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# The differential expression of protease activated receptors contributes to functional differences between dark and fair keratinocytes

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ABSTRACT

*Background:* Dark skin has different properties in comparison to fair skin. Melanocytes have been shown to partly contribute to these differences, however, the involvement of keratinocytes from dark or fair skin is not well demonstrated.
*Objectives:* This study investigated the proliferation and barrier function of dark keratinocytes (DK) and fair keratinocytes (FK), and the role of protease activated receptor (PAR)1 and PAR2.
*Methods:* DK and FK were isolated from human neonatal foreskins. Cells were treated with PAR1/PAR2 agonists or antagonists, proliferation was measured by BrdU assay; permeability by the flux of FITC-dextran; protein expression by immunostaining or western blot.
*Results:* When compared to FK, DK proliferated significantly slower; had higher cell permeability; expressed less phosphorylated (P)-ERK/ERK, caspase-14, E-cadherin, tissue growth factor (TGF)-β3 and PAR1; and expressed more PAR2, and matrix metalloproteinase (MMP)-9. Activation of PAR1 or inhibition of PAR2 stimulated cell proliferation and ERK activation, and in concordance inhibition of PAR1 or activation of PAR2 suppressed cell proliferation and ERK activation in both DK and FK. Inhibition of PAR2 decreased and inhibition of PAR1 increased cell permeability. In foreskin sections, the epidermis of dark foreskin expressed less caspase-14 and the same level but different distribution of E-cadherin, when compared to fair foreskin.
*Conclusions:* These data highlight functional differences in proliferation and barrier integrity between HK and FK that are partly associated with their differential expression of PAR1 and PAR2.


## 1. Introduction

The skin is a multilayered organ that protects the organism against external aggression. The outermost layer of the skin is the epidermis, which is the major barrier of the skin [1], and is continuously renewed by the proliferation of keratinocytes at the basal layer. Melanocytes also reside within the epidermis. The production and distribution of melanin synthesized by melanocytes determine the skin color. It is well documented that dark skin (African descent) is more resistant to the damaging effects of UV radiation [2], has later onset and less skin wrinkling and sagging signs, decreased skin surface pH [3–5], and lower rates of basal/squamous cell carcinomas and melanomas [6], when compared to fair skin (Caucasian descent). However, trans-epidermal water loss is greater, abnormal scarring such as hypertrophic scar, keloid disease and striae distensae are more common or severe in dark skin [7].

Although melanocytes and their synthesized melanin contribute to functional differences in these two colored skins, dark keratinocytes (DK) and fair keratinocytes (FK) also show intrinsic differences in skin function [8,9]. For example: keratinocyte growth factor-promoted melanosome transfer is more significant in FK compared to DK [10]; PAR2, which is expressed on keratinocytes [11], but not on melanocytes [12], is more highly expressed and activated in dark skin when compared to fair skin [13]. Inhibition of PAR2 activation reduces melanosome transfer and distribution, leading to a dose-dependent skin lightening in vivo [14–16]. However, the mechanistic differences between DK and FK are not well understood.

PARs are G-coupled receptors that belong to a family of four members (PAR1-4). PAR1 is mainly activated by thrombin; and PAR2 by trypsin and mast cell tryptase, but not by thrombin. Activation of PARs occurs in, and contributes to the

*Abbreviations:* DK, dark keratinocytes; FK, fair keratinocytes; PAR, protease activated receptor; P, phosphorylated; MMP, matrix metalloproteinase; ELISA, enzyme-linked immunosorbent assay.

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pathogenesis of various acute and chronic inflammatory conditions [17,18]. In skin, both PAR1 and PAR2 are expressed in epidermis and dermis [19,20]. PAR1 activation promotes the proliferation of human keratinocytes [21] and dermal fibroblasts [22], however, the functional consequence of PAR1 expression in the skin still remains elusive. In contrast, ample evidence shows that PAR2 affects keratinocyte proliferation and differentiation, maintains the epidermal barrier, regulates inflammation [23–27] and pain perception [28], and has a tumor-protective role in the skin [29].

In this study, we investigated the functional differences between DK and FK and the contribution of PAR1 and PAR2.

## 2. Materials and methods

### 2.1. Keratinocyte isolation and culture

Human keratinocytes were isolated from dark (African descent, scored with Fitzpatrick's skin type 6) or fair (Caucasian descent, scored with Fitzpatrick's skin type 2) neonatal foreskins after routine circumcision in local hospitals and cultured as described previously [30]. When greater than 70% confluent, cells were trypsinized and seeded into either 24 or 96-well culture plates, or 8-well permanox™ slides for further treatment/analysis.

Usage of human skin tissues was in accordance with the ethics committee of Local Health District. Studies were based on skin color alone as the neonates from whom the specimens were obtained were de-identified to the researchers. Four dark and four fair foreskins were used.

### 2.2. BrdU cell proliferation and MTT assays

Cell proliferation was detected by BrdU Cell Proliferation assay kit (Merck Millipore, Merck KGaA, Darmstadt, Germany) according to the manufacturer's instructions; cell viability was measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The concentrations of PAR1 or PAR2 agonist/antagonist and FITC-dextran used in this study did not affect cell viability.

