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The sterile alpha-motif (SAM) domain of p63 binds in vitro monoasialoganglioside (GM1) micelles

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ABSTRACT

The transcription factor p63 plays pivotal roles in epidermal barrier formation and in embryonic development. The protein structures of TAp63 and ΔNp63α isoforms include a C-terminal steril alpha-motif (SAM) involved in protein–protein interaction. Identification of p63 SAM domain interactors could lead to the explanation of novel mechanisms of regulation of p63 activity, possibly relevant in the physiological role of p63 and in genetic disorders associated with mutations of the p63 gene. In this work, we have performed a biochemical analysis of p63 SAM domain preferences in lipid binding. We have identified the ganglioside GM1 as a high affinity interactor, capable of modulating p63 transcriptional ability exclusively on epidermal target genes. In agreement with these data we report a consistent expression profile and localization analysis of p63 and GM1 in primary keratinocytes and in human epidermal biopsies. Therefore, we propose a potential biological role of p63–GM1 interaction in regulation of p63 during epidermal differentiation.

1. Introduction

p63 is a transcription factor required for epidermal barrier formation and embryonic development [1–7]. It is structurally related to p53 and p73 and shares significant amino acid identity with them in the transactivation, DNA-binding, and oligomerization domains. Having two transcriptional promoters, p63 expresses various isoforms including TAp63 and ΔNp63, with the latter lacking the N-terminal transactivation domain (TA). Moreover, an alternative splicing allows maturation of three C-terminal variants (αβ and γ isoforms). The alpha isoforms contain a sterile alpha motif (SAM) at the C-terminal, which is important for protein–protein interaction [8,9]. The three-dimensional structure of the p63 SAM domain is composed of five alpha-helices that are structurally very similar to the cognate p73 SAM domain [10] and to the prototype SAM domain of the ephrin B2 receptor [11]. The function of the SAM domain does not appear to be limited to oligomer formation and can be variable depending on different protein interactions [12]. p73 SAM binds both zwitterionic (phosphatidylcholine, PC) and anionic (phosphatidic acid, PA) lipids, and was proposed a role of SAM lipid binding in p73 regulation [13]. Recently, SAM domains in the Smaug family, a translational repressor, have also been found as part of RNA binding modules that recognizes RNA hairpins with a specific sequence loop [14,15].

In contrast to p53, p63 is rarely mutated in human cancers, but mutations of the p63 gene have been associated with several genetic disorders known as ectodermal dysplasia syndromes (EDs). EDs include ectrodactyly-ectodermal dysplasia-cleft lip/palate (EEC), ankyloblepharon-ectodermal dysplasia-clefting (AEC) syndrome, split hand-foot malformation (SHFM) and limb-mammary syndrome (LMS). The position of the mutations is correlated to the abnormal phenotype observed. Mutations included in the SAM domain are often associated to AEC syndrome, where patients present skin erosion, eyelid, nail and tooth defects, but the absence of limb malformation [16–19].

Gangliosides, a family of glycosphingolipids containing one or more sialic acid residues (Fig. 1A), have been implicated with various cellular functions including growth, differentiation, cell-to-cell interaction and signal transduction [20]. They have generally been assumed to be localized primarily in the plasma membrane but evidence suggests that they also occur in intracellular compartments, such as the Golgi apparatus, endoplasmic reticulum and

Abbreviations: SAM, sterile alpha motif; PA, phosphatidic acid; PC, phosphatidylcholine; GM, ganglioside; PS, phosphatidylserine; SM, sphingomyelin; HEKn, human epithelial keratinocytes normal.

