

Δ Np63 α Acts as a Lineage-Survival Oncogene in Squamous Cell Carcinoma

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Received date: January 24, 2017; Accepted date: February 22, 2017; Published date: February 27, 2017

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Commentary

The transcription factor p63 is a p53 family member involved in numerous biological processes, including ectodermal development, skin homeostasis, female germline protection and carcinogenesis. Of the multiple isoforms found in higher vertebrates, Δ Np63 α is the predominant one expressed in the epidermis. Lacking the N-terminal, but containing the C-terminal transactivation domain, Δ Np63 α has both transcriptional repressor and transactivation properties, positively and negatively regulating a plethora of genes, involved in proliferation, stemness, cell death, inflammation and differentiation [1].

Two decades following the discovery of TP63, Δ Np63 α has been proven to be a crucial player in Squamous Cell Carcinoma (SCC) development and treatment. Particularly because the majority of SCCs overexpress Δ Np63 α , often as a result of amplification of the TP63 genomic region [2,3]. In agreement, numerous *in vitro* studies highlight Δ Np63 α as a potential oncogene, due to its ability to bypass oncogene-induced senescence, inhibit apoptosis or induce angiogenesis. On the other hand, observations in patients and genetic mouse models with single-allelic loss of p63 reveal an inverse correlation between Δ Np63 α levels and tumor invasiveness and metastasis [2,3]. In addition, tumor Δ Np63 α levels were shown to correlate both with better or worse therapeutic responses, adding additional complexity on the diverse regulatory actions of Δ Np63 α [3]. However, it is clear that, depending on the stage of the tumor and the type of tissue, Δ Np63 α is able to affect tumorigenesis in diverse ways. Over the years, the crucial role of Δ Np63 α during development and homeostasis has prompted the generation of transgenic mouse models capable of studying the influence of Δ Np63 α during the different stages of carcinogenesis. Previously reported Δ Np63 α transgenic mice succumb to spontaneous inflammation early in life, preventing the use of long-term cancer models [4].

In a study recently published in the Journal of Investigative Dermatology, we reported a Keratin 5-Cre-recombinase-controlled ROSA26 promoter-based transgenic Δ Np63 α mouse model [5]. We demonstrated that these epidermal Δ Np63 α -overexpressing mice develop only mild spontaneous phenotypes: epidermal hyperplasia, which gets milder with age, minor hair defects and occasional epidermal cyst development, but no spontaneous inflammation. We explain this difference with the transgenic mice developed by Romano and colleagues by the moderate Δ Np63 α overexpression levels in our model due to the relatively weak ROSA26 promoter, avoiding drastic developmental and inflammatory phenotypes. This allowed us to apply the 7,12-dimethylbenz[a]anthracene (DMBA)- based carcinogenesis model and found that Δ Np63 α -overexpressing mice developed SCCs much faster and in greater numbers compared to wild-type littermates. No significant differences in DMBA- induced cytotoxicity were

observed between transgenic and wild-type epidermis. Instead, isolated keratinocytes from Δ Np63 α -overexpressing mice showed a delay in cellular senescence and enhanced stem cell survival, compared to wild-type keratinocytes (Figure 1).

