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Amino-terminal residues of ΔNp63, mutated in ectodermal dysplasia, are required for its transcriptional activity

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ABSTRACT

p63, a member of the p53 family, is a crucial transcription factor for epithelial development and skin homeostasis. Heterozygous mutations in TP63 gene have been associated with human ectodermal dysplasia disorders. Most of these TP63 mutations are missense mutations causing amino acidic substitutions at p63 DNA binding or SAM domains that reduce or abolish the transcriptional activity of mutants p63. A significant number of mutants, however, resides in part of the p63 protein that apparently do not affect DNA binding and/or transcriptional activity, such as the N-terminal domain. Here, we characterize five p63 mutations at the 5′ end of TP63 gene aiming to understand the pathogenesis of the diseases and to uncover the role of ΔNp63α N-terminus residues in determining its transactivation potential.

1. Introduction

Humans heterozygous mutations in the TP63 gene are associated to a wide spectrum of autosomal dominant syndromes with ectodermal dysplasias (ED)-like syndromes, including: Rapp-Hodgkin Syndrome (RHS), Ankyloblepharon-Ectodermal defects-cleft lip/palate syndrome (AEC), Ectrodactyly-Ectodermal dysplasia and cleft lip/palate syndrome (EEC), Acro-Dermato-ungual-Lacrimal-Tooth syndrome (ADULT), Limb Mammary syndrome (LMS) and Split Hand/Foot Malformation (SHFM) [1,2]. Affected individuals present, with different severity, developmental orofacial, skin and limbs defects. Point mutations associated to these pathologies have been mapped in the TP63 gene coding region and generate amino acidic substitutions in the p63 protein. p63, a member of the tumour suppressor p53 family, is a transcription factor with a well established role in embryonic development [3,4], epithelia differentiation [5–8] and in the maintaining of epithelial stem cells proliferative potential [9–12]. TP63 expression is controlled by two different promoters giving rise to two classes of transcripts, known as TAp63 (codified from the P1 promoter) and ΔNp63 isoforms (codified from the P2 promoter). Both of them consist of conserved domains, but TAp63 isoforms own a N-terminal transactivation domain (aa 1-107), which is absent in ΔNp63 isoforms. The mRNAs of both isoforms undergo also to alternative splicing events at their 3′ ends generating distinct alternative splicing variants called α, β and γ [13]. The C-terminal full-length α-isoforms contains a Sterile Alpha Motif (SAM, aa 541-607), which mediates protein–protein interactions [3], and a Transactivation Inhibitory domain (TI, aa 610-680). TI interaction with TA domain masks residues required for transactivation, decreasing TAp63α transactivation potential [14]. All p63 isoforms share a DNA Binding Domain (DBD, aa 170-372) and an Oligomerization Domain (OD, aa 394-443). ΔNp63 isoforms lack the N-terminal transactivation

Abbreviations: ED, Ectodermal Dysplasia; AEC, Ankyloblepharon-Ectodermal defects-cleft lip/palate syndrome; EEC, Ectrodactyly-Ectodermal dysplasia and cleft lip/palate syndrome; ADULT, Acro-Dermato-ungual-Lacrimal-Tooth syndrome; LMS, Limb Mammary syndrome; RHS, Rapp-Hodgkin Syndrome; SAM, Sterile Alpha Motif; ChIP, Chromatin ImmunoPrecipitation; RE, responsive element.

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domain (TA) but present their own transcriptional activity and a specific subset of target genes as described in several studies [15,16]. This observation suggests the presence of a second transactivation domain (TA2 domain), still poorly mapped and characterized: some evidences support its presence at ΔNp63 N-terminus, specifically in the first 26 amino acids [17], while other localized TA2 between amino acids 410 and 512, in the region comprised between p63 OD and SAM domains [15].

In this study, we aim to characterize mutations causing amino acid substitutions or premature translation termination at ΔNp63α N-terminus. We considered the N6H substitution associated to ADULT syndrome [18], the G76W associated with LMS and the p.Gln9fsX23, p.Gln11X, p.Gln16X that introduce premature stop codons in the ORF and protein synthesis restarting at methionine 26 [17]. The latter three substitutions were found in individuals affected by RHS and AEC syndromes [19]. Phenotypic description and their clinical features were described, but the molecular mechanisms involved in the pathogenesis have not yet been fully understood. We evaluated mutant proteins expression, cellular localization, DNA binding and transactivation potential. We attempted also to define ΔNp63α TA2 domain; our data indicate that it resides within the first N-terminus 25 amino acids of ΔNp63α, this region also affects mutants p63 protein accumulation.

2. Materials and methods

2.1. Cell culture, transfection and constructs

H1299 and HEK293E cells were grown and transfected as previously described [8]. pGl3 reporter vectors containing K14, BPAG1 and Envoplakin promoters and pcDNA-HA ΔNp63α WT constructs were described [20,5] Mutations were introduced by PCR. Primers used are available on request. pEGFP-C1 was cotransfected to normalize for transfection efficiency.

2.2. Luciferase assay

1.2×10^5 H1299 cells were seeded in 12-well dishes 24 h before transfection. Transfections and luciferase assay were performed as previously described [5].

