Annals of Anatomy 213 (2017) 25–32

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat

RESEARCH ARTICLE

# Platelet-released growth factors induce psoriasin in keratinocytes: Implications for the cutaneous barrier

Andreas Bayer[a,*,1], Justus Lammel[b], Sebastian Lippross[d], Tim Klüter[d], Peter Behrendt[d], Mersedeh Tohidnezhad[c], Thomas Pufe[c], Jochen Cremer[a], Holger Jahr[e], Franziska Rademacher[b], Regine Gläser[b], Jürgen Harder[b,1]

[a] Department of Heart- and Vascular Surgery, University Hospital of Schleswig-Holstein, Campus Kiel, Arnold-Heller Straße 3, Haus 26, 24105 Kiel, Germany
[b] Department of Dermatology, University Hospital of Schleswig-Holstein, Campus Kiel, Schittenhelmstr. 7, 24105 Kiel, Germany
[c] Institute of Anatomy and Cell Biology, RWTH University of Aachen, Wendlingweg 2, 52072 Aachen, Germany
[d] Department of Traumatology, University Hospital of Schleswig-Holstein, Campus Kiel, Arnold-Heller Straße 3, Haus 18, 24105 Kiel, Germany
[e] Department of Orthopedics, Aachen University Hospital, Pauwelsstr. 30, 52074 Aachen, Germany

ARTICLE INFO

*Article history:*
Received 1 March 2017
Received in revised form 9 April 2017
Accepted 20 April 2017

*Keywords:*
Wound healing
Antimicrobial peptides
Cutaneous innate defense
Psoriasin (S100A7)

ABSTRACT

Millions of patients around the world suffer minor or major extremity amputation due to progressive wound healing complications of chronic or infected wounds, the therapy of which remains a challenge. One emerging therapeutic option for the treatment of these complicated wounds is the local application of an autologous thrombocytes concentrate lysate (*e.g.* platelet-released growth factors ((PRGF)) or Vivostat PRF®) that contains a multitude of chemokines, cytokines and growth factors and is therefore supposed to stimulate the complex wound healing process. Although PRGF and Vivostat PRF® are already used successfully to support healing of chronic, hard-to-heal and infected wounds the underlying molecular mechanisms are not well understood.

Psoriasin, also termed S100A7, is a multifunctional antimicrobial protein expressed in keratinocytes and is involved in various processes such as wound-healing, angiogenesis, innate immunity and immune-modulation. In this study, we investigated the influence of PRGF on psoriasin expression in human primary keratinocytes *in vitro* and the influence of Vivostat PRF® on psoriasin expression in experimentally generated skin wounds *in vivo*.

PRGF treatment of primary keratinocytes caused a significant concentration- and time-dependent increase of psoriasin gene and protein expression *in vitro* that were partially mediated by the epidermal growth factor receptor (EGFR) and the interleukin-6 receptor (IL-6R). In accordance with these cell culture data, Vivostat PRF® induced a significant psoriasin gene and protein expression when applied to artificially generated skin wounds *in vivo*. The observed psoriasin induction in keratinocytes may contribute to the wound healing-promoting effects of therapeutically used thrombocyte concentrate lysates.



## 1. Introduction

In the early stages of the physiological wound healing process thrombocytes perform a major function. After tissue injury they enter the tissue defect and release a multitude of chemokines, cytokines and growth factors (Eppley et al., 2004; Weibric et al., 2002; Yazawa et al., 2003; Anitua et al., 2004; van den Dolder et al., 2006) thus initializing and organizing the complex process of human wound healing. As platelet-released growth factors (PRGF) is a thrombocyte concentrate lysate containing these wound healing relevant proteins, it has the capacity to stimulate cell proliferation and tissue regeneration, to modify cell and tissue differentiation and to support angiogenesis *in vitro* (Yamaguchi et al., 2012; Graziani et al., 2006; Rughetti et al., 2008; Kawasumi et al., 2008; Arpornmaeklong et al., 2004; Kilian et al., 2004; Kakudo et al., 2011, 2008; Spreafico et al., 2009). In line with these known characteristics of thrombocyte concentrate lysates, it is not surprising that their preparations for *in vivo* use (*e.g.* Vivostat PRF®) have already revealed promising effects in promoting the wound

