Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 361 (2007) 1–6
BBRC
www.elsevier.com/locate/ybbrc

Expression of GATA-3 in epidermis and hair follicle: Relationship to p63

Anissa Chikh [a], Emre Sayan [b], Sebastien Thibaut [c], Anna Maria Lena [a], Sabrina DiGiorgi [a], Bruno A. Bernard [c], Gerry Melino [b,*], Eleonora Candi [a,*]

[a] Department of Experimental Medicine and Biochemical Sciences, University of Rome "Tor Vergata", via di Tor Vergata 135, 00133 Rome, Italy
[b] Medical Research Council, Toxicology Unit, University of Leicester, Lancaster Road, Leicester LE1 9HN, UK
[c] L'Oreal Recherche, 14, rue Theodore de Banville, 75017 Clichy, France

Received 31 May 2007
Available online 2 July 2007

Abstract

The epidermis is a multi-layered stratified epithelium continuously renewed by differentiating keratinocytes that develops by the action of p63, a member of the p53 family. The TP63 contains two promoters, resulting in the expression of different proteins, containing (TAp63) or not (ΔNp63) an amino-terminal transactivation domain, which contribution in skin formation is not fully understood. We found that p63 binds and transactivate GATA-3 promoter, which in turn transactivate IKKα, two pivotal regulators of epithelial development. Indeed, GATA-3 is a regulator of cell lineage in skin and hair follicles formation. To further study the relationship between GATA-3 and p63 isoforms here we investigated their expression during keratinocyte differentiation, in human epidermis and hair follicle.

Keywords: p63; p53 family; GATA-3; IKKα; Hair follicle; Epidermis

p63, a member of p53 gene family, plays a key role in regulating epithelial development and maintenance of squamous epithelia [1]. Data from p63 knock out mice suggest that p63 is critically required for limb, skin and craniofacial development. Indeed, these mice display defects in their skin and their derivatives, and die shortly after birth due to lack of epidermal barrier [2,3]. Recently, it has been shown that p63 specifically functions to maintain the extraordinary proliferative capacity of the epithelial stem cells of stratified epithelia [4]. In addition p63, and particularly the ΔNp63 isoform, is important for thymic development [4,5]. p63 is expressed in at least six different isoforms, these multiple isoforms are the result of two different promoters, one preceding the first exon and the second within the third intron; transcription from these promoters give rise to TA- or ΔN-amino termini of p63, respectively. In addition, alternative splicing of the C-termini generate isoforms α, β and γ.

The ΔNp63α isoform is the main isoform expressed at all embryonic stages during epithelial, tooth and hair development, accounting for 100% of all p63 expressed up to E9 and 99% at E13; accordingly, TAp63 expression starts at E13 and accounts for 1% of total p63 protein expressed at this time [6]. ΔNp63 is the main isoform expressed in the basal layer of the adult epidermis and it is important for the formation of the basal layer, whereas TAp63 acts synergistically and/or subsequently to ΔNp63 to contribute to the differentiation program [7–9].

p63 reveals a remarkable structural similarity to p53 reaching 66% identity in the DNA binding domain, suggesting that, p63 can regulate target genes transcription by binding to p53-like responsive element, in addition to p63-specific responsible elements that have been recently identified [7,10–12]. By micro array analysis [7,13,14] we found out that several GATA family members are up regulated by p63 [14].

The GATA family is divided into two subfamilies, based on the tissue distribution of each GATA family member: GATA-1, -2, and -3 and GATA-4, -5, and -6. GATA-1, -2, and -3 are predominantly associated with the haematopoietic cell lineage; whereas GATA-4, -5, and

* Corresponding authors. Fax: +39 06 20427290.
E-mail addresses: melino@uniroma2.it (G. Melino), candi@uniroma2.it (E. Candi).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.06.069

-6 are mainly associated with organ development such as liver, lung, pancreas, gut, and heart [15]. In addition to the haematopoietic system, GATA-3 displays temporally and spatially, a developmental dynamic expression pattern in the embryo. The expression pattern of GATA-3 changes in the differentiating organs and tissues, and during the gestation, GATA-3 is restricted to the central and peripheral nervous systems, the kidney, the adrenal gland and the primitive thymus [16]. Embryos deficient in GATA-3 do not survive beyond E11.0, showing massive internal bleeding, malformations of the brain and spinal cord, aberrations in foetal liver haematopoiesis and marked growth retardations [17]. The cause of lethality seems related to be due to the noradrenalin deficiency, which is critical for maintaining cardiac homeostasis, a consequence of the heart failure [18].