2.3. Western blot and antibodies

Cells were lysed and analyzed by WB as previously described [8]. Antibody used: anti-HA (Covance, 1/1000 dilution), anti-GFP (BD, 1/300 dilution), anti-β actin (Sigma, 1/5000 dilution) and anti mouse IgGs (Biorad, 1:10,000 dilution).

2.4. Chromatin immunoprecipitation assay

1.5×10^6 HEK 293E cells were transfected as described. To perform ChIP, MAGnify Chromatin Immunoprecipitation System (Invitrogen) was used according to manufacturer instructions. Immunoprecipitation was performed using 5 μg of anti-HA antibody (Abcam). Rabbit IgGs were used as negative control. Primers used to amplify p63-binding site in ZNF750 promoter were previously described [21].

2.5. Immunofluorescence and confocal analysis

Cells were fixed in 4% buffered formaldehyde solution, washed and permeabilized with PBS/0.5% TritonX-100. Blocking was performed using 5% goat serum in PBS. Antibody used: anti-p63 (Santa Cruz, 1/1000 dilution), anti-phalloidin Alexa Fluor 488 (Invitrogen, 1/1000 dilution), goat anti-mouse IgGs Alexa Fluor 568 (Invitrogen, 1/1000 dilution). DAPI was used for nuclear DNA counterstaining. Slides were analyzed as previously described [5].

3. Results

3.1. ΔNp63α-N6H, -G21W and -Δ1-25 mutant proteins are expressed at different levels and localize in the nucleus

Five mutations at the 5′ end of TP63 gene were associated with human ectodermal dysplasia syndromes. The two missense mutations, N6H and G21W (the G21W counting from ΔNp63 start codon is identical to G76W, counting from TAp63 start codon; hereinafter G21W), are known to cause ADULT [18] and LMS [17] syndromes respectively. N6H is a ΔNp63 isoform specific substitution, while G21W affects all p63 isoforms. The others, are nonsense mutations, p.Gln9fsX23, p.Gln11X and p.Gln16X, associated with RHS or AEC syndromes [19]. They insert premature termination codons in p63 mRNA giving rise to translation re-initiation at the next downstream methionine codon (M26), generating N-terminal truncated forms of ΔNp63 (Δ1-25) (Fig. 1A). We generated different vectors that drive the expression of N-terminal HA-tagged p63 WT and mutant proteins. Fig. 1B shows a western blot performed using HEK-293E total protein extracts collected 24 h after transfection with the described constructs. Cells were co-transfected with the same amount of GFP expression vector and anti-GFP antibody demonstrated comparable transfection efficiency in samples. β-actin was used as loading control. The anti HA antibody, and the relative densitometric analysis (Fig. 1C), show that expression of ΔNp63α-N6H and ΔNp63α-G21W mutants is reduced from 30 to 70% compared to WT protein, while Δ1-25 variant level is 2.4 fold higher than WT, suggesting an increased protein accumulation or a reduced degradation of this truncated protein, similarly to the p63 transcriptional defective mutants (EEC and AEC) [20]. In order to assess the correct subcellular localization of these mutant proteins, immunofluorescence staining of HEK-293E cells after transfection with the same constructs was performed. In Fig. 1D, p63 staining is shown in red, actin (cytoskeleton) in green and nuclear DNA (DAPI) in blue. All ΔNp63α variants tested (WT, N6H, G21W and Δ1-25) co-localize with DAPI staining (Merge) demonstrating their correct nuclear import and localization.

This finding suggests also a correct protein folding of the mutant forms. Altogether, the data indicate that mutants are expressed at protein level and localized in the correct subcellular compartment.

3.2. ΔNp63α-N6H, -G21W and -Δ1-25 physically binds p63 responsive elements in different promoters

To demonstrate ΔNp63-N6H, -G21W and -Δ1-25 ability to bind p63 responsive elements (RE) we performed a chromatin immunoprecipitation (ChIP) after transient transfections of HEK-293E cells. ChIP was performed using an anti-HA antibody and was followed by specific PCR reactions to amplify previously selected p63 responsive element (RE) present in ZNF-750 promoter [21].

Fig. 2A shows the fragments obtained after specific PCR reactions. Cells were transfected with an empty vector as transfection negative control. ChIPs with anti-rabbit IgGs were used as immunoprecipitation negative control. The presence of ZNF750 promoter specific fragment in all the HA-immuno-precipitated DNAs, except for the empty vector sample, demonstrate that all ΔNp63α mutants are able to bind this known p63 binding site. In order to evaluate the transactivation potential of ΔNp63α-N6H, -G21W and -Δ1-25 mutants compared to WT p63 protein, luciferase reporter assays were performed. Three different ΔNp63α epithelial responsive promoters were used: keratin 14 (K14), envoplakin (EVPL) and bullous pemphigoid antigen 1 (BPAG1) promoters [5,22,23], and