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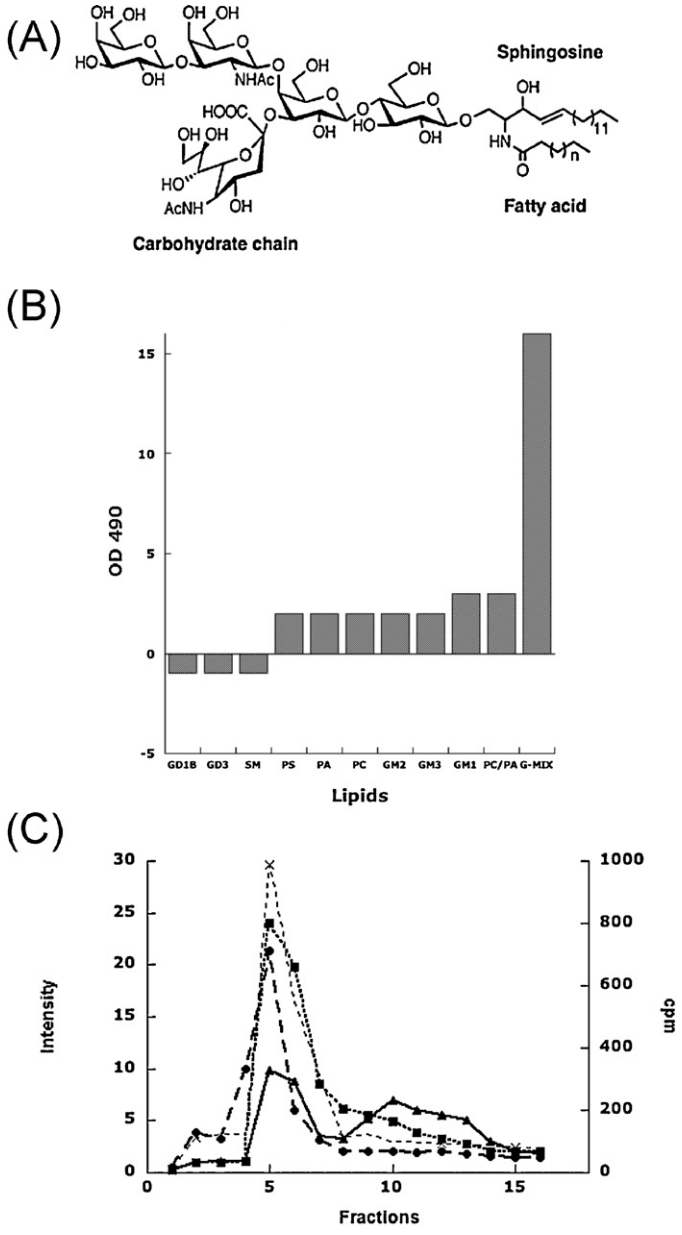

Fig. 1. In vitro binding of p63 SAM to gangliosides. (A) Structure of GM1. (B) ELISA assay to test p63 SAM domain affinity for phospholipids and sfingolipids. GangMix, gangliosides mixture from ox brain; GD3, GD1B, di-sialogangliosides; PC/PA, phosphatidylcoline/phosphatidic acid; GD, mono-sialoganlgiosides (GM1, GM2 and GM3); PC, phosphatidylcoline; PA, phosphatidic acid; PS, phosphatidylserine; SM, sphingomyelin. (C) Sephadex G-100 gel filtration to purify complexes of the SAM domain with different lipids. The eluted fractions were collected and tested both for radioactivity (indicating lipid presence) and Trp fluorescence (indicating the presence of the SAM domain). Typical elution profiles for the recombinant SAM domain alone or with liposomes made of PC/PA, Ganglmix, or GM1 are shown. The fraction of SAM domain bounded with micelles varies depending on the nature of lipids being fully sequestered by GM1 micelles but only partially by Ganglmix (70%) and PC + PA (35%). Typical elution profiles for the liposomes alone (cross) or for recombinant SAM domain incubated with liposomes made with PC/PA (triangles), Ganglmix (squares), or GM1 (circles). The figure shows one representative experiment of three.

nucleus [21–23]. Lipid analysis supported the idea that nuclear envelope is the site of an active autonomous ganglioside metabolism, which is regulated independently from that of the plasma membrane [24] in both neural [25] and extra-neural tissue and cells [26]. It has been proposed in neuroblastoma cells that some differentiation stimuli activate a nuclear neuraminidase able to convert the GD1α present in the nuclear envelope into GM1 that, in turn, would influence the overall cell Ca^{2+} homeostasis enhancing the nuclear Na^{+}/Ca^{2+} exchanger (NCX) [27]. Finally, has been proposed that lipids do not belong only to the nuclear envelope, but are also inside the nucleus forming proteo-lipid complexes [28]. Using immunogold electron microscopy analysis Parkinson et al. demonstrated the presence of GM1 in mouse epithelial cells heterochromatin [22]. Immunochemical study also suggested that in rat cortical neurons, the ganglioside GD3 binds to heterochromatin inside the nucleus, and there is also evidence that its levels increase after stimulation of cells with beta-amyloid peptide [29].

In this paper we have carried out an extensive study of the p63 SAM domain preferences in lipid binding. Our results indicate that p63 SAM is able to bind GM1 with high affinity, and this interaction modulates p63 transcriptional ability.