In addition to the immune system, GATA-3 is expressed in the upper basal layers (spinous) of the epidermis and in the inner root sheat (IRS) of the hair follicle [19,20]. Interestingly, it has been shown that GATA-3 is a regulator of cell lineage in skin and hair follicles formation [19]. Indeed, GATA-3-null embryos show aberrations in hair follicle morphogenesis that include not only structural defects in the IRS and in the hair shaft, but also molecular defects in cell lineage determination [19]. Beside its role in cell lineage determination, GATA-3 is required to establish the epidermal barrier and survive in ex-utero environment, by direct transactivation of the lipid acyltransferase gene, AGPAT5 [20]. In addition, we found at molecular level that GATA-3 is a p63 target gene [14]. In order to further investigate the role of GATA-3 in epidermal and hair maturation, we investigated the expression of GATA-3 in normal human epidermis and human hair follicle in comparison to TAp63 and ΔNp63 isoforms.

Materials and methods

Antibody production. The N-terminal part of TAp63, which is not shared with ΔNp63, (amino acids 1–69) was cloned into the pET101/D vector with an N-terminal six histidine tag. Recombinant proteins were expressed in Escherichia coli (BL21) and purified under denaturing conditions using Ni–NTA resin. Proteins were then refolded and buffer exchanged to PBS using NAP buffer exchange columns (Amersham). Finally, proteins were concentrated to 0.5 mg/ml using Centripreps (Millipore). Antisera were generated by subcutaneous injection of recombinant proteins emulsified with Titermax adjuvant (Stratech Scientific, UK) into New Zealand White rabbits at 4-week intervals. Serum was collected prior to the first immunization (pre-immune serum) and after the third immunization with peptide conjugate. The immune sera obtained only recognized TAp63 but not ΔNp63; hereof referred to as anti-TAp63-sp.

Cell culture and transfection. Sub confluent HEK293 cells in 10-cm dishes were transfected with 15 μg of total DNA (HA-tagged TAp63 and ΔNp63 vectors) using 30 μl of lipofectamine 2000 reagent (Invitrogen). Cells were harvested 24 h after transfection and lysed in RIPA buffer with protease inhibitors (Sigma, St. Louis, MI, USA) and 1 mM DTT (Sigma).

Solubilization of proteins and Western blots. To extract proteins from E19.5 embryonic skin, embryos were incubated at room temperature (RT) for 24 h in 100 mM Tris–HCl, pH 8.5, 1% SDS, 20 mM DTT, and 5 mM EDTA; 50 μg of extracted proteins was separated on SDS–PAGE and transferred onto polyvinylidendifluoride membranes; blots were kept in blocking solution for 2 h. Blots were incubated for 2 h with shaking at RT with the following primary antibodies: monoclonal anti-p63 (H-137,1:200 dilution; Ab-4, 1:100 dilution), polyclonal anti-HA (Y-11, Santa Cruz, CA, USA, 1:100 dilution), polyclonal anti-K10; polyclonal, anti-loricrin and anti-filaggrin (Babco, 1:300 dilution), anti GATA-3 (Santa Cruz, dilution 1:50). Normalization was achieved with a polyclonal anti-tubulin (H-235, Santa Cruz, 1:1000 dilution) or with a goat anti-actin antibody (C-11, Santa Cruz, 1:1000 dilution). After three washes in PBS–Tween 20 (0.05%), HRP-conjugated secondary antibodies (Bio-Rad, goat anti-mouse, 170–5047 and goat anti-rabbit, 170–5046; and Santa Cruz, bovine anti-goat, sc-2384) were added (1:10 000–20,000 dilution in blocking solution). Proteins were detected using the ECL method.

Immunofluorescence and confocal analysis. Plastic surgery samples of human scalp skin were obtained from informed volunteers according to hospital ethical committee practice. Samples were cut into small pieces containing about 6 intact follicles. Unfixed tissue samples were embedded in Tissue-Tek OCT compound (Miles, Naperville, IL, USA), quick frozen over dry ice, and stored at −80 °C until used for immuno-fluorescence. Longitudinal frozen sections (10-μm thick) of hair follicles and human skin were prepared on a cryostat CM3050 (Leica, Rueil-Malmaison, France) whose chamber temperature was set at −40 °C. Sections were then air dried and stored at +4 °C overnight before being processed as previously described [21]. Briefly, samples were fixed in acetone at −20 °C for 10 min followed by several washes in phosphate-buffered saline (PBS). Primary antibodies, were diluted in 0.05% PBS–Tween containing 10% of normal serum corresponding to the conjugated secondary antibody species, and applied for 30 min at room temperature (dilutions: GATA-3, HG3-35 Santa Cruz, 1:50; p63 (H137), Santa Cruz, 1:100; TAp63-sp 1:100). Fluorescent species-specific secondary antibodies were then applied for 30 min at room temperature (dilutions: FITC, Jackson, 1:100; Cv3, Jackson, 1:300. Stainings were analysed with a Zeiss Axioscop microscope (Carl Zeiss, Oberkochen, Germany), and multi-fluorescent detections were analysed with a confocal laser-scanning microscope LSM 510 (Carl Zeiss, Oberkochen, Germany). Data were processed with Zeiss software allowing a fine analysis of labelling co-distribution.