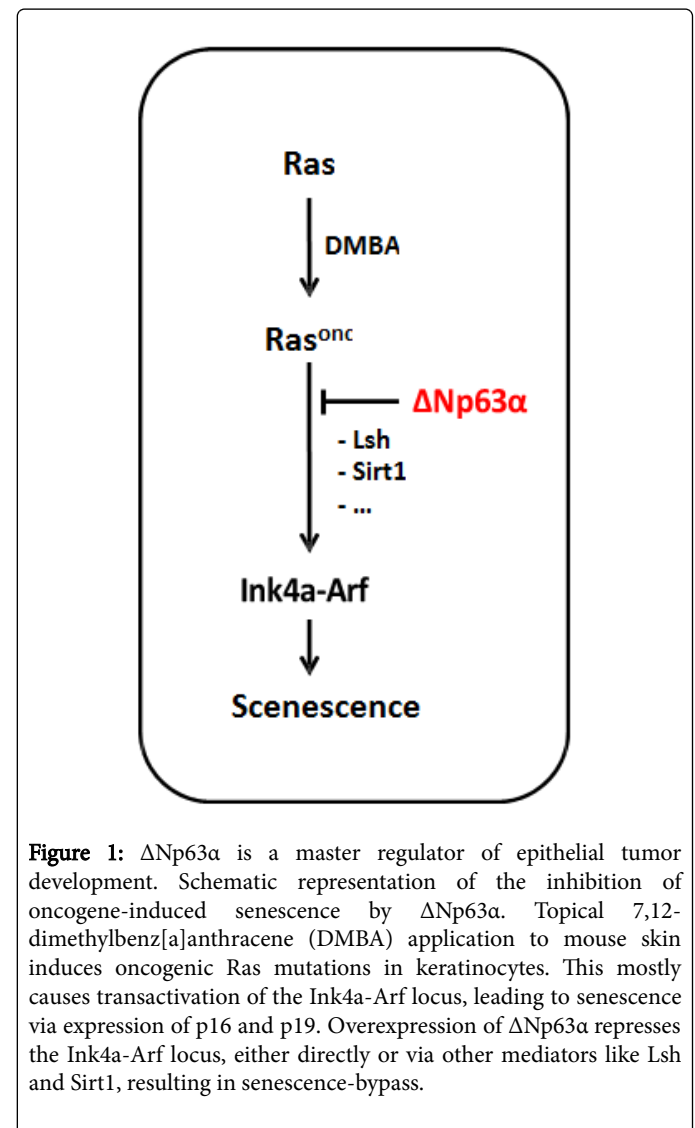


Figure 1: Δ Np63 α is a master regulator of epithelial tumor development. Schematic representation of the inhibition of oncogene-induced senescence by Δ Np63 α . Topical 7,12-dimethylbenz[a]anthracene (DMBA) application to mouse skin induces oncogenic Ras mutations in keratinocytes. This mostly causes transactivation of the Ink4a-Arf locus, leading to senescence via expression of p16 and p19. Overexpression of Δ Np63 α represses the Ink4a-Arf locus, either directly or via other mediators like Lsh and Sirt1, resulting in senescence-bypass.

These observations are in line with previously published reports identifying Δ Np63 α as a mediator in oncogene-induced senescence [6,7]. We found that Δ Np63 α overexpression resulted in higher levels of the chromatin remodelers Lymphoid-specific helicase (Lsh), known

to repress p16Ink4a expression, and Sirtuin 1 (Sirt1) (Figure 1) [6,8]. Consequently, Δ Np63 α transgenic keratinocytes showed delayed upregulation of p16Ink4a and p19Arf compared with wild-type keratinocytes. This could explain the higher yield of skin tumors observed in p63 transgenic mice using the DMBA model. The precise molecular mechanisms mediating senescence-bypass by Δ Np63 α remain unknown, but increasing evidence suggests a complex interplay with microRNAs [9]. It is tempting to speculate that Δ Np63 α is involved in cancer stem cell regulation. Indeed, we showed that Δ Np63 α overexpression results in a higher percentage of CD34-positive hair follicle stem cells [5]. Given the importance of CD34-positive cells for the development of tumors in the DMBA carcinogenesis model, Δ Np63 α could facilitate tumor formation by increasing the self-renewal potential of epithelial cancer stem cells [10]. Mechanistically this could occur by maintaining the quiescent state of stem cells or by promoting asymmetric stem cell division, a type of cell division resulting in long-lived classical stem cells and a short-lived population of transit-amplifying cells. In relation to this, it has been shown that inhibition of SMAD signaling in Δ Np63 α expressing cells is associated with epithelial basal cell identity [11]. Instead, Δ Np63 α might suppress the differentiation capacity of tumor stem cells. In view of our observation that Δ Np63 α overexpressing epidermis contains more C34-positive stem cells, promotion of stem cell division or suppression of stem cell differentiation are the two most plausible mechanisms at work in our model. The molecular pathways implicating p63 in epithelial cancer stem cell maintenance have been thoroughly reviewed by Melino and colleagues [1].