2. Materials and methods

2.1. Enzyme-linked immunosorbent assay (ELISA)

ELISA-SAM binding to various lipids was evaluated by ELISA, as described previously [27]. In brief, the wells of microtiter plates were coated with phospholipid antigen (10 μM) in ethanol by evaporation at room temperature. After blocking the wells with Tris-buffered saline (TBS, 10 mM Tris–HCl, pH 7.4, 150 mM NaCl) containing 30 mg/ml bovine serum albumin (BSA), the wells were incubated with various concentrations of SAM domains in TBS containing 10 mg/ml BSA (1% BSA-TBS) for 2 h at room temperature. After washing the wells with TBS, the bound SAM was detected by incubating the wells with anti-p63 antiserum, diluted 1/1000 with 1% BSA-TBS for 2 h at room temperature, followed by incubation (2 h at room temperature) with biotinylated anti-rabbit IgG and peroxidase-conjugated streptavidin. The intensity of the color developed with o-phenylenediamine as the substrate was measured using an ELISA reader (Bio-Rad, Hercules, CA). All the chemicals used were from Sigma–Aldrich (St. Louis, MO).

2.2. Gel filtration and fluorescence measurement

GM1 and other gangliosides were dissolved in water above their micellar critical concentration. SAM (final concentration 7–10 μM) and different concentrations of GM1 were incubated for 30 min in 250 μl of Phosphate Buffer Saline (PBS) + 2 mM of DTT. The reaction mixture was applied to a Sephadex G-75 gel filtration column (Amersham Pharmacia Biotech, Piscataway, NJ) to separate the SAM–lipid complex to the unbound protein. The column was eluted with PBS-DTT at a flow rate of 0.5 ml/min. A few nCi of [14C]-palmitic acid (Amersham Pharmacia Biotech, Piscataway, NJ) were added to the gangliosides to determine the elution time of micelles after analysis of radioactivity in the different fractions. Protein elution was tracked by analysis of intrinsic Trp fluorescence (Ex. 220 Em. 346). All the chemicals used were from Sigma–Aldrich (St. Louis, MO).

2.3. Non-denaturing acrylamide gel electrophoresis

SAM domain (final concentration 7–10 μM) and different concentrations of GM1 were incubated for 30 min in 250 μl of Phosphate Buffer Saline (PBS) + 2 mM of DTT. The reaction mixture was resolved by polyacrylamide gel electrophoresis prepared run in non-denaturating conditions (Bio-Rad, Hercules, CA). The polyacrylamide gel was blotted onto a Hybond PVDF membrane (GE Healthcare, Hatfield, UK). Membranes were blocked with PBST 5% non-fat dry milk, incubated with anti-p63 primary antibodies (Ab4, NeoMarkers, Fremont, CA) for 2 h at room temperature, washed and hybridized with peroxidase-conjugate secondary

antibody for 1 h (mouse, Bio-Rad, Hercules, CA). Detection was performed with the ECL chemiluminescence kit (Perkin Elmer, Norwalk, CT). All the chemicals used were from Sigma–Aldrich (St. Louis, MO).

2.4. Immunofluorescence and confocal analysis

Human skin biopsies were embedded in the frozen specimen medium Criomatix (Shandon, Pittsburgh, PA). Microwave-assisted antigen retrieval was performed in 0.01 M sodium citrate (pH 6) for three cycles of 5 min. Nonspecific antigens were blocked by incubation in 5% goat serum in PBS for 1 h in a humidified atmosphere at room temperature. Subsequently, sections were incubated for 1 h with the following primary antibodies: anti-K14 (Covance, Princeton, NJ; 1/1000 dilution), anti-GM1 (Abcam, Cambridge, UK; 1/200 dilution), anti-p63 (Ab4; NeoMarkers Fremont, CA; 1/500 dilution), then washed three times with PBS and incubated for 1 h with 488- or 568-Alexa Fluor secondary antibodies (Invitrogen, Park Paisley, UK; dilution 1/1000). Immunofluorescence on HEKn was performed by fixing in 2% paraformaldehyde (10 min). After 0.5% Triton X100 permeabilization (10 min) and 5% goat serum PBS blocking (1 h), cells were incubated for 1 h with primary antibodies. Cells were then washed three times with PBS and incubated for 1 h with 488- or 568-Alexa Fluor secondary antibodies. After two washes in PBS, the tissue sections or cells were counterstained with DAPI to highlight nuclei. Slides were then mounted by using Prolong Antifade kit (Invitrogen, Park Paisley, UK), and fluorescence was evaluated by confocal microscopy (Nikon Instruments, Melville, NY; C1 on Eclipse Ti; EZC1 software) fitted with an argon laser (488-nm excitation), He/Ne laser (542-nm excitation), and UV excitation at 405 nm.

