sampath D, Rao VA, Plunkett W. Mechanisms of apoptosis induction by nucleoside analogs. *Oncogene*. 2003 Dec 8;22(56):9063–74.
- 17 Rheinwald JG, Beckett MA. Tumorigenic keratinocyte lines requiring anchorage and fibroblast support cultured from human squamous cell carcinomas. *Cancer Res*. 1981 May; 41(5):1657–63.
- 18 Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J Cell Biol*. 1988 Mar;106(3):761–71.
- 19 Tebbe B, Wu S, Geilen CC, Eberle J, Kodelja V, Orfanos CE. L-ascorbic acid inhibits UVA-induced lipid peroxidation and secretion of IL-1 α and IL-6 in cultured human keratinocytes in vitro. *J Invest Dermatol*. 1997 Mar; 108(3):302–6.
- 20 Faurschou A, Gniadecki R, Wulf HC. Infliximab inhibits DNA repair in ultraviolet B-irradiated premalignant keratinocytes. *Exp Dermatol*. 2008 Nov;17(11):933–8.
- 21 Aubin F, Gheit T, Prétet JL, Tommasino M, Mougin C. Effect of infliximab on the UVB-induced apoptosis of keratinocytes infected by HPV38 E6/E7. *ISRN Dermatol*. 2013;2013: 907189.
- 22 Fecker LF, Stockfleth E, Braun FK, Rodust PM, Schwarz C, Köhler A, et al. Enhanced death ligand-induced apoptosis in cutaneous SCC cells by treatment with diclofenac/hyaluronic acid correlates with downregulation of c-FLIP. *J Invest Dermatol*. 2010 Aug;130(8): 2098–109.
- 23 Kurbanov BM, Fecker LF, Geilen CC, Sterry W, Eberle J. Resistance of melanoma cells to TRAIL does not result from upregulation of antiapoptotic proteins by NF- κ B but is related to downregulation of initiator caspases and DR4. *Oncogene*. 2007 May 17;26(23): 3364–77.
- 24 Lehman TA, Modali R, Boukamp P, Stanek J, Bennett WP, Welsh JA, et al. p53 mutations in human immortalized epithelial cell lines. *Carcinogenesis*. 1993 May;14(5):833–9.
- 25 Vaccariello M, Javaherian A, Wang Y, Fusenig NE, Garlick JA. Cell interactions control the fate of malignant keratinocytes in an organotypic model of early neoplasia. *J Invest Dermatol*. 1999 Sep;113(3):384–91.
- 26 Ridd K, Dhir S, Smith AG, Gant TW. Defective TPA signalling compromises HaCat cells as a human in vitro skin carcinogenesis model. *Toxicol In Vitro*. 2010 Apr;24(3):910–5.
- 27 Zimmermann M, Koreck A, Meyer N, Basinski T, Meiler F, Simone B, et al. TNF-like weak inducer of apoptosis (TWEAK) and TNF- α cooperate in the induction of keratinocyte apoptosis. *J Allergy Clin Immunol*. 2011 Jan;127(1):200–7.
- 28 Southall MD, Isenberg JS, Nakshatri H, Yi Q, Pei Y, Spandau DF, et al. The platelet-activating factor receptor protects epidermal cells from tumor necrosis factor (TNF) α and TNF-related apoptosis-inducing ligand-induced apoptosis through an NF- κ B-dependent process. *J Biol Chem*. 2001 Dec 7; 276(49):45548–54.
- 29 Qin JZ, Bacon PE, Chaturvedi V, Bonish B, Nickoloff BJ. Pathways involved in proliferating, senescent and immortalized keratinocyte cell death mediated by two different TRAIL preparations. *Exp Dermatol*. 2002 Dec;11(6): 573–83.
- 30 Wood J, Pring M, Eveson JW, Price N, Proby CM, Hague A. Co-overexpression of Bag-1 and heat shock protein 70 in human epidermal squamous cell carcinoma: bag-1-mediated resistance to 5-fluorouracil-induced apoptosis. *Br J Cancer*. 2011 Apr 26;104(9):1459–71.
- 31 Lamberti M, Porto S, Zappavigna S, Stiuso P, Tirino V, Desiderio V, et al. Levofolene modulates apoptosis induced by 5-fluorouracil through autophagy inhibition: clinical and occupational implications. *Int J Oncol*. 2015 May;46(5):1893–900.
- 32 Liang L, Cheng C, Hu G, Wang X, Liu J, Yan Z, et al. TWEAK promotes the proliferation of squamous cell carcinoma cells through activating cIAP1 signals. *Front Oncol*. 2020;10: 439.
- 33 Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. *Cell*. 1996 Jan 26;84(2):299–308.
- 34 Liu ZG, Hsu H, Goeddel DV, Karin M. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF- κ B activation prevents cell death. *Cell*. 1996 Nov 1;87(3):565–76.
- 35 Tobin D, van Hogerlinden M, Toftgård R. UVB-induced association of tumor necrosis factor (TNF) receptor 1/TNF receptor-associated factor-2 mediates activation of Rel proteins. *Proc Natl Acad Sci U S A*. 1998 Jan 20; 95(2):565–9.
- 36 Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol*. 2001 Sep;11(9):372–7.
- 37 Selimovic D, Wahl RU, Ruiz E, Aslam R, Flanagan TW, Hassan SY, et al. Tumor necrosis factor- α triggers opposing signals in head and neck squamous cell carcinoma and induces apoptosis via mitochondrial- and non-mitochondrial-dependent pathways. *Int J Oncol*. 2019 Dec;55(6):1324–38.
- 38 Hersch EM, Metch BS, Muggia FM, Brown TD, Whitehead RP, Budd GT, et al. Phase II studies of recombinant human tumor necrosis factor α in patients with malignant disease: a summary of the Southwest Oncology Group experience. *J Immunother*. 1991 Dec; 10(6):426–31.
- 39 Wolk K, Haugen HS, Xu W, Witte E, Waggie K, Anderson M, et al. IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN- γ are not. *J Mol Med*. 2009 May;87(5):523–36.
- 40 Ryffel B, Brockhaus M, Dürmüller U, Gudat F. Tumor necrosis factor receptors in lymphoid tissues and lymphomas. Source and site of action of tumor necrosis factor α . *Am J Pathol*. 1991 Jul;139(1):7–15.
- 41 Cheng H, Zhan N, Ding D, Liu X, Zou X, Li K, et al. HPV Type 16 infection switches keratinocytes from apoptotic to proliferative fate under TWEAK/Fn14 interaction. *J Invest Dermatol*. 2015 Oct;135(10): 2427–36.
- 42 Peleva E, Exton LS, Kelley K, Kleyn CE, Mason KJ, Smith CH. Risk of cancer in patients with psoriasis on biological therapies: a systematic review. *Br J Dermatol*. 2018 Jan; 178(1):103–13.