### 2.3. Western blot

Western blot was performed as described previously [31]. The primary antibodies used were: PAR1 and PAR2 (Santa Crutz Technology), caspase-14, E-cadherin, Akt and phosphorylated (P)-Akt, ERK and P-ERK (Cell Signaling Technology). Immunoreactivity was detected using the ECL detection system (Amersham). Anti-human β-actin antibody was included to normalize against unequal loading.

### 2.4. Immunofluorescent/immunohistochemical staining

Keratinocytes in permanox slides were fixed with 1% paraformaldehyde. Human foreskins were fixed with 10% phosphate buffered saline buffered formalin. Paraffin-embedded tissue sections were de-paraffinised and subjected to immunostaining using mouse anti-human caspase-14 and rabbit anti-human E-cadherin antibodies, followed by fluorescent-conjugated second antibodies and counterstained with DAPI. Slides were observed

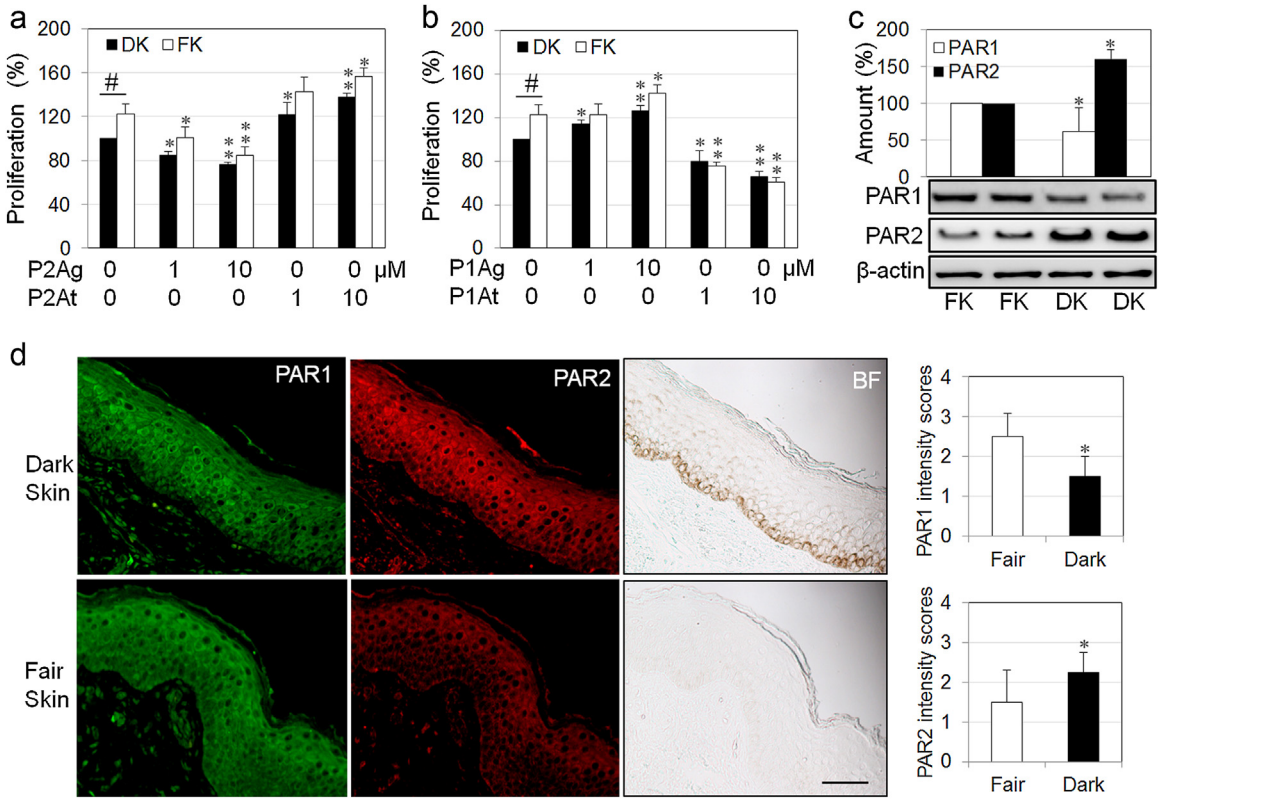


**Fig. 1.** The proliferation of DK and FK and its association with PAR1 and PAR2.
Cell proliferation of DK and FK in normal culture conditions or treated with PAR2 (a) or PAR1 (b) agonist (Ag)/antagonist (At) for 72 h, detected by BrdU proliferation assay. P1: PAR1, P2: PAR2. c) The expression of PAR1 and PAR2 by DK and FK, detected by western blot and semi-quantitated by image analysis software. β-actin was used as a loading control. Data on graph are shown as mean ± SD (n = 3). d) PAR1 and PAR2 in epidermis of fair and dark skin detected by immunofluorescent staining and semi-quantified as described in method section. Scale bar: 100 μm. # $P \leq 0.05$, when DK compared to FK; * $P < 0.05$ and ** $P < 0.01$ when compared to each non-treated normal culture conditions. Images represent one of four experiments.