2.5. Cell cultures and immunoprecipitation

Human primary epidermal keratinocytes from neonatal foreskin, HEKn (Invitrogen, Park Paisley, UK), were grown in EpiLife medium, supplemented with Human Keratinocyte Growth Supplement (HKGS) (Invitrogen, Park Paisley, UK). Human primary keratinocytes were plated on collagen-coated dishes. HaCat cell line was grown in DMEM-F12 (1:1) medium, 10% FBS, 100 U penicillin and 100 μg streptomycin (Invitrogen, Park Paisley, UK).

Protein concentrations of cell extracts were determined by the Bradford assay using bovine serum albumin as the standard. For immunoprecipitation, total cell extracts were incubated with specific antibody anti-GM1 (Abcam, Cambridge, UK) for 2 h at 4 °C and then immunoprecipitated with proteinG-plus agarose (Calbiochem, San Diego, CA) overnight at 4 °C. Immunoprecipitates were washed five times, denatured in Laemmli buffer for 5 min at 100 °C and then resolved in SDS-polyacrylamide gel. SDS-PAGE was blotted onto Hybond PVDF membrane (GE Healthcare, Hatfield, UK), and membranes were blocked and incubated with primary antibodies anti-p63 (NeoMarkers, Fremont, CA) or anti-SUMO (Sigma–Aldrich, St. Louis, MO). After washes membranes were incubated with a peroxidase-conjugate secondary antibody for 1 h (rabbit and mouse, Bio-Rad, Hercules, CA). Detection was performed with the ECL chemoluminescence kit (Perkin Elmer, Waltham, MA).

2.6. Plasmids and luciferase reporter assay

The K14, Bax and Gadd45 luciferase plasmids and all the p63 expressing constructs have already been described [30]. For luciferase reporter assays, HEK293 cells were cultured in 12-well dishes and transfections were performed with Effectene (Qiagen, Milan, IT), according to manufacturer recommendations. We used a 1:3 ratio of reporter plasmid (containing the firefly luciferase gene under the control of either the K14, Bax and Gadd45 promoters) to the given expression vectors encoding allp63 isofoms and mutants. In all cases, 10 ng of Renilla Luciferase Vector (pRL-CMV; Promega, Madison, WI) were co-transfected to control transfection efficiency. GM1 was added to the cell cultures at a final concentration of 100 μM. Twenty-four hours after transfection, luciferase activities in cellular extracts were measured by using a Dual Luciferase Reporter Assay System (Promega, Madison, WI); light emission was measured over 10 s using an OPTOCOMP I luminometer.

3. Results

3.1. Specific binding of p63 SAM to gangliosides in vitro

ELISA assay (Fig. 1B) shows that recombinant p63 SAM domain, in vitro purified, has a high affinity for both phospholipids and sfingolipids, which varies according to the following order: gangliosides mixture from ox brain (GangMix) > phosphatidylcoline/phosphatidic acid (PC/PA) > mono-sialoganlgiosides (GM1, GM2 and GM3) > phosphatidylcoline (PC) > phosphatidic acid (PA) > phosphatidylserine (PS). The nature of SAM–lipid interaction has also been investigated using liposomes or micelles made up by lipids that in ELISA showed the highest affinity for SAM i.e. GangMix, GM1 and PC/PA. Complexes of the SAM domain with different lipids have been purified using sephadex G-100 gel filtration. Briefly, the presence of aggregate lipids carrying a radio-labelled fatty acid as a tracer and SAM domain was evaluated in the eluted fractions testing both radioactivity (indicating lipid presence) and Trp fluorescence (indicating the presence of the protein). The elution profile of labelled lipid micelles showed a sharp peak of radioactivity corresponding to the void volume of the column, while the elution profile of SAM showed a peak of tryptophan fluorescence delayed when compared to void volume (data not shown). Finally, the elution profile of SAM after incubation with lipids shows that a portion of the fluorescence, which varies depending on the type of lipid, is present in the void volume, indicating the formation of stable complexes of SAM–lipids. Fig. 1C shows typical elution profiles for the SAM domain in the presence of liposomes made with PC/PA (triangles), Ganglmix (squares), or GM1 (circles). The SAM amount in the different fractions has been assessed by evaluation of tryptophan fluorescence intensity; liposomes amount in the different fractions is expressed as cpm of tritiated palmitic acid trapped in the vesicles. Liposomes made from different lipids (cross) eluted in the same fractions (typically 3–6) corresponding to the void volume of the column. The fraction of SAM domain bounded with micelles varies depending on the nature of lipids being fully sequestered by GM1 micelles but only partially by Ganglmix (70%) and PC + PA (35%) (Fig. 1C).