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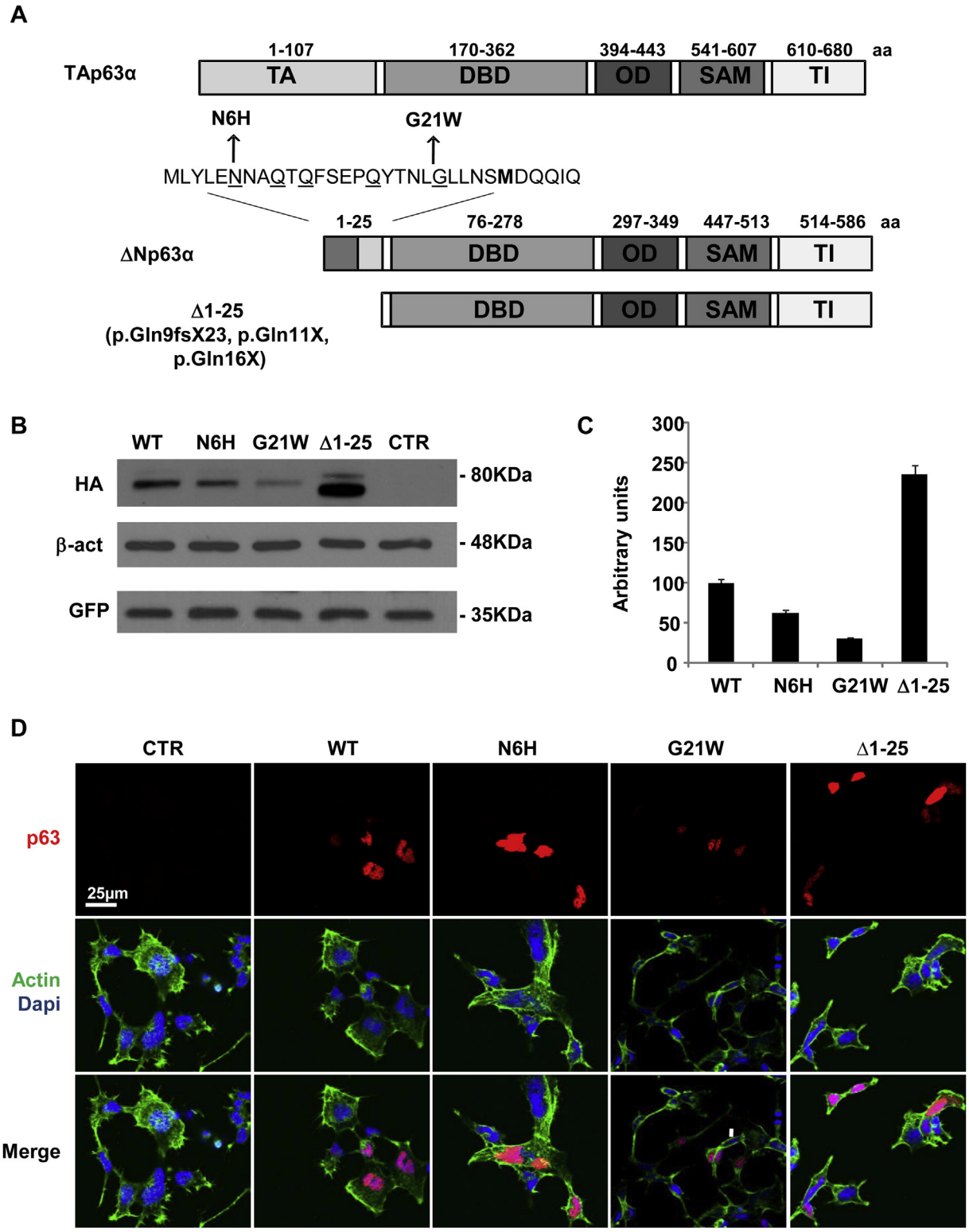

Fig. 1. ΔNp63α-N6H, -G21W and -Δ1-25 are expressed and are located in cell nucleus. A) Schematic representation of ΔNp63α domains. N6H, G21W, p.Gln9fsX23, p.Gln11X, p.Gln16X mutations are underlined, metionine 26 is in bold. TA, Transactivation Domain; DBD, DNA Binding Domain; OD, Oligomerization Domain; SAM, Sterile Alpha Motif domain; TI, Transactivation Inhibitory domain. B) Western blot of HEK-293E cells lysed 24 h after transfection with WT and mutants HA-tagged cDNA expression vectors. β-actin was used as loading control, GFP as transfection efficiency control. One of three independent experiments is shown. C) Densitometric quantification of western blot signals. The histogram shows the mean ± s.d. of three independent experiments. D) Subcellular localization of ΔNp63α and its mutant forms by confocal microscopy. p63 is in red, actin is in green and nuclei are in blue. Merge staining shows co-localization of DAPI and p63 staining. Scale bars: 25 μm.

