How does ΔNp63α drive cancer?

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"...just as every cancer differs in its mutational status and cell behavior, each epithelial cell type and carcinoma may respond differently to ΔNp63α overexpression."

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ΔNp63α is a pleiotropic oncogene in diverse cancer types

ΔNp63α is a member of the p53 family of transcriptional regulators that is required for the proliferation of epithelial stem cells and basal keratinocytes [1]. It is frequently overexpressed in carcinomas of multiple origins, including squamous cell carcinomas (SCCs) [2,3], basal breast carcinomas [4] and others. In addition, ΔNp63α expression has been described as an indicator of poor prognosis for a number of different tumor types [4]. Thus, the oncogenic role of ΔNp63α has been well established. However, the effects of ΔNp63α expression on cancer and epithelial cell proliferation are varied. In normal keratinocytes, loss of ΔNp63α expression results in cell cycle arrest and/or senescence [5,6]. In cancer cells of various origins, loss of ΔNp63α expression results in cell cycle arrest [2], apoptosis [3,5,7,8] and/or an increased sensitivity to apoptosis-inducing chemotherapeutics [9,10]. Furthermore, ΔNp63α expression can promote anchorage-dependent and -independent growth, motility and invasion in head and neck SCC (HNSCC) and pancreatic cancer cells [10,11]. Therefore, although ΔNp63α generally promotes cancer cell proliferation, it is important to recognize that the effects of ΔNp63α expression on cancer cell behavior are varied and cell-type specific.

ΔNp63α positively & negatively regulates transcription in a cell-type specific manner

ΔNp63α has been shown to function both as a transcriptional activator and as a transcriptional repressor within the p53 network [7]. Transcription initiation at an alternative promoter of the *Tp63* locus and alternative splicing at the 3′ end of the gene results in expression of this isoform, which lacks the canonical N-terminal TA domain [1] and contains a C-terminal transcriptional inhibition domain [12], likely contributing to ΔNp63α's role as a transcriptional repressor. Conversely, a putative C-terminal TA2 domain may allow it to function as a transcriptional activator in some contexts [13].

Two elegant studies using normal human keratinocytes [14] and ME180 cervical carcinoma cells [7] have paired a genome-wide analysis of p63 binding sites (using chromatin immunoprecipitation [ChIP]; specifically ChIP-sequencing and ChIP-on-chip, respectively) with analysis of gene expression following p63-knockdown (via microarray) to identify putative direct transcriptional targets of ΔNp63α. These studies determined that the direct targets of ΔNp63α are surpassed in a number by genes whose expression is modulated by the loss of ΔNp63α in *trans*, without direct p63 binding, and that ΔNp63α can both positively and negatively affect expression of its target genes. Interestingly, a number of these direct target genes play a role in regulating cell growth, differentiation and death.

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ΔNp63α is a potent transcriptional repressor of a number of antiproliferative p53 target genes, including *p21* (*CDKN1A*), *14–3–3σ* (*SFN*), *PUMA* (*BBC3*) and *NOXA* (*PMAIP1*) [3,5,15]. However, it is important to note that ΔNp63α affects expression of each of these genes in a cell-type specific manner. For instance, knockdown of ΔNp63α in normal keratinocytes results in derepression of *p21*, causing cell cycle arrest and/or senescence, whereas in JHU-029

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HNSCC cells, *p21* expression is unaffected and the BCL-2 family member *PUMA* is induced, triggering apoptosis [5]. Furthermore, none of these antiproliferative genes are affected in a number of other SCC cell lines [2,9]. Instead, loss of ΔNp63α results in upregulation of the proapoptotic factor *IGFBP3* in a variety of SCC cells [2,9,11]. Finally, novel antiproliferative ΔNp63α-repressed target genes, such as *SAMD9L* [2] and the microRNAs miR-138, -181a, -181b and -130b [6], continue to be identified. Taken together, it is readily apparent that ΔNp63α can effectively promote cancer cell proliferation through the transcriptional repression of multiple factors, although the specific effects of ΔNp63α vary across cell types.