3.2. GM1 induces changes in tridimensional structure of p63 SAM domain

An indication of SAM three-dimensional structure modifications in response to lipids binding was performed by evaluating changes of intrinsic tryptophan fluorescence. SAM domain posses only a Trp residue (Trp563), that is partly buried in the hydrophobic core of the protein in the middle of helix 5 [31]. Increasing amounts of lipid (PA/PC or GM1) induce a parallel increase of Trp fluorescence of the SAM domain and a shift of maximum emission (Fig. 2A). The fluorescence increase of Trp (less quenched) and the red shift of the emission peak both indicate that lipid-binding promoted a change in the conformation of the SAM domain involving an increase of solvent-exposure of the Trp moiety. To investigate the entity shift of Trp emission we

(A)

	Buffer	PC/PA	Gang Mix	GM1
$\lambda(I_{max})$ Trp	345	353	347	357
$\Delta\lambda$	-	8	2	12

(B)

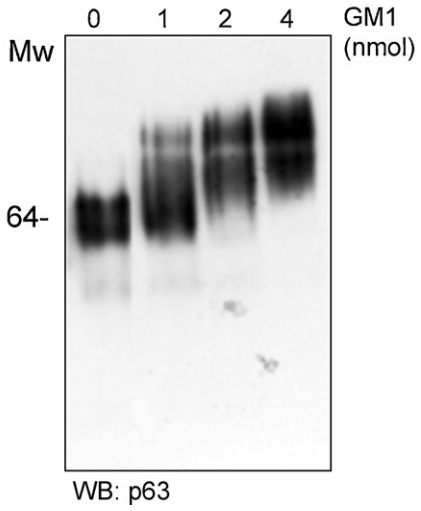

Fig. 2. GM1 and changes in p63 SAM domain structure. (A) Measurements of the p63 SAM domain intrinsic tryptophan fluorescence in the native protein and in the protein eluted with phospholipids and ganglioside micelles after gel filtration. (B) Native gel electrophoresis analysis shows an altered migration of the SAM domain as a function of increasing GM1 concentration. SAM domain bands were revealed by anti-p63 immunoblotting.

compared the fluorescence emission spectra of tryptophan in the native protein and in the protein eluted with phospholipids and ganglioside micelles after gel filtration, a condition where the whole SAM domain is bounded to the lipid. In the SAM domain/GM1 micelles, the wavelength of maximum emission underwent a red shift from 346.6 to 352.4 at pH 6.0 (Fig. 2A). A similar shift was observed also in the case of SAM–PC/PA and SAM–GangMix but the shift values appeared less evident (Fig. 2A), indicating that phospholipid binding induces a smaller change in the SAM domain with respect to the gangliosides.

To confirm the recombinant SAM domain–GM1 interaction, we performed a native gel electrophoresis analysis. Fig. 2B shows an altered migration of the recombinant SAM domain on native gel electrophoresis, as a function of increasing GM1 concentration. SAM domain bands, revealed by anti-p63 immunoblotting, show a band shift in their gel migration, suggesting a dose-dependent interaction between the protein domain and the ganglioside GM1.

3.3. p63 SAM domain interacts with GM1 in human epidermal keratinocytes

To evaluate the biological relevance of p63 SAM domain and GM1 interaction, we performed an immunofluorescence analysis on human skin and in vitro keratinocytes to analyze the possible co-localization of p63 and GM1. As shown in Fig. 3A primary human keratinocytes, HEKn, GM1 is present in the nuclear membrane, according to Wu et al. [32]. Immunostaining for p63 reveals the typical localization of ΔNp63 in the nucleus of proliferating keratinocytes. GM1 presence in the nuclear membrane suggests a possible physiological interaction between the nuclear transcription factor p63 and GM1 according our preliminary in vitro study. When the cells are confluent GM1 expression in down-regulated as well as during calcium-induced differentiation. K14 staining is shown as control. Immunofluorescence analysis on human epidermal biopsies shows a clear co-localization of p63 and GM1 in nucleus of basal proliferating cells (Fig. 3B). The expression profiles of GM1 in the epidermis show a clear prevalence of the lipid in the proliferating cells, thus supporting the hypothesis of its involvement in the regulation of p63 in proliferating keratinocytes.