* Corresponding author.
*E-mail address:* andreas.bayer@uksh.de (A. Bayer).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.aanat.2017.04.002
0940-9602/

healing process (Steenvoorde et al., 2008) and have been described as a biomimetic approach to enhance surgical wound healing (Fernandez-Moure et al., 2017). Despite these principal properties of PRGF and the observed beneficial effects of an application of thrombocyte concentrate lysates such as Vivostat PRF® on the human wound healing process, little is known about possible mechanisms involved. Recently, we have found that PRGF and Vivostat PRF® induced the expression of terminal differentiation marker in keratinocytes (unpublished submitted data). We have also shown that PRGF and Vivostat PRF® induced the antimicrobial peptides human beta-defensin-2 (hBD-2) (Bayer et al., 2016) and human beta-defensin-3 (hBD-3) (unpublished submitted data) in primary human keratinocytes *in vitro* and *in vivo*. The question remains as to whether PRGF and Vivostat PRF® are able to induce further antimicrobial peptides in human keratinocytes demonstrating their positive effect on the epithelial skin barrier and their relevance for the wound healing process. Psoriasin (or S100A7) is such an antimicrobial peptide that was initially isolated from keratinocytes of psoriasis patients (Celis et al., 1990; Madsen et al., 1991). It is expressed by keratinocytes of healthy humans (Wittersheim et al., 2013) as well as by epithelial cells of patients with inflammatory or malignant diseases of the skin, the breast, the bladder and urinary tract, the tongue, the nose, the ocular surface and the oral cavity (Garreis et al., 2011; Laudien et al., 2011; Gambichler et al., 2009; Meyer et al., 2008; Ostergaard et al., 1997; Celis et al., 1996; Moubayed et al., 2007; Zhou et al., 2008; Moog-Lutz et al., 1995). Psoriasin restricts the outgrowth of *Escherichia coli* on human skin (Gläser et al., 2005) and acts as a major *E. coli*-cidal factor in the female genital tract (Mildner et al., 2010). It is induced in human keratinocytes after microbial (*e.g. E.coli* or *Pseudomonas aeruginosa*) (Meyer-Hoffert et al., 2011; Gläser et al., 2005) or physical (Gläser et al., 2009a,b; Harder et al., 2010) threats. In this context, a strong psoriasin induction has been observed at the margins of chronic wounds that are supposedly colonized by pathological microorganisms (Dressel et al., 2010) or after superficial skin injury (Harder et al., 2010). Moreover, psoriasin has been proven to display chemoattractant properties (Jinquan et al., 1996) and to regulate keratinocyte differentiation after skin injury (Lei et al., 2017). In conclusion, psoriasin is a multifunctional protein with strong antimicrobial properties assumed to be a key molecule of the cutaneous barrier (Gläser et al., 2011). Because of its proposed relevance for the human skin barrier and wound healing process we decided to analyze whether PRGF influences psoriasin expression in human primary keratinocytes *in vitro* and whether Vivostat PRF® affects psoriasin expression in experimentally wounded skin *in vivo*.