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two cell cycle and proliferation associated promoters p21 and Growth Arrest and DNA-damage-inducible protein 45 (GADD45) [24]. Fig. 2B–D show the results obtained as the mean of three independent experiments for epithelial promoters. ΔNp63α WT activates K14 promoter 11 fold over control, EVPL 6 fold and BPAG1 29 fold. ΔNp63α-N6H displays a transactivation potential similar to WT protein activating the three promoters considered 9, 6.1 and 27 fold over control respectively. ΔNp63α-G21W, expressed at the lower level compared to WT protein, maintains its transcriptional potential, being able to transactivate 13, 11 and 23 fold K14, EVPL and BPAG1 promoters. Similar results were obtained for p21 and GADD45 promoters. ΔNp63α WT activates p21 promoter 2.9 fold over control and GADD45 31.8 fold (Fig. 2E and F). ΔNp63α-N6H is able to activate the two promoters 3.1 and 29.2 fold over control, respectively. Finally, ΔNp63α-G21W transactivates 5.6 and 61.5 fold over control p21 and GADD45 promoters, respectively. In all the assays performed, the ΔNp63α-Δ1-25 deletion mutant is not able to transactivate the considered promoters, displaying relative luciferase activity similar to empty vector control.

Our findings suggest that the first 25 amino acids of ΔNp63α are required for transcriptional activity but are not required for DNA binding.

3.3. Mapping ΔNp63α N-terminal transactivation sequences

The first 25 amino acids of ΔNp63α, highly conserved among different species (Supplementary Fig. 1), include two regions: the first 14 amino acids represent a ΔNp63 specific sequence, while the following 12 are common to all p63 isoforms. In order to map at protein primary structure the ΔNp63α N-terminal domain, two vectors deleting the two new HA-tagged deletion mutants were generated. Fig. 3A shows a schematic representation of the two mutants: ΔNp63α-Δ1-14 e ΔNp63α-Δ15-25. After transient transfections, the expression of the mutant forms was evaluated by western blot analysis (Fig. 3B and C) demonstrating that they were expressed at protein level. Interestingly, ΔNp63α-Δ1-14 and ΔNp63α-Δ1-25 mutants accumulated in the cell at protein level (about 50% higher than the ΔNp63α-WT), while ΔNp63α-Δ15-25 protein level was similar to WT. These results suggest that, similarly to DNA binding domain- and SAM-domain mutants [30] N-terminal mutants affect protein stability, and that the first 14 N-terminus residues of ΔNp63α could be extremely relevant for the protein folding and/or for the interaction with other proteins. Confocal analysis of transfected cells (Fig. 3D) demonstrates the expected nuclear localization of the Δ1-14 e Δ15-25 mutants. To assess the transactivation potential of these mutants luciferase reporter assays were performed as described before, on K14, EVPL and BPAG1 promoters. Both the deletion mutants display a reduced transcriptional activity compared to ΔNp63α. In fact, WT protein transactivates K14, ENV and BPAG1 promoters about 19-, 3.4- and 48-fold over the control (empty vector), respectively. Instead, ΔNp63α-Δ15-25 was able to activate the same promoters 10, 1.3 and 11 fold over control, while ΔNp63α-Δ1-14 presents the lowest transcriptional activity to 6 and 12 fold over control, for K14 and BPAG1 promoters respectively, while resulted inactive for ENPL promoter.

These results demonstrate that both stretch of amino acids, 1–14 and 15–25, are required for ΔNp63α full transactivation activity, strongly indicating that the ΔNp63 TA2 domain is contained in the first N-terminal 1–25 amino acids.

4. Discussion

p63 belongs to the p53 family of transcription factors, which includes also p73 and p53 itself [25]. Being discovered much earlier,

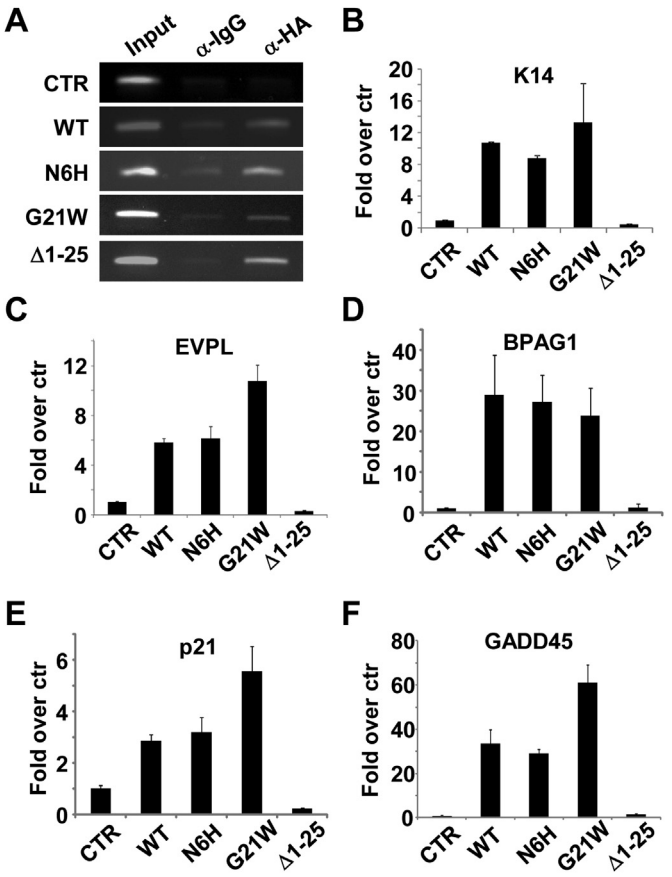

Fig. 2. ΔNp63α-N6H, ΔNp63α-G21W and ΔNp63α, ΔNp63α-Δ1-25 binding to ZNF750 p63 responsive element and quantification of their transactivation activity. A) ChIP analysis of the ZNF750 promoter region known to bind p63. ChIP was performed on ΔNp63α, ΔNp63α-N6H, ΔNp63α-G21W and ΔNp63α-Δ1-25 transfected HEK-293E cells. B–F) Relative luciferase activity expressed as fold over control (CTR) show the ability of ΔNp63α-WT and its mutant forms to transactivate Keratin 14 (K14), Envoplakin (EVPL), BPAG1, p21 and GADD45 promoters. Results are shown as mean ± s.d. of three independent experiments.