To conclude the SAM domain–GM1 interaction analysis, we performed a co-immunoprecipitation experiment that confirmed the interaction in the cellular environment. Co-immunoprecipitation analysis on protein extracts from the HaCat cell line demonstrated the interaction between the lipid and the endogenous transcription factor as shown by immunoblot anti-p63 (Fig. 3C, upper panel). Immunoprecipitation showed two different bands of p63, interacting with GM1. By western blot analysis with the anti-SUMO antibody, we identify the highest band as a sumoylated p63 form (Fig. 3C, lower panel).

3.4. GM1 induces changes in p63 transcriptional ability

To understand the biological meaning of the p63–GM1 interaction, we evaluated p63's transcriptional ability in the presence of GM1. We analyzed three different known p63 responsive promoters, K14, Bax and Gadd45, by measuring luciferase reporter plasmid activity in the HEK293 cell line. As expected, K14 was induced by each ΔNp63 isoform, while among the TAp63 isoforms only TAp63β showed a significant induction (Fig. 4A). By adding GM1 to the cell culture, we found a significant increase in the transcriptional ability of alpha isoforms ΔN and TAp63 (10- to 23-fold increase, respectively, and from 1- to 4.5-fold increase), while no relevant effects were evident in the SAM domain-lacking isoforms, beta and gamma, supporting our hypothesis of a specific interaction between GM1 and the SAM domain (Fig. 4A). Analyzing Bax and Gadd45 promoter revealed an expected induction by the pro-apototic isoforms TAp63 (Fig. 4B and C). Interestingly, analyzing effects of the transcriptional activity on Bax and Gadd45 promoters, we found that GM1 did not have any significant effect, suggesting the possibility that the specific interaction between the transcription factor and the lipid is promoter-dependent.

4. Discussion

The SAM domain is present in many proteins of different origins and probably mediates the hetero- and homo-dimers formation as well as heterotypic interactions with other proteins not containing SAM domains [8]. Since a molecular partner which regulates SAM activity was not found, we investigated the hypothesis that lipids represent its natural target. Here we report a detailed analysis of the SAM domains affinity for different lipids as well as the changes of SAM structure induced by lipid binding. It has been reported in a previous paper that p73-SAM, a domain structurally similar to p63-SAM, is able to bind artificial membranes composed of PC/PA undergoing conformational change [13]. Even p63-SAM domain shows a high affinity for PC/PA, but it also shows affinity for other negatively charged lipids such as gangliosides (Fig. 1). The presence of net negative charge in the polar head is an important factor in determining the affinity of SAM for lipids but probably it is not the exclusive determinant. First, both the negatively charged PS and PA alone exhibit a lower affinity for SAM than zwitterionic lipids such as PC. Second, the number of residues of charged sialic