Since no spontaneous skin tumors arise in Δ Np63 α transgenic skin, Δ Np63 α differs from classical oncogenes, like members of the Ras subfamily. However, Δ Np63 α overexpression significantly facilitates SCC development initiated by oncogenic HRas. In line with this, the high frequency of TP63 amplification in human SCC demonstrates an addiction to high Δ Np63 α expression levels within a large subset of these tumors [3]. This suggests that SCC cells rely to a great extent on Δ Np63 α -dependent proliferation and survival mechanisms, which are embedded into the lineage precursor program. Indeed, Δ Np63 α is a master regulatory transcription factor associated with keratinocyte lineage development and homeostasis. Δ Np63 α -deficient mice fail to develop a mature stratified epidermis, due to impaired self-renewal potential and differentiation [12]. Taken together, by controlling keratinocyte lineage survival during development and facilitating epidermal tumorigenesis triggered by classical oncogenes, Δ Np63 α can be identified as a lineage-survival oncogene in SCC [13]. Another example of an amplified lineage-survival oncogene in SCC is the high mobility group transcription factor SOX2 [14]. Interestingly, Δ Np63 α and Sox2 were shown to cooperatively regulate gene expression essential for SOX2-amplified SCC growth and survival [15]. Whether the increased SCC development in our transgenic Δ Np63 α overexpressing mouse model is dependent on cross-talk between Δ Np63 α and Sox2 is currently not clear. On the other hand, one can postulate that lineage-independent mechanisms push tumors towards poorly differentiated aggressive malignancies. Indeed, clinical studies clearly correlate loss of Δ Np63 α expression with invasive and metastatic behavior [3]. In agreement, a study in Cell Stem Cell, published by Latil et al. at the same time as our study, shows that Δ Np63 α is associated with well-differentiated SCCs [16]. Δ Np63 α protein levels are increased in tumor cells expressing epithelial markers such as Keratin 14, Epcam and E-cadherin and Δ Np63 α binding sites in target gene promoters are more into an open chromatin structure (Figure 2). In addition, Δ Np63 α engages in repressing epithelial-to-

mesenchymal transition (EMT) genes through micro-RNA induction. Importantly, sustained expression of Δ Np63 α in Lrg5CreER/KRasG12D/p53fl/fl transgenic mice, which normally develop epithelial, mesenchymal and mixed tumors, resulted in higher proportions of well-differentiated SCCs (Figure 2), supporting the concept of Δ Np63 α dictating lineage-dependency.