Results and discussion

Generation and characterization of TAp63-sp polyclonal antibody

The N-terminal part of human TAp63, which is not shared with ΔNp63, (amino acids 1–69) was cloned into the pET101/D vector, expressed in Escherichia coli (BL21) and purified (Fig. 1A). Purified protein was used to inject rabbits at 4-week intervals. An affinity column purified the immune sera obtained, the antibody obtained was then characterized by Western blot and immunofluorescence (Fig. 1). Hemagglutinin-tagged (HA-tagged) TAp63α, β, γ, and ΔNp63α, β, γ constructs were transiently transfected in HEK-293 cell line. Following transfection, total proteins were extracted and subjected to Western blotting with the new polyclonal anti-TAp63 (named: TAp63-sp) and anti-HA antibodies (Fig. 1B). The anti-TAp63-sp antibody detected the proteins at the expected sizes only in TAp63α, β, or γ transfected cells but did not detected any band in the ΔNp63 α, β, or γ transfected cells, showing the specificity of the new polyclonal antibody generated (Fig. 1B) within TA and ΔN isoforms. In order to evaluate the specificity and the affinity of the anti-TAp63-sp polyclonal antibody within the other p53 family members, HEK-293 cells were transiently transfected with

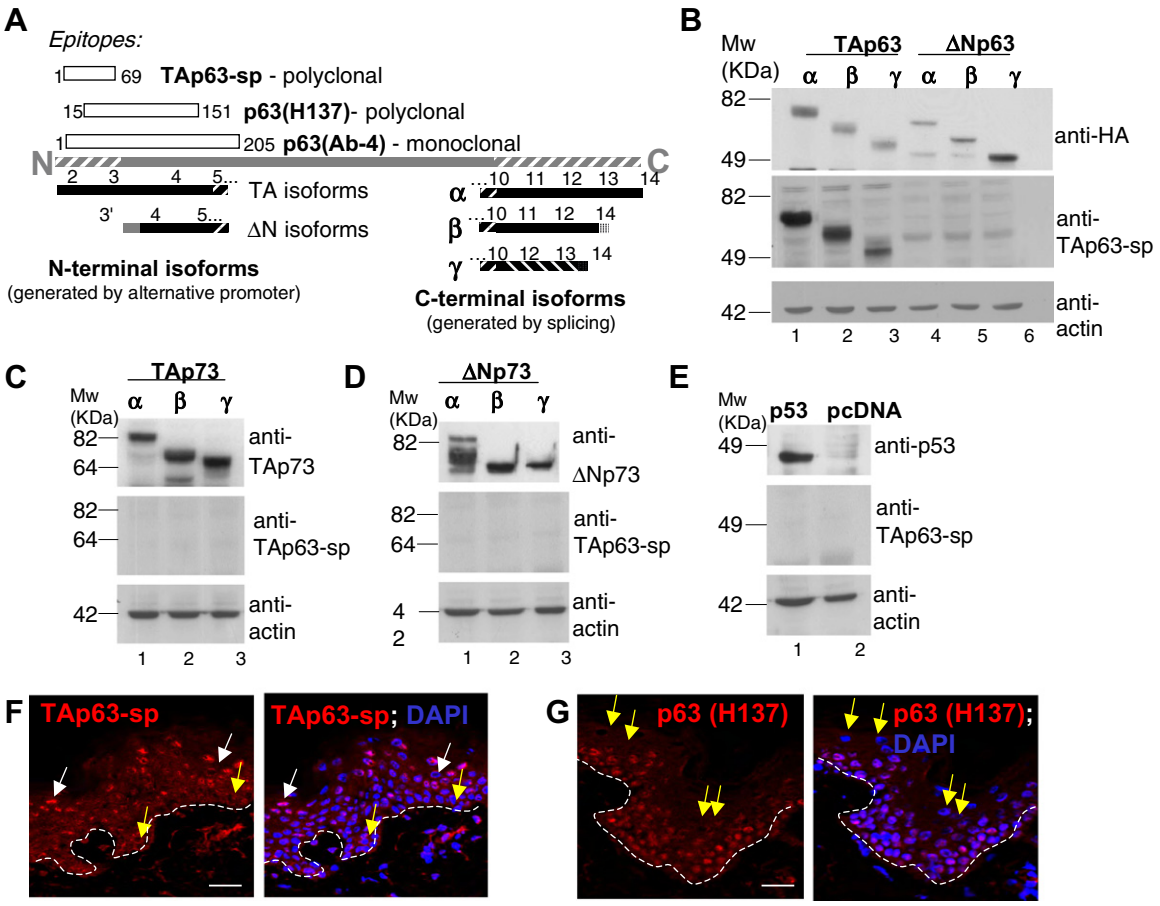

Fig. 1. Characterization of the specific anti-TAp63-sp antibody. (A) Schematic representation of the p63 molecule indicating the location of the epitopes recognised by the antibodies used in this study. (B) HA-tagged TAp63 and ΔNp63 isoforms were transiently transfected in HEK 293 cells. Protein extracts were analysed by Western blotting using anti-HA (upper panel) and anti-TAp63-sp (middle panel) antibodies. The expression of all p63 isoforms was shown by anti-HA staining. As loading control we have used the actin staining. The specificity of the new antibody was tested against p53 (E) and p73 isoforms (C and D). In both cases TAp73, ΔNp73 isoforms, and p53 were transiently transfected in HEK 293 cells. Protein extracts were analysed by Western blotting using anti-p73 (C and D, upper panels) and anti-p53 (C, upper panel) and TAp63-sp antibodies. The anti-TAp63-sp that we have generated does not recognise p53 and p73 isoforms (middle panels). As loading control we have used the actin staining. Human skin cryo-sections were used to test the anti-TAp63-sp antibody as compared