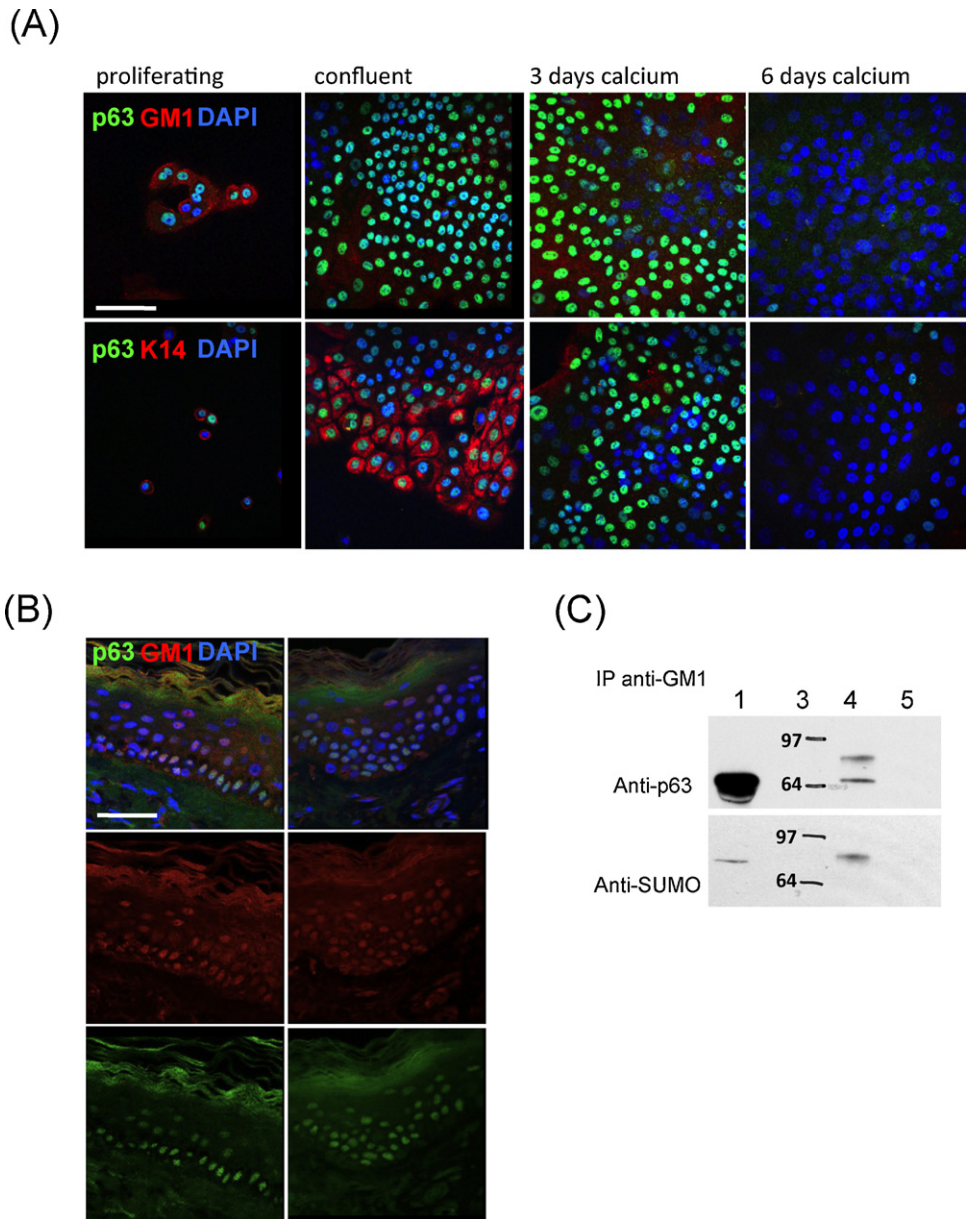

Fig. 3. p63 SAM domain and GM1 in human epidermal keratinocytes and skin. (A) Primary human keratinocytes, HEKn, expresses both GM1 and p63 in proliferating condition. GM1 is detected at the nuclear membrane and its expression decreases in confluent cells and cell treated with calcium. K14 staining was included as marker of proliferating keratinocytes. (B) Co-localization of p63 and GM1 in the nucleus of basal proliferating cells in human epidermis. The immunofluorescences were performed on two different donors. Bars indicate 500 μm. (C) Upper panel: co-immunoprecipitation analysis on protein extracts from the HaCat cell demonstrated a direct interaction between the lipid and the endogenous transcription factor as shown by immunoblot anti-p63. IP: anti-GM1. Lower panel: western blot analysis with the anti-SUMO antibody identify the highest band as a sumoylated p63.

acid present in the gangliosides does not correlate with the affinity for the domain (monosialogangliosides > disialogangliosides). Third, SAM affinity varies greatly with respect to molecules showing identical number but different molecular topologies of sialic acid (GM1 > GM3 > GM2). Finally, tryptophan fluorescence analysis clearly demonstrates that the amount of negative charges on the polar head of lipids is not related to the structural changes of the domain after lipid binding (GM1 > brain gangliosides mixture = PC/PA). We conclude that the SAM–lipid binding is not merely an electrostatic process, but that SAM is able to recognize a precise molecular geometry of oligosaccharide moieties of the ganglioside molecule and that PC/PA mixture, most likely, can mimic the charge topology of the GM1 polar head.

Data obtained with ELISA indicate that GM1 shows the highest affinity among gangliosides, but conversely that the brain ganglioside mixture shows a higher affinity for SAM with respect to GM1. On the other hand, the change of fluorescence intensity as well as of maximum emission of tryptophan indicates that p63 SAM undergoes a structural change more marked after GM1 then after ganglioside mixture binding. We are unable to understand this apparent paradox, probably residing in the nature of the arrangement in the ELISA microwells that greatly varies for different lipids.