p53 is the best studied member of the family, showing a very complex gene activation program from autophagy [26], mitochondria and ROS regulation [27], metabolism [28], DNA damage repair [29,30], stemness and lineage determination [31]. Despite so many years, many questions remain still open in order to fully understand the biological role and function of this transcription factor. This complexity raise from different angles, including for example its stability and degradation [32,33], its connection to micro-RNA [34,35] or its splicing isoforms [36,37]. In keeping with the progress in understanding p53 biology, significant progress is also under way on its potential therapeutic application [38,39]. Although being identified much later, already now, p63 and p73 show their complexity and interaction with p53 [40–42]; where p63 function is highly relevant in skin formation and homeostasis [43] as well as in cancer [41,44]. Altogether, with the above structural and functional similarities, we can always use the information gained on p53 as a starting point for the understanding of the data on p63. By molecular dynamics experiments of p53 on the C-α after 1sq fit to C- α for 800ns, we have recently shown several strong long distant molecular interactions, including the presence of multiple long residence hydrogen bonds between the N-terminal and DBD domains. In addition, we also detected long residence hydrogen bonds between N-terminal and C-terminal domains [45]. These data clearly indicate that the N-terminal domain can stably engage fluctuation of the C-α that allow several hydrogen bonds of

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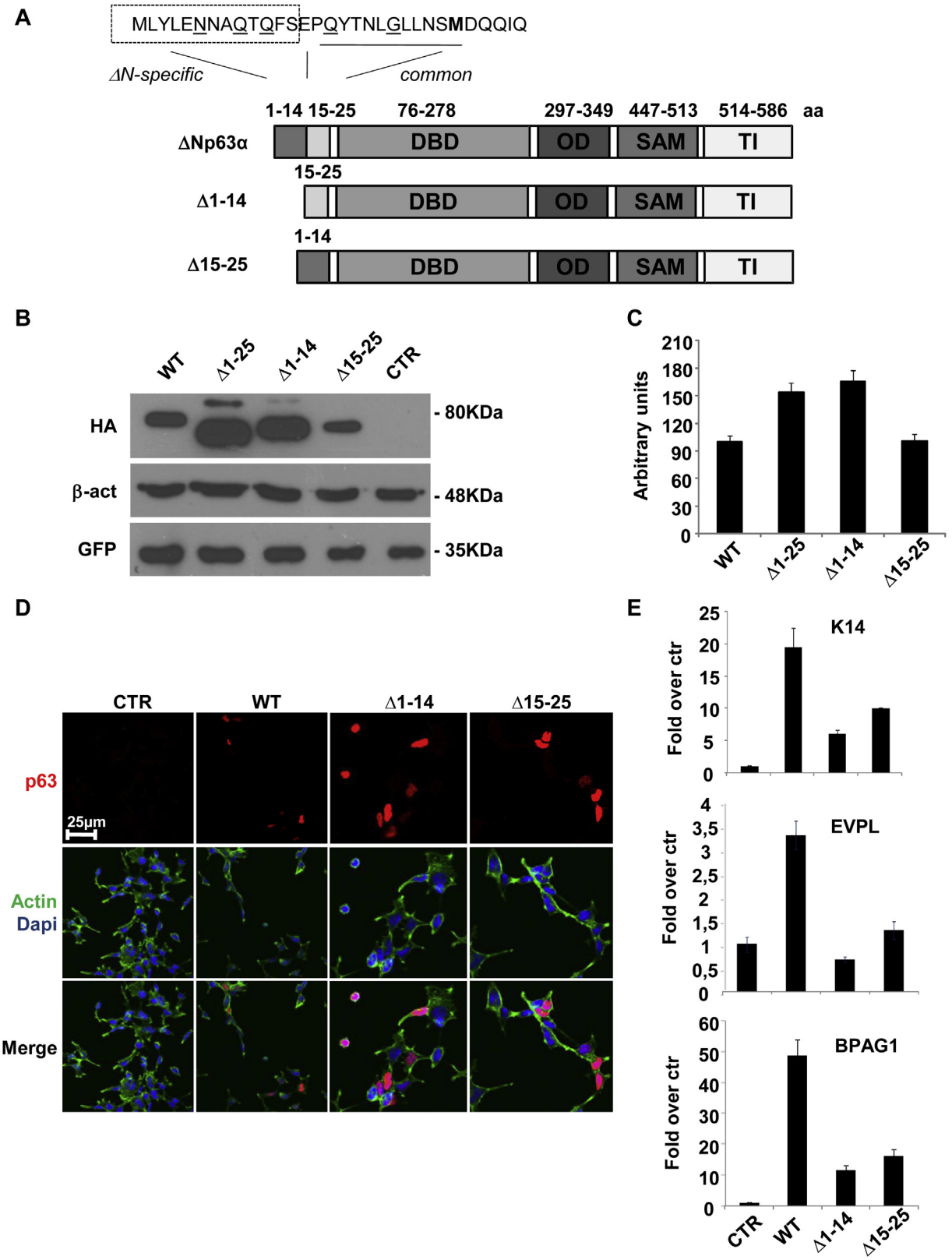

