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Commentary OMICS International

ΔNp63α Acts as a Lineage-Survival Oncogene in Squamous Cell Carcinoma

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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, ΔNp63α is the predominant one expressed in the epidermis. Lacking the N-terminal, but containing the C-terminal transactivation domain, ΔNp63α 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, ΔNp63α has been proven to be a crucial player in Squamous Cell Carcinoma (SCC) development and treatment. Particularly because the majority of SCCs overexpress ΔNp63α, often as a result of amplification of the TP63 genomic region [2,3]. In agreement, numerous *in vitro* studies highlight ΔNp63α 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 ΔNp63α levels and tumor invasiveness and metastasis [2,3]. In addition, tumor ΔNp63α levels were shown to correlate both with better or worse therapeutic responses, adding additional complexity on the diverse regulatory actions of ΔNp63α [3]. However, it is clear that, depending on the stage of the tumor and the type of tissue, ΔNp63α is able to affect tumorigenesis in diverse ways. Over the years, the crucial role of ΔNp63α during development and homeostasis has prompted the generation of transgenic mouse models capable of studying the influence of ΔNp63α during the different stages of carcinogenesis. Previously reported ΔNp63α 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 ΔNp63α mouse model [5]. We demonstrated that these epidermal ΔNp63α-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 ΔNp63α 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 ΔNp63α-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 ΔNp63α-overexpressing mice showed a delay in cellular senescence and enhanced stem cell survival, compared to wild-type keratinocytes (Figure 1).

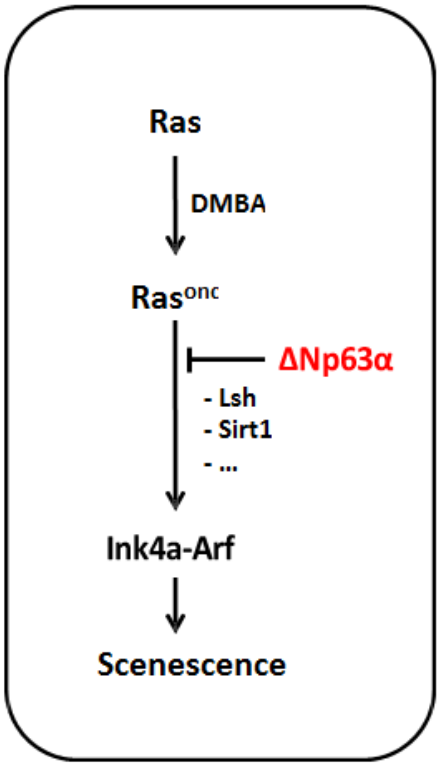

Figure 1: ΔNp63α is a master regulator of epithelial tumor development. Schematic representation of the inhibition of oncogene-induced senescence by ΔNp63α. Topical 7,12-dimethylbenz[a]anthracene (DMBA) application to mouse skin induces oncogenic Ras mutations in keratinocytes. This mostly causes transactivation of the Ink4a-Arf locus, leading to senescence via expression of p16 and p19. Overexpression of ΔNp63α 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 ΔNp63α as a mediator in oncogene-induced senescence [6,7]. We found that ΔNp63α 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, ΔNp63α 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 ΔNp63α remain unknown, but increasing evidence suggests a complex interplay with microRNAs [9]. It is tempting to speculate that ΔNp63α is involved in cancer stem cell regulation. Indeed, we showed that ΔNp63α 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, ΔNp63α 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 ΔNp63α expressing cells is associated with epithelial basal cell identity [11]. Instead, ΔNp63α might suppress the differentiation capacity of tumor stem cells. In view of our observation that ΔNp63α 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 ΔNp63α transgenic skin, ΔNp63α differs from classical oncogenes, like members of the Ras subfamily. However, ΔNp63α 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 ΔNp63α expression levels within a large subset of these tumors [3]. This suggests that SCC cells rely to a great extent on ΔNp63α- dependent proliferation and survival mechanisms, which are embedded into the lineage precursor program. Indeed, ΔNp63α is a master regulatory transcription factor associated with keratinocyte lineage development and homeostasis. ΔNp63α-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, ΔNp63α 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, ΔNp63α 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 ΔNp63α overexpressing mouse model is dependent on cross-talk between ΔNp63α 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 ΔNp63α 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 ΔNp63α is associated with well-differentiated SCCs [16]. ΔNp63α protein levels are increased in tumor cells expressing epithelial markers such as Keratin 14, Epcam and E-cadherin and ΔNp63α binding sites in target gene promoters are more into an open chromatin structure (Figure 2). In addition, ΔNp63α engages in repressing epithelial-to-mesenchymal transition (EMT) genes through micro-RNA induction. Importantly, sustained expression of ΔNp63α 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 ΔNp63α dictating lineage-dependency.

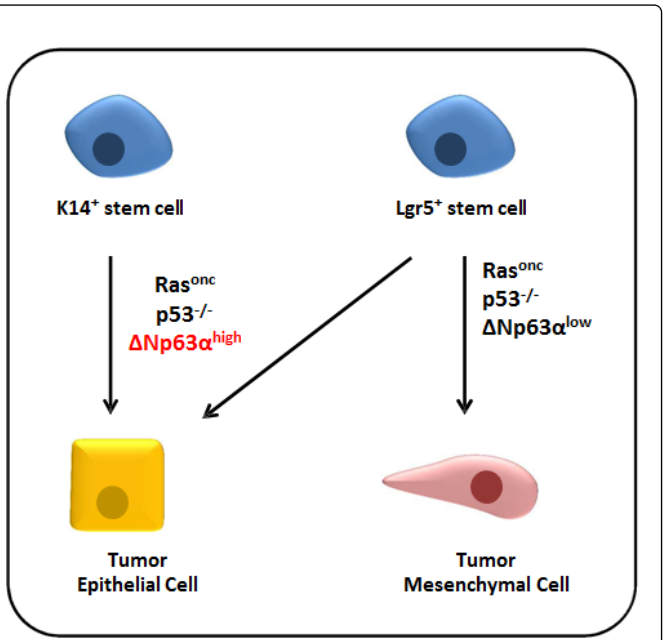

Figure 2: ΔNp63α is a master regulator of epithelial tumor development. Schematic representation of ΔNp63α dictating epidermal lineage-specificity. Genetic skin cancer models with transgenic oncogenic Ras expression and p53 loss targeted either in interfollicular epidermal Keratin-14-positive (K14+) 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 ΔNp63α 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 ΔNp63α in the cellular response to DNA damage-inducing agents used as chemotherapeutics have led to conflicting observations. High ΔNp63α protein levels have been correlated with a favorable outcome to platinum-based chemotherapy [18]. In agreement, we and others found that ΔNp63α 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 ΔNp63α is phosphorylated and subsequently degraded upon DNA damage [18]. Given the poised anti-apoptotic role of ΔNp63α, this would imply that ΔNp63α-expressing tumor cells are rendered sensitive to apoptosis upon DNA damage partly as a result of loss of ΔNp63α expression. In contrast, some reports state that ΔNp63α 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 ΔNp63α controls cell death.

In conclusion, our new ΔNp63α transgenic mouse model provides *in vivo* proof that increased ΔNp63α levels facilitate cutaneous SCC development and dictate lineage-specificity. A challenge for the future is to define how ΔNp63α influences patient prognosis and treatment, for which this transgenic model could present an interesting tool.

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