to the p63 (H137) commercially available antibody. (F) By immunofluorescence positive staining is seen using anti-TAp63-sp in the nuclei of the keratinocytes. TAp63 positive nuclei are detected mostly in the upper layers of the epidermis (as indicated by the white arrow), while many nuclei in the basal layer are TAp63 negative (yellow arrow). (G) The nuclei stained by anti-p63(H137) are partially overlapping with the one stained with TAp63-sp antibody, indeed the p63(H137) detects p63 into the nuclei of almost all the epidermal layers (basal and upper layers). The staining was more intense in the basal layer, where 100% of the nuclei were positive, here the ΔNp63 isoform is the more abundant protein. Bars: 30 μm.

non-tagged TAp73α, β, γ, ΔNp73α, β, γ, or non-tagged p53 plasmids. Total proteins were extracted as described in Materials and methods, run on SDS–page gel, transferred to PVDF membrane and blotted with anti-TAp63sp, anti-TAp73, anti-ΔNp73, and DO-1 (anti-p53) antibodies (Fig. 1C–E). As shown in Fig. 1C–E, the TAp63-sp antibody did not detect p73 isoforms and p53 at all, demonstrating that its specificity for TAp63 isoforms.

The new antibody generated, TAp63-sp, was also used to detect the endogenous TAp63 proteins by staining human skin cryo-sections; the results were compared with the one obtained using anti-63 (H137, Fig. 1G, and Ab-4 clone, not shown) antibodies, which recognises both TAp63 and ΔNp63 isoforms (Fig. 1F and G). These two commercially available antibodies, can detect all p63 isoforms (both TAp63 and ΔNp63, see Fig. 1A for epitope mapping). By immunofluorescence positive staining was seen using anti-TAp63-sp in the nuclei of the keratinocytes: TAp63 positive nuclei were detected mostly in the upper layers of the epidermis (Fig. 1G, as indicated by the white arrow), whereas many nuclei in the basal layer are TAp63 negative (Fig. 1G, yellow arrow). The specificity of the staining observed was also validated by using pre-immune serum that did not detect any staining at all (not shown).

The staining pattern obtained using anti-p63(H137) was partially overlapping with the one obtained using TAp63-sp antibody; indeed the p63(H137) detected p63 into the nuclei of the keratinocytes of the basal and spinous layers. Several nuclei of the keratinocytes of the granular layer are negative (Fig. 1G, yellow arrows). In conclusion, the