## 2. Methods

### 2.1. Preparation of PRGF

PRGF was isolated from supernatants of several freshly donated human thrombocyte concentrates donated by the Institute of Transfusion Medicine, University Hospital of Schleswig-Holstein, Campus Kiel and prepared as previously described (Bayer et al., 2016). We centrifuged the freshly donated human thrombocyte concentrates for 10 min at 2000 × *g*, washed the thrombocyte pellet twice with a sodium citrate buffer (0.11 mM, ph 5.5, 37 °C) and centrifuged again for 10 min at 2000 × *g*. Afterwards, we resuspended the thrombocytes in half the volume of the initial thrombocyte concentrate volume using Keratinocyte Growth Medium 2 (Promocell, Heidelberg, Germany) without supplements. The resuspended thrombocytes were stored on ice, lysed by ultrasound and stored at −80 °C for 24 h. Subsequently, we thaw the suspension, repeated the ultrasound procedure and stored the suspension again at −80 °C for 24 h. The next day the suspension was thawed and centrifuged for 1 min at 18.000 × *g*. The supernatant is the PRGF which was stored in aliquots at −20 °C.

### 2.2. Culture and stimulation of primary human keratinocytes

We used foreskin-derived primary human keratinocytes pooled from different individuals (Promocell, Heidelberg, Germany) and cultured them in Keratinocyte Growth Medium 2 (KGM-2, Promocell, Heidelberg, Germany) at 37 °C in a humidified atmosphere with 5% $CO_2$. The keratinocytes were seeded in 12-well tissue culture plates (BD Biosciences, Franklin Lakes, New Jersey) for stimulatory experiments. When the cell confluence reached 90–100% we thawed the frozen PRGF and diluted it to the indicated concentrations with KGM-2 for keratinocyte stimulation. For EGFR- or IL-6 receptor blocking experiments we employed the EGFR-blocking antibody cetuximab (Merck, Darmstadt, Germany) or the IL-6 receptor blocking antibody tocilizumab (Hoffmann-La Roche, Basel, Switzerland) at concentrations of 20 μg/ml and 50 μg/ml, respectively. IL-6 and TGF-alpha were purchased from Peprotech (Hamburg, Germany) and used at a concentration of 50 ng/ml for keratinocyte stimulation. After stimulation, we harvested the supernatants for ELISA and washed the cells with 1 ml per well of PBS before RNA isolation.

### 2.3. RNA-isolation and cDNA synthesis

We harvested keratinocytes from one well of a 12-well plate and lysed them using 500 μl Crystal RNAmagic reagent. Afterwards, total RNA was prepared according to the supplier's protocol (Biolab-Products, Bebensee, Germany) and RNA quantity and quality was estimated photometrically by a NanoDrop device (Peqlab, Erlangen, Germany). A 1 μg aliquot of total RNA was reversely transcribed to cDNA using oligo-dT-primers and 50 Units Maxima Reverse Transcriptase (Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's protocol.

### 2.4. Real-time PCR

We performed real-time PCR analyses in a fluorescence-temperature cycler (StepOne Plus, Life Technologies) as previously described (Roth et al., 2014). The following intron spanning primers for psoriasin were used: 5′-AGA CGT GAT GAC AAG ATT GAG-3′ (forward primer) and 5′-TGT CCT TTT TCT CAA AGA CATC-3′ (reverse primer). Standard curves were generated by serial dilutions of cDNA. All quantifications were normalized to the house keeping gene RPL38 (ribosomal protein L38) using the primer pair: 5′-TCA AGG ACT TCC TGC TCA CA-3′ (forward primer) and 5′-AAA GGT ATC TGC TGC ATC GAA-3′ (reverse primer). Relative expression is given as a ratio between psoriasin and RPL38 gene expressions.

### 2.5. ELISA

We determined secreted psoriasin protein levels by ELISA as described (Gläser et al., 2009a) with a detection range of the ELISA of 0.6–40 ng/ml.

### 2.6. Analyses of the influence of Vivostat PRF® on the psoriasin expression of keratinocytes in vivo

We performed an *in vivo* study with five male test persons in which we set bilateral gluteal wounds by punch biopsy (Ø 4 mm) after local anesthesia. After generation, the wounds were immediately treated with either NaCl 0.9% as a control (left) or Vivostat PRF® (right) and closed by a polyurethane dressing (Biatain® "Schaumverband selbst-haftend", Coloplast, Hamburg, Germany).