The finding that the SAM domain shows a high affinity for GM1 in vitro, and its ability to recognize small differences between the molecular geometry of different gangliosides suggests an in vivo role for this interaction. Gangliosides are involved in a variety of differentiation signals and modulate several proteins in different cell regions, including the nucleus in both neural [25] and extraneural tissue and cells [26]. Ganglioside profiles in nuclei and whole cells in several cell lines such as neuroblastoma, glioma [25] and hepatoma [26] vary according to different stimuli, suggesting that the nucleus is the site for an active autonomous

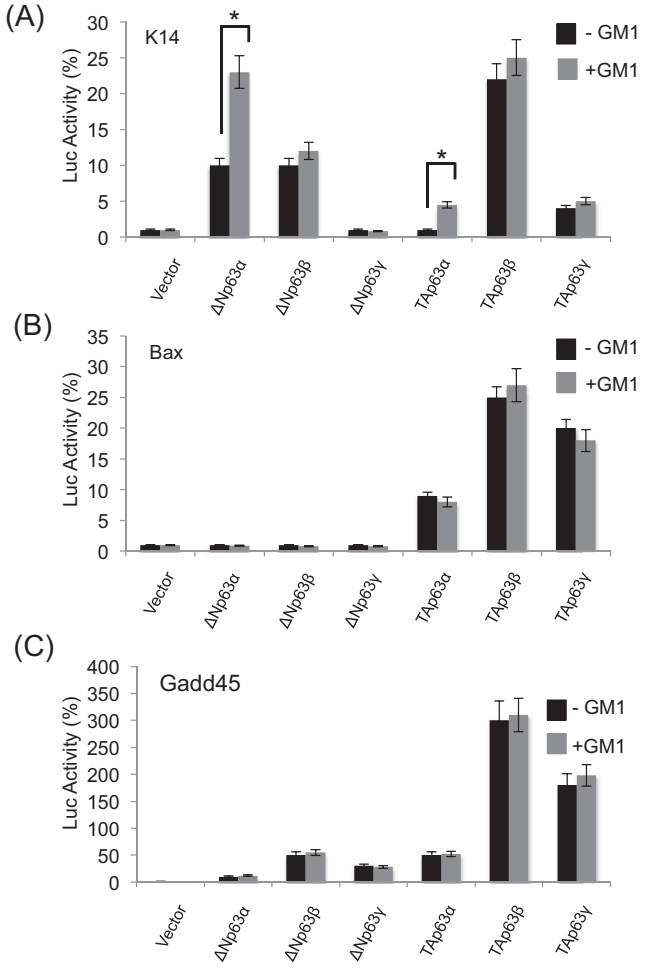

Fig. 4. GM1 affects p63 transcriptional ability evaluated by luciferase-assay. (A) K14, (B) Bax and (C) Gadd45 promoters cloned in the luciferase reporter vector were used individually or co-transfected in HEK293 cells with an empty expression vector (pcDNA-HA) as control (vector) or one expressing the indicated ΔNp63 and TAp63 proteins in the presence or absence of GM1. All luciferase assays were normalized for transfection efficiency with a Renilla reporter vector. The results shown are representative of at least three independent experiments performed each time in triplicate, and error bars indicate the standard deviation. Statistical significance was evaluated by Student *t* test **p*-value <0.01.

lipid metabolism, which is regulated independently from that of the plasma membrane [24]. We found that ganglioside-p63 in vivo interaction exerts a role in the modulation of p63 transcriptional ability. GM1 addiction increases transcriptional activity of p63α isoforms, but in a promoter-specific manner. This effect is probably associated with the interaction between GM1 and the SAM domain and is exclusively related to transcription of the K14 promoter. In the modulation of Bax and Gadd45 promoters, results were not affected by GM1 presence. These results suggest that probably the regulation by GM1 on p63 activity is restricted to epidermal-specific target genes, in which the SAM domain including the isoform ΔNp63α plays a pivotal role during epidermal morphogenesis. This is also in agreement with the expression profiles of p63 and GM1 during keratinocyte differentiation and strongly supports the hypothesis of a biological role of this interaction in the epidermis.

In conclusion our work reports the potential interaction between the GM1 ganglioside and the SAM domain of p63, proposing a biological role of this phenomenon in the regulation of p63 activity during epidermal differentiation.

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