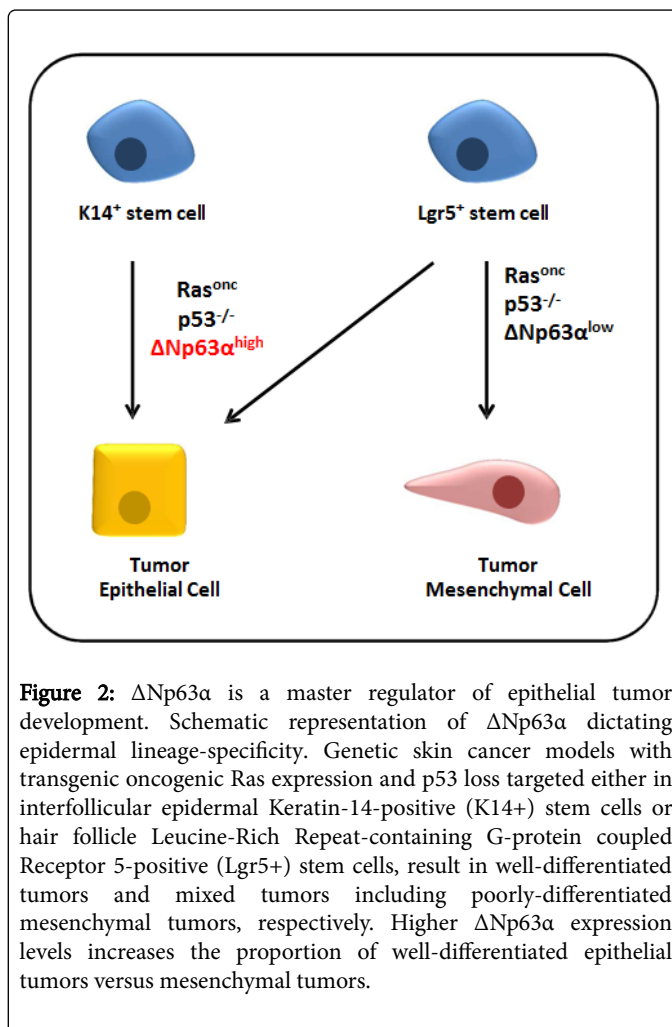


Figure 2: Δ Np63 α is a master regulator of epithelial tumor development. Schematic representation of Δ Np63 α dictating epidermal lineage-specificity. Genetic skin cancer models with transgenic oncogenic Ras expression and p53 loss targeted either in interfollicular epidermal Keratin-14-positive (K14+) stem cells or hair follicle Leucine-Rich Repeat-containing G-protein coupled Receptor 5-positive (Lgr5+) stem cells, result in well-differentiated tumors and mixed tumors including poorly-differentiated mesenchymal tumors, respectively. Higher Δ Np63 α expression levels increases the proportion of well-differentiated epithelial tumors versus mesenchymal tumors.

Over 1 million people worldwide die each year from squamous cell carcinomas (SCCs) and treatment options for SCC include surgery, radiotherapy or chemotherapy [17]. While regional tumor spread is primarily managed by surgery in combination with radiotherapy, chemotherapy is mostly considered for distant metastasis. Over the years, studies on the role of Δ Np63 α in the cellular response to DNA damage-inducing agents used as chemotherapeutics have led to conflicting observations. High Δ Np63 α protein levels have been correlated with a favorable outcome to platinum-based chemotherapy [18]. In agreement, we and others found that Δ Np63 α overexpression resulted in a higher ratio of mitotically active cells, which are more vulnerable to common chemotherapeutic compounds [5,19]. On the other hand, multiple studies have shown that Δ Np63 α is phosphorylated and subsequently degraded upon DNA damage [18]. Given the poised anti-apoptotic role of Δ Np63 α , this would imply that Δ Np63 α -expressing tumor cells are rendered sensitive to apoptosis upon DNA damage partly as a result of loss of Δ Np63 α expression. In contrast, some reports state that Δ Np63 α overexpression results in

decreased cell survival, independent of p53 [20,21]. In general, insufficient clinical evidence exists to support *in vitro* findings, regarding the molecular mechanisms how Δ Np63 α controls cell death.

In conclusion, our new Δ Np63 α transgenic mouse model provides *in vivo* proof that increased Δ Np63 α levels facilitate cutaneous SCC development and dictate lineage-specificity. A challenge for the future is to define how Δ Np63 α influences patient prognosis and treatment, for which this transgenic model could present an interesting tool.

Acknowledgements

This research has been supported by the Flanders Institute for Biotechnology (VIB); Belgian grants: Interuniversity Attraction Poles, IAP7/32; Stichting tegen Kanker (2010-162 and FAF-F/2016/868); Flemish grants: FWO-Vlaanderen (G.0544.11) and a Methusalem grant (BOF09/01M00709) from the Flemish Government to Peter Vandenabeele; an a UGent grant (GOA-01G01914).

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