Fig. 3. Mapping ΔNp63α N-terminus transactivation activity. A) Schematic representation of ΔNp63α domains. Metionine 26 is in bold. TA, Transactivation Domain; DBD, DNA Binding Domain; OD, Oligomerization Domain; SAM, Sterile Alpha Motif domain; TI, Transactivation Inhibitory domain. B) Western blot of HEK-293E lysed 24 h after transfection with WT and deletion mutant HA-tagged cDNAs expression vectors. β-actin was used as loading control, GFP as transfection efficiency control. One of three independent experiments is shown. C) Densitometric quantification of western blot signals. The histogram shows the mean ± s.d. of three independent experiments. D) Subcellular localization of ΔNp63α and its mutant forms by confocal microscopy. p63 is in red, actin is in green and nuclei are in blue. Merge staining shows co-localization of DAPI and p63 staining. Scale bars: 25 μm. E) Relative luciferase activity expressed as fold over control (CTR) show the ability of ΔNp63α-WT and its mutant forms to transactivate K14, EVPL and BPAG1 promoters. Results are shown as mean ± s.d. of three independent experiments.

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the N-terminal domain with other long distant domains. It is therefore conceivable that also for p63 there could be long distant molecular interactions of the N-terminal domain that affect the transcriptional function of p63 itself.

Here, were characterized five ectodermal dysplasia ΔNp63α mutants derived from point mutations leading to amino acid substitutions or premature translation termination at the N-terminus of ΔNp63α. We studied N6H substitution, responsible of ADULT syndrome [18], G21W, associated with LMS [17] and p.Gln9fsX23, p.Gln11X, p.Gln16X, linked to RHS and AEC syndromes [19]. These N-terminus ΔNp63 mutants have been here characterized for two main reasons: to study the molecular mechanisms underlying the pathology and to investigate the role of ΔNp63α N-terminal residues in determining its transactivation activity. Indeed, ΔNp63 isoforms, despite the absence of the N-terminal transactivation domain present in TAp63 isoforms, own a transactivating potential, therefore a second transactivation domain (TA2) should be present. Several studies have attempted to map this hypothetical TA2 domain, but results obtained are not fully coherent. Some evidences exist in favor of its presence in the first 25 amino acids of the ΔN isoforms [17,46], while others localize it between the isomerization and the SAM domains [15]. The effects of these mutations were assessed investigating on the protein expression levels, subcellular localization, ability to bind DNA and transactivate known ΔNp63α target promoters. We demonstrated that ΔNp63α-N6H, -G21W and -Δ1-25 mutants are expressed at different levels, localize in nucleus and are able to physically bind a p63 responsive element. Similarly to WT-ΔNp63α, ΔNp63α-N6H and ΔNp63α-G76W mutants transactivate efficiently target promoters, while the deleted Δ1-25 variant lost this potential indicating that the N-terminal 1–25 amino acids of ΔNp63α are involved in transactivation activity, possibly including the entire or part of the ΔNp63 TA2 domain. Taking into account the importance of the amino acids 1–25 for the ΔNp63 isoforms, two different deletion mutants were performed: ΔNp63α-Δ1-14, unique ΔNp63 residues, and ΔNp63α-Δ15-25, common to all p63 isoforms. Both ΔNp63α-Δ1-14 and ΔNp63α-Δ15-25 variants show a reduced transactivation potential, demonstrating that a putative TA2 domain reside within the first 25 amino acids of ΔNp63. As matter of fact, both deletions reduced the activity on K14, ENV and BPAG1 promoters, but did not abolish it. This work strongly support the hypothesis of a TA2 domain at the N-terminus of the protein and suggest that amino acidic residues both ΔN specific (1–14) and common (15–25) to all p63 isoform, are required for transcriptional activity or contribute to it. Further studies will be required to demonstrate in a direct way that this amino acidic sequence is necessary and sufficient to activate ΔNp63 transcription.

We also showed that the first 14 amino acids, ΔNp63 specific, are involved in determining ΔNp63 protein accumulation in the cells, as shown by the higher levels of ΔNp63α-Δ1-14 and ΔNp63α-Δ15-25 proteins. This region could be determining a conformational long-distant interaction allowing an induced-fit mechanism that affects the DBD function, or, alternatively, could be interacting directly with the transcriptional machinery. These results could parallel data shown in previous studies in which we detected increased stability in DBD mutants (EEC) and SAM domain mutants (AEC), indicating that alteration in transcription and/or protein stability could be part of the ectodermal dysplasia syndromes pathogenic mechanisms [20].