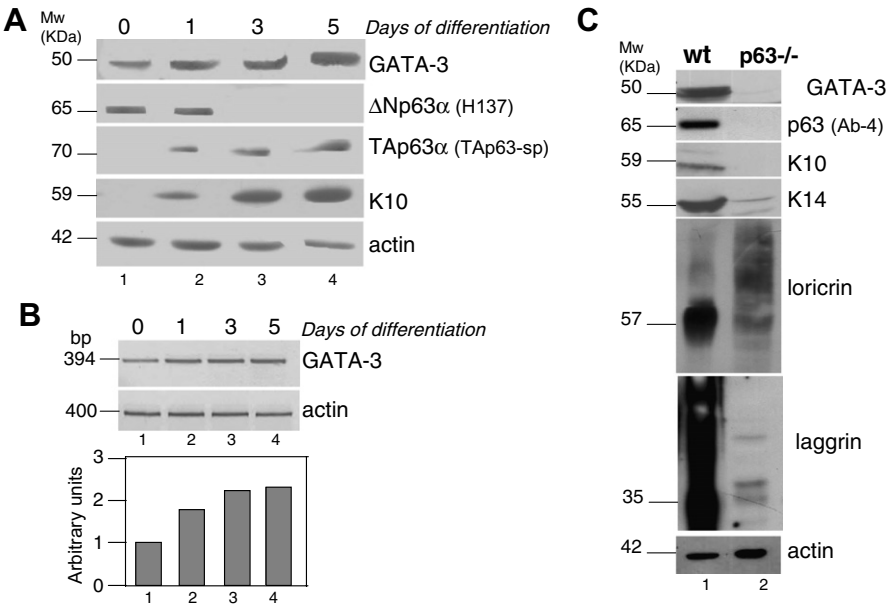

Fig. 2. Expression of GATA-3 during keratinocytes differentiation. (A) Keratinocytes were induced to differentiate by calcium addition (1.2 mM) for 1–5 days. Proteins were extracted and Western blotting analysis were performed using anti-GATA-3, anti-TAp63-sp, anti-p63(H137), anti-K10, and anti-actin (the latter as loading control). K10 increases as indicating that the cells undergoes to terminal differentiation. (B) Semiquantitative real time PCR performed on differentiating HaCat, shows a small increase of the GATA-3 mRNA levels. (C) Expression of epidermal differentiation proteins by Western blot in wt and p63−/− mice. The epidermal proteins were extracted in wt and p63−/− mice by incubation of E18.5 embryos for 24 h in buffer (1% SDS, 20 mM DTT) to solubilise the outermost epidermal layers; proteins were quantified, electrophoresed and blotted. GATA-3 protein is not detected in p63−/− embryos. As control, Western blot for K10, K14, loricrin, and filaggrin, was performed. Loading control, actin.

p63(H137) staining was more intense in the basal layer, where 100% of the nuclei were positive (Fig. 2G), while TAp63 (TAp63-sp) was mainly observed in the granular layer. This result further support that ΔNp63 is pivotal in the basal layer for the maturation of the epidermis [6,7,22].

GATA-3 is regulated during normal human keratinocyte differentiation

In addition to the hemapoietic lineages, GATA-3 is also a regulator of cell lineage determination in skin [19].

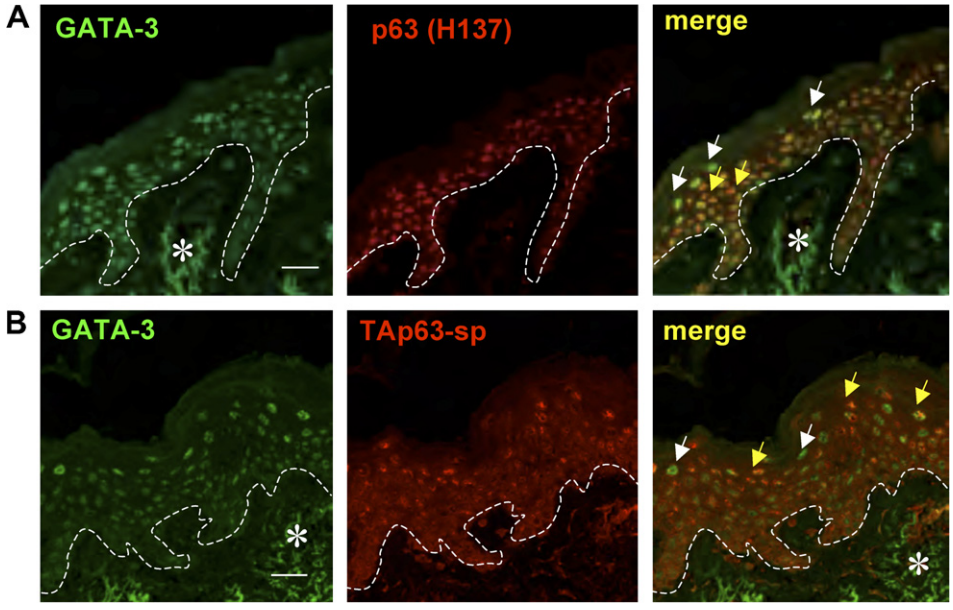

Fig. 3. GATA-3 partially colocalises with p63 isoforms in human interfollicular epidermis. GATA-3 is expressed in human skin and partially co-localizes with TAp63 and ΔNp63. Frozen sections of normal human skin are subjected to immunofluorescence with anti-p63(H137) and anti-GATA-3 (A) or anti-TAp63-sp and anti-GATA-3 (B). Immunofluorescence shows a partial nuclear localization of GATA-3 and p63 both in nuclei located in the basal and in the upper layers, indicating that GATA-3 colocalize both with TAp63 and ΔNp63 isoforms. (white arrows: nuclei positive only for GATA-3; yellow arrows: nuclei positive for both GATA -3 and p63). Bars: 30 μm.