> "*...ΔNp63α may modulate the chromatin landscape of its target genes.*"

Although the vast majority of studies have focused on the role of ΔNp63α-repressed target genes in cancer cell proliferation and survival, it is important to consider the oncogenic effect of its positively regulated targets as well. ΔNp63α induces expression of factors, such as epidermal growth factor receptor, which can increase the proliferation of pancreatic cancer cells [10]. Keratin 6, keratin 14 [16] and aquaporin 3 [11] are highly expressed by undifferentiated basal epithelial cells, and upregulation of these factors by ΔNp63α may also allow SCC cells and/or cancer stem cells to avoid differentiation. Furthermore, heat shock protein HSP70 is a potent antiapoptotic factor that is upregulated by ΔNp63α in head and neck cancer cells [17]. Therefore, the positive transcriptional effects of ΔNp63α can also promote cancer cell growth and it is likely that this also occurs in a cell-type specific manner.

ΔNp63α employs diverse mechanisms of transcriptional regulation

Despite the important role that ΔNp63α plays in regulating the proliferation and survival of epithelial stem cells and cancer cells, remarkably little is known about the mechanisms by which it modulates expression of its target genes. For example, although the DNA-binding and TA2 domains are required for ΔNp63α-dependent transcriptional activation of the *ATM* promoter in non-small-cell lung cancer cells [18], it is not known exactly how ΔNp63α activates transcription of the hundreds of target genes seemingly being directly transactivated by it. More specifically, nothing is known about the coactivators that ΔNp63α would employ at these target loci, either via the TA2 domain or other transactivation domains that have not yet been identified.

More information is available about ΔNp63α-mediated transcriptional repression. Thus far, three different mechanisms have been identified that are used to varying degrees in different cell types. First, ΔNp63α may directly antagonize TAp73 in select HNSCC cells; not only does ΔNp63α hetero-oligomerize with TAp73β [3,5], thereby preventing p73 from transactivating its target genes, but ΔNp63α levels also exceed that of TAp73 in most cells of epithelial origin [3,5], allowing ΔNp63α to occupy enhancer sites as homotetramers, thereby preventing p73 occupancy. Second, ΔNp63α may modulate the chromatin landscape of its target genes. ΔNp63α may physically interact with the histone deacetylases HDAC1 and HDAC2, and recruit them to p53/p63/p73 enhancer sites [8], subsequently mediating the deacetylation of histones H3 and H4 at those enhancer sites [8,19]. The HDAC-mediated repression of ΔNp63α target genes has been shown to occur in both keratinocytes and HNSCC cells [8,19]. Finally, we have recently shown that ΔNp63α physically interacts with members of the SRCAP chromatin remodeling complex, and through exchange of histone H2A with histone variant H2A.Z at enhancer sites and target gene promoters, ΔNp63α creates a chromatin environment that is repressive to transcription [2]. We have shown that the repressive effects of H2A.Z occur in lung SCC cells as well as immortalized keratinocytes and HNSCC cells of various origins [2]. However, the degree to which ΔNp63α uses these three mechanisms to repress its target gene expression varies highly between cell types. ΔNp63α represses *p21* expression in a manner independent of p73 in primary keratinocytes [5], whereas in H226 lung SCC cells, repression of ΔNp63α target genes is independent of both p73 and histone deacetylation [Gallant-Behm CL, Espinosa JM. DNp63a utilizes multiple mechanisms to repress transcription in squamous cell carcinoma cells (2012), Submitted]. Therefore, just as every cancer differs in its mutational status and cell behavior, each epithelial cell type and carcinoma may respond differently to ΔNp63α overexpression.

Future studies

As we have discussed here, ΔNp63α undoubtedly serves as a potent oncogene for many cancers of epithelial origin, including SCCs, basal cell carcinomas, non-small-cell lung cancer among others [2–4]. Although ΔNp63α overexpression

is crucial for continued cell proliferation in these differing cell types, the means by which ΔNp63α promotes proliferation varies highly between cell types [2,3,5,8,19]. In order to better understand the role of ΔNp63α in cancer cell proliferation, it behooves us to define:

- How exactly ΔNp63α targets the genes it does and why those target genes differ between cell types;
- Which ΔNp63α target genes allow for rapid cancer cell proliferation and avoidance of cell cycle arrest, senescence and/or apoptosis;
- How ΔNp63α mechanistically recruits chromatin remodelers, such as HDAC1/2 and the SRCAP complex, to its target genes and;
- What additional mechanisms may allow ΔNp63α to activate or repress transcription of its target genes.

Only when these questions are answered can we begin to develop novel therapeutics to treat ΔNp63α-overexpressing cancers in patients.

Disclaimer

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