While the biochemical characterization of ΔNp63α-Δ1-25 variant reveals that p.Gln9fsX23, p.Gln11X, p.Gln16X alterations result in inactive protein, studies on ΔNp63α-N6H and ΔNp63α-G76W mutants do not shed light on the possible pathogenetic mechanism underling these genomic alterations, as they, at least in our experimental conditions, are expressed, localize in the nucleus, bind DNA and transactivate p63 promoters.

In conclusion, these data indicate that ΔNp63 N-terminal residues are required for transcriptional activity being part or containing TA2 domain, although the amino acid substitutions studied (N6H, G21W), residing in this area, do not compromise ΔNp63 function. Further investigations are needed to fully elucidate the molecular mechanisms and/or interactors involved in human ectodermal dysplasia syndromes caused by N6H and G76W p63 variants.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.09.111.

Transparency document

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References

[1] H. Vanbokhoven, G. Melino, E. Candi, et al., p63, a story of mice and men, J. Investig. Dermatol. 131 (2011) 1196–1207.
[2] T. Rinne, B. Hamel, H. van Bokhoven, et al., Pattern of p63 mutations and their phenotypes-update, Am. J. Med. Genet. A 140 (2006) 1396–1406.
[3] A. Yang, M. Kaghad, Y. Wang, 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.
[4] A.A. Mills, B. Zheng, X.J. Wang, et al., p63 is a p53 homologue required for limb and epidermal morphogenesis, Nature 398 (1999) 708–713.
[5] E. Candi, A. Rufini, A. Terrinoni, et al., Differential roles of p63 isoforms in epidermal development: selective genetic complementation in p63 null mice, Cell Death Differ. 13 (2006) 1037–1047.
[6] E. Candi, I. Amelio, M. Agostini, et al., MicroRNAs and p63 in epithelial stemness, Cell Death Differ. 22 (2015) 12–21.
[7] E. Candi, R. Schmidt, G. Melino, The cornified envelope: a model of cell death in the skin, Nat. Rev. Mol. Cell Biol. 6 (2005) 328–340.
[8] A.M. Lena, R. Cipollone, I. Amelio, et al., Skn-1a/Oct-11 and ΔNp63α exert antagonizing effects on human keratin expression, Biochem. Biophys. Res. Commun. 401 (2010) 568–573.
[9] F. McKeon, p63 and the epithelial stem cell: more than status quo? Genes Dev. 18 (2004) 465–469.
[10] E. Candi, A. Rufini, A. Terrinoni, et al., DeltaNp63 regulates thymic development through enhanced expression of FgfR2 and Jag2, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 11999–12004.
[11] M. Senoo, F. Pinto, C.P. Crum, et al., p63 is essential for the proliferative potential of stem cells in stratified epithelia, Cell 129 (2007) 523–536.
[12] C. Blanpain, E. Fuchs, p63: revving up epithelial stem-cell potential, Nat. Cell Biol. 9 (2007) 731–733.
[13] G. Melino, X. Lu, M. Gasco, et al., Functional regulation of p73 and p63: development and cancer, Trends Biochem. Sci. 28 (2003) 663–670.
[14] G.B. Deutsch, E.M. Zielonka, D. Coutandin, et al., DNA damage in oocytes induces a switch of the quality control factor TAp63α from dimer to tetramer, Cell 144 (2011) 566–576.
[15] P. Ghioni, F. Bolognese, P.H. Duijf, et al., Complex transcriptional effects of p63 isoforms: identification of novel activation and repression domains, Mol. Cell Biol. 22 (2002) 8659–8668.
[16] T. Rinne, E. Bolat, R. Meijer, et al., Spectrum of p63 mutations in a selected patient cohort affected with ankyloblepharon-ectodermal defects-cleft lip/palate syndrome (AEC), Am. J. Med. Genet. A 149A (2009) 1948–1951.
[17] H.G. Brunner, B.C. Hamel, H. Van Bokhoven, The p63 gene in EEC and other syndromes, J. Med. Genet. 39 (2002) 377–381.
[18] J. Amiel, G. Bougeard, C. Francannet, et al., TP63 gene mutation in ADULT syndrome, Eur. J. Hum. Genet. 9 (2001) 642–645.
[19] T. Rinne, S.E. Clements, E. Lamme, P.H. Duijf, A novel translation re-initiation mechanism for the p63 gene revealed by amino-terminal truncating mutations in Rapp-Hodgkin/Hay-Wells-like syndromes, Hum. Mol. Genet. 17 (2008) 1968–1977.
[20] G. Browne, R. Cipollone, A.M. Lena, et al., Differential altered stability and transcriptional activity of ΔNp63 mutants in distinct ectodermal dysplasias, J. Cell Sci. 124 (2011) 2200–2207.
[21] G.L. Sen, L.D. Boxer, D.E. Webster, et al., ZNF750 is a p63 target gene that induces KLF4 to drive terminal epidermal differentiation, Dev. Cell 22 (2012) 669–677.
[22] M. Osada, H.L. Park, Y. Nagakawa, et al., Differential recognition of response elements determines target gene specificity for p53 and p63, Mol. Cell Biol. 25 (2005) 6077–6089.