However, the role of GATA-3 in epidermis, as well as its epidermal target genes, has not been fully established so far. Very recently, we have demonstrated that IkB kinase-α (IKKα), which is very important in keratinocyte differentiation and skeletal and craniofacial morphogenesis [23], has four putative binding sites located on its proximal promoter and that GATA-3 is able to transactivate IKKα by luciferase assay [14]. We have also demonstrated by chromatin immunoprecipitation that both TAp63 and ΔNp63 bind GATA-3 promoter, which contains a p53-like responsive element, and that they are able to transactivate GATA-3 in a luciferase assay [7]. In order to further study the relationship between GATA-3 and p63 isoforms we decided to investigate the expression of these transcription factors during keratinocyte differentiation and in epidermis. Normal human epidermal keratinocytes, NHEK, were differentiated by calcium addition (1.2 mM), cells were collected, proteins extracted and Western blot was performed. The results obtained showed a significative accumulation of GATA-3 protein during keratinocyte differentiation, parallel to and increase for TAp63 and keratin 10 (K10; the latter reported as differentiation positive control) (Fig. 2A). As expected the ΔNp63α decreases while the cells undergo to differentiation (Fig. 2A). Semi-quantitative RT-PCR analysis (Fig. 2C) revealed two fold increase of the induction of GATA-3 mRNA during keratinocyte differentiation.

To further confirm that GATA-3 is downstream of p63 as indicated *in vitro* by us [14], we decided to check *in vivo* expression of GATA-3 in the epidermis of p63−/− mice. Using epidermal proteins extracted from E19.5 embryos wt and p63−/− [7], we observed by Western blotting the absence of GATA-3 protein in absence of p63 (Fig. 2C). As control we included epidermal markers such us K14, K10, loricrin and filaggrin.

Expression of GATA-3 and p63 proteins in human epidermis and hair follicle

To analyse the pattern distribution of GATA-3 and p63 isoforms, we investigated their expression in normal human skin. GATA-3 is located both in basal and suprabasal epidermal layer (Fig. 3A and B). As shown in the "merge" panels, GATA-3 co-localizes with both ΔNp63 (H137; yellow arrows) and with TAp63 (TAp63-sp; yellow arrows). This co-localization is partial, since nuclei positive only for GATA-3 are also evident (white arrows) in the upper layer of the epidermis, indicating that other transcription factors contribute in the expression of GATA-3.

We investigated the expression of GATA-3 and p63 isoforms in different hair follicle compartments (Fig. 4A and B). GATA-3 labelling confirmed a specific nuclear distribution from the early onset of the inner root sheath (IRS) (Fig. 4A and B; left panels). p63 labelling obtaining using both anti-TAp63-sp and anti-p63 (H137/Ab-4) is more diffuse but preferentially observed in the nuclei around the dermal papilla and in the outer root sheath (ORS). Analysis by laser confocal microscopy confirmed the indirect immuno-fluorescence results. Intensities of both channels were analyzed and two distinct populations of spots were distributed. Only occasional spots were found in the area where both intensities were maximal (Fig. 4A and B, right panels). These spots were selected for white colour, and revealed that scattered IRS cells co-expressed p63 and GATA-3. These results clearly evidenced that GATA-3 and p63 proteins are only partially co-localizing in the human hair follicle. GATA-3 is specific for the IRS differentiation program while p63 is mainly detected in the hair shaft and the ORS cells. Nevertheless, we detected some IRS cells exhibiting a distinct amount of p63 protein.

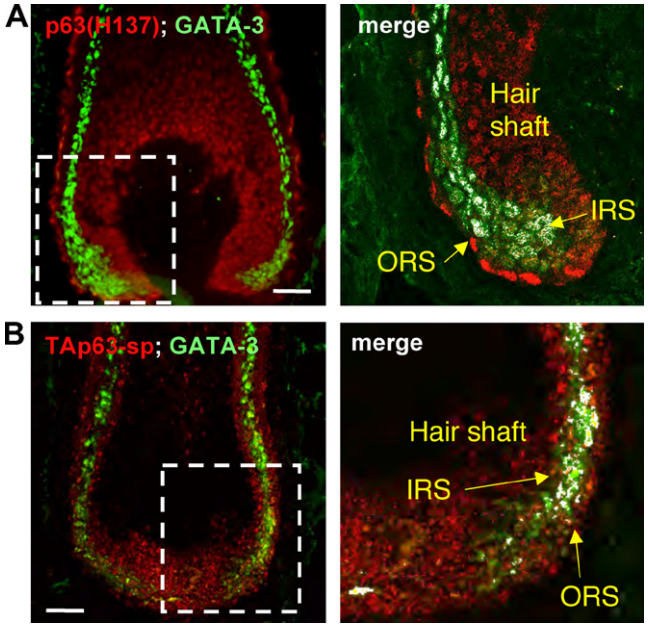

Fig. 4. Expression of GATA-3 and p63 isoforms in the different hair follicle compartments. (A) GATA-3 presents a nuclear distribution in the inner root sheath (IRS) compartment of the hair follicle. Anti-p63(H137) staining is observed in the nuclei around the dermal papilla and in the outer root sheath (ORS). GATA-3 and p63 co-localize in a very limited portion of the IRS as indicated by the white spots. (B) TAp63-sp antibody staining is more diffuse in comparison to H137 antibody. Also in this case GATA-3 and TAp63 co-localize in a very limited portion of the IRS as shown by the white spots. Left panels are details of right panels (indicated with dashed line). Bars: 100 μm.

Conclusion

The characterization of TAp63-sp, which reacts specifically with TAp63 isoforms, compared to the commercial antibodies anti-p63 (H137/Ab-4), which recognises all p63 isoforms, allowed us to investigate which p63 isoform is associated with GATA-3 expression *in vivo*. In our previous studies, was reported that GATA-3 is a p63 target gene *in vitro* [14]. In this study, we illustrated that in a p63 null embryo, GATA-3 is not detectable by Western blot in the outer part of the embryo, suggesting that *in vivo* p63 contribute to GATA-3 transcription. Finally, we demonstrated that in the interfollicular epidermis and in the IRS of the

hair follicle GATA-3 partially co-localizes with both TAp63 and ΔNp63 isoforms.

Acknowledgments

The work discussed in this manuscript has been supported by the Medical Research Council (G.M.) and by grants from Telethon (GGPO4110 to G.M.), AIRC (2743), EU (QLK-CT-2002-01956–Impaled; LSGBH-2005-019067–Epistem; Blandino-2004-LSHC-CT-2004-503576–Active p53), Genomica Funzionale COMETA, FIRB-2001-RBNE01KJHT_004 (Marino) & RBNE01NWCH_008 (Rotilio), MIUR, PRIN, MinSan to G.M.

References

[1] A. Yang, M. Kaghad, Y. Wang, E. Gillett, M.D. Fleming, V. Dotsch, et al., p63, a p53 homolog at 3q27-29, encodes multiple products with transactivating, death-inducing, and dominant-negative activities, Mol. Cell 2 (1998) 305–316.

[2] A. Yang, R. Schweitzer, D. Sun, M. Kaghad, N. Walker, R.T. Bronson, et al., p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development, Nature 398 (1999) 714–718.

[3] A.A. Mills, B. Zheng, X.J. Wang, H. Vogel, D.R. Roop, A. Bradley, p63 is a p53 homologue required for limb and epidermal morphogenesis, Nature 398 (1998) 708–713.

[4] M. Senoo, F. Pinto, C.P. Crum, F. McKeon, p63 is essential for the proliferative potential of stem cells in stratified epithelia, Cell 4 (2007) 523.

[5] E. Candi, A. Rufini, A. Terrinoni, A. Giamboi-Miraglia, A.M. Lena, R. Mantovani, R. Knight, G. Melino, DeltaNp63 regulates thymic development through enhanced expression of FgfR2 and Jag2, Proc. Natl. Acad. Sci. USA (in press).

[6] J. Laurikkala, M.L. Mikkola, M. James, M. Tummers, A.A. Mills, I. Thesleff, p63 regulates multiple signalling pathways required for ectodermal organogenesis and differentiation, Development 133 (2006) 1553–1563.

[7] E. Candi, A. Rufini, A. Terrinoni, D. Dinsdale, M. Ranalli, A. Paradisi, V. De Laurenzi, L.G. Spagnoli, M.V. Catani, S. Ramadan, R.A. Knight, G. Melino, Differential roles of p63 isoforms in epidermal development: selective genetic complementation in p63 null mice, Cell Death Differ. 13 (2006) 1037–1047.

[8] M.I. Koster, D. Dai, D.R. Roop, Conflicting roles for p63 in skin development and carcinogenesis: are the mouse models at fault? Cell Cycle 6 (2007) 269–273.