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[23] M. Osada, Y. Nagakawa, H.L. Park, et al., p63-specific activation of the BPAG-1e promoter, J. Investig. Dermatol. 125 (2005a) 52–60.
[24] F. Bernassola, A. Oberst, G. Melino, et al., The promyelocytic leukaemia protein tumour suppressor functions as a transcriptional regulator of p63, Oncogene 24 (2005) 6982–6986.
[25] G.P. Zambetti, Expanding the reach of the p53 tumor suppressor network, Cell Death Differ. 21 (2014) 505–506.
[26] H.U. Simon, S. Yousefi, I. Schmid, et al., ATG5 can regulate p53 expression and activation, Cell Death Dis. 5 (2014) e1339.
[27] Y. Ci, K. Shi, J. An, et al., ROS inhibit autophagy by downregulating ULK1 mediated by the phosphorylation of p53 in selenite-treated NB4 cells, Cell Death Dis. 5 (2014) e1542.
[28] J. Xu, J. Wang, Y. Hu, et al., Unequal prognostic potentials of p53 gain-of-function mutations in human cancers associate with drug-metabolizing activity, Cell Death Dis. 5 (2014) e1108.
[29] B.C. Nair, S.R. Krishnan, G.R. Sareddy, et al., Proline, glutamic acid and leucine-rich protein-1 is essential for optimal p53-mediated DNA damage response, Cell Death Differ. 21 (2014) 1409–1418.
[30] T.J. Phesse, K.B. Myant, A.M. Cole, et al., Endogenous c-Myc is essential for p53-induced apoptosis in response to DNA damage in vivo, Cell Death Differ. 21 (2014) 956–966.
[31] J. Kim, M. Nakasaki, D. Todorova, et al., p53 Induces skin aging by depleting Blimp1+ sebaceous gland cells, Cell Death Dis. 5 (2014) e1141.
[32] J. Liu, C. Zhang, X.L. Wang, et al., E3 ubiquitin ligase TRIM32 negatively regulates tumor suppressor p53 to promote tumorigenesis, Cell Death Differ. 21 (2014) 1792–1804.
[33] H.H. Zhang, S.Z. Li, Z.Y. Zhang, et al., Nemo-like kinase is critical for p53 stabilization and function in response to DNA damage, Cell Death Differ. 21 (2014) 1656–1663.
[34] M.E. Fiori, C. Barbini, T.L. Haas, et al., Antitumor effect of miR-197 targeting in p53 wild-type lung cancer, Cell Death Differ. 21 (2014) 774–782.
[35] Y. Hoffman, D.R. Bublik, Y. Pilpel, et al., miR-661 downregulates both Mdm2 and Mdm4 to activate p53, Cell Death Differ. 21 (2014) 302–309.
[36] V. Marcel, K. Fernandes, O. Terrier, et al., Modulation of p53β and p53γ expression by regulating the alternative splicing of TP53 gene modifies cellular response, Cell Death Differ. 21 (2014) 1377–1387.
[37] T.L. Slatter, N. Hung, S. Bowie, et al., Δ122p53, a mouse model of Δ133p53α, enhances the tumor-suppressor activities of an attenuated p53 mutant, Cell Death Dis. 6 (2015) e1783.
[38] J. Cheng, Y.H. Fan, X. Xu, et al., A small-molecule inhibitor of UBE2N induces neuroblastoma cell death via activation of p53 and JNK pathways, Cell Death Dis. 5 (2014) e1079.
[39] M. Rossi, B. Rotblat, K. Ansell, et al., High throughput screening for inhibitors of the HECT ubiquitin E3 ligase ITCH identifies antidepressant drugs as regulators of autophagy, Cell Death Dis. 5 (2014) e1203.
[40] M.P. Fatt, G.I. Cancino, F.D. Miller, et al., p63 and p73 coordinate p53 function to determine the balance between survival, cell death, and senescence in adult neural precursor cells, Cell Death Differ. 21 (2014) 1546–1559.
[41] J. Wu, S. Liang, J. Bergholz, et al., ΔNp63α activates CD82 metastasis suppressor to inhibit cancer cell invasion, Cell Death Dis. 5 (2014) e1280.
[42] A. Kostecka, A. Sznarkowska, K. Meller, et al., JNK-NQO1 axis drives TAp73-mediated tumor suppression upon oxidative and proteasomal stress, Cell Death Dis. 5 (2014) e1484.
[43] P. Burnley, M. Rahman, H. Wang, et al., Role of the p63-FoxN1 regulatory axis in thymic epithelial cell homeostasis during aging, Cell Death Dis. 4 (2013) e932.
[44] Z. Salah, T. Bar-mag, Y. Kohn, et al., Tumor suppressor WWOX binds to ΔNp63α and sensitizes cancer cells to chemotherapy, Cell Death Dis. 4 (2013) e480.
[45] G. Chillemi, P. Davidovich, M. D'Abramo, et al., Molecular dynamics of the full-length p53 monomer, Cell Cycle 12 (2013) 3098–3108.
[46] M. Dohn, S. Zhang, X. Chen, p63alpha and DeltaNp63alpha can induce cell cycle arrest and apoptosis and differentially regulate p53 target genes, Oncogene 20 (2001) 3193–3205.

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