[9] E. Candi, D. Dinsdale, A. Rufini, P. Salomoni, R.A. Knight, M. Mueller, P.H. Krammer, G. Melino, TAp63 and ΔNp63 in cancer and epidermal development, Cell Cycle 6 (2007) 274–285.

[10] D.K. Carroll, J.S. Carroll, C.O. Leong, F. Cheng, M. Brown, A.A. Mills, J.S. Brugge, L.W. Ellisen, p63 regulates an adhesion programme and cell survival in epithelial cells, Nat. Cell Biol. 8 (2006) 551–561.

[11] M. Osada, Y. Nagakawa, H.L. Park, K. Yamashita, G. Wu, M.S. Kim, A. Fomenkov, B. Trink, D. Sidransky, p63-specific activation of the BPAG-1e promoter, J. Invest. Dermatol. 125 (2005) 52–60.

[12] M. Osada, H.L. Park, Y. Nagakawa, K. Yamashita, A. Fomenkov, M.S. Kim, G. Wu, S. Nomoto, B. Trink, D. Sidransky, Differential recognition of response elements determines target gene specificity for p53 and p63, Mol. Cell Biol. 25 (2005) 6077–6089.

[13] O. Gressner, T. Schilling, K. Lorenz, E. Schulze Schleithoff, A. Koch, H. Schulze-Bergkamen, A.M. Lena, E. Candi, A. Terrinoni, M.V. Catani, M. Oren, G. Melino, P. H Krammer, W. Stremmel, M. Muller, TAp63alpha induces apoptosis by activating signaling via death receptors and mitochondria, EMBO J. 24 (2005) 2458–2471.

[14] E. Candi, A. Terrinoni, A. Rufini, A. Chikh, A.M. Lena, Y. Suzuki, B.S. Sayan, R.A. Knight, G. Melino, p63 is upstream of IKKα in epidermal development, J. Cell Sci. 119 (2006) 4617–4622.

[15] K.H. Lieuw, G. Li, Y. Zhou, F. Grosveld, J.D. Engel, Temporal and spatial control of murine GATA-3 transcription by promoter-proximal regulatory elements, Dev. Biol. 188 (1997) 1–16.

[16] K.M. George, M.W. Leonard, M.E. Roth, K.H. Lieuw, D. Kioussis, F. Grosveld, J.D. Engel, Embryonic expression and cloning of the murine GATA-3 gene, Development 120 (1994) 2673–2686.

[17] P.P. Pandolfi, M.E. Roth, A. Karis, M.W. Leonard, E. Dzierzak, F.G. Grosveld, J.D. Engel, M.H. Lindenbaum, Targeted disruption of the GATA3 gene causes severe abnormalities in the nervous system and in fetal liver haematopoiesis, Nat. Genet. 11 (1995) 40–44.

[18] K.C. Lim, G. Lakshmanan, S.E. Crawford, Y. Gu, F. Grosveld, J.D. Engel, Gata3 loss leads to embryonic lethality due to noradrenaline deficiency of the sympathetic nervous system, Nat. Genet. 25 (2000) 209–212.

[19] C.K. Kaufman, P. Zhou, H.A. Pasolli, M. Rendl, D. Bolotin, K.C. Lim, X. Dai, M.L. Alegre, E. Fuchs, Gata-3: an unexpected regulator of cell lineage determination in skin, Genes Dev. 17 (2003) 2108–2122.

[20] C. de Guzman Strong, P.W. Wertz, C. Wang, F. Yang, P.S. Meltzer, T. Andl, S.E. Millar, I.C. Ho, S.Y. Pai, J.A. Segre, Lipid defect underlies selective skin barrier impairment of an epidermal-specific deletion of Gata-3, J. Cell Biol. 175 (2006) 661–670.

[21] S. Thibaut, E. Candi, V. Pietroni, G. Melino, R. Schmidt, B.A. Bernard, Transglutaminase 5 expression in human hair follicle, J. Invest. Dermatol. 125 (2005) 581–585.

[22] K.E. King, R.M. Ponnamperuma, T. Yamashita, T. Tokino, L.A. Lee, M.F. Young, et al., deltaNp63alpha functions as both a positive and a negative transcriptional regulator and blocks in vitro differentiation of murine keratinocytes, Oncogene 22 (2003) 3635–3644.

[23] Y. Hu, V. Baud, M. Delhase, P. Zhang, T. Deerinck, M. Ellisman, R. Johnson, M. Karin, Abnormal morphogenesis but intact IKK activation in mice lacking the IKKalpha subunit of IkappaB kinase, Science 284 (1999) 316–320.