ABSTRACT

p63 (also known as TP63) is a transcription factor of the p53 family, along with p73. Multiple isoforms of p63 have been discovered and these have diverse functions encompassing a wide array of cell biology. p63 isoforms are implicated in lineage specification, proliferative potential, differentiation, cell death and survival, DNA damage response and metabolism. Furthermore, p63 is linked to human disease states including cancer. p63 is critical to many aspects of cell signaling, and in this Cell science at a glance article and the accompanying poster, we focus on the signaling cascades regulating TAp63 and ΔNp63 isoforms and those that are regulated by TAp63 and ΔNp63, as well the role of p63 in disease.

KEY WORDS: Cancer, Development, Isoforms, p63, Stemness, Differentiation, Signal transduction

Introduction

p63, also known as Trp63 (transformation related protein 63), TP63 (tumor protein 63) or AIS (amplified in squamous cell carcinoma), is a member of the p53 family of transcription factors, which also includes p73 (Botchkarev and Flores, 2014; Soares and Zhou, 2018). Despite initial ideas that p63 evolved to modulate p53 function, p63 has been shown to be the primordial family member of the p53/p63/p73 gene family (Amelio et al., 2012). Several
isoforms of p63 exist, and these are linked to numerous biological functions, including development and differentiation, senescence, proliferation, stem cell maintenance, aging, and apoptosis (Galoczova et al., 2018; Yoh and Prywes, 2015; Keyes and Mills, 2006; Gonfloni et al., 2015). p63 is an important player in embryonic epidermal development and in epidermal keratinocyte morphogenesis, proliferation and differentiation, where it directly transactivates a plethora of target genes involved in cellular proliferation, differentiation and adhesion (Kouwenhoven et al., 2015; Moll and Slade, 2004; Boughner et al., 2018; van Bokhoven et al., 2011; Westfall and Pietenpol, 2004). Additionally, p63 plays a role in the modulation of the chromatin landscape in epidermal keratinocytes by directly regulating chromatin-modulating factors and by engaging and opening chromatin regions (Qu et al., 2018, Pattison et al., 2018). The existence of multiple isoforms with distinct, often opposing, functions allows p63 to have a wide array of effects on essential cellular functions (Westfall and Pietenpol, 2015; Moll and Slade, 2004; Boughner et al., 2018; van Bokhoven et al., 2011; Westfall and Pietenpol, 2004) (see Box 1 and poster). Different p63 isoforms have been shown to impact various cells, tissues and diseases (Soares and Zhou, 2018). Because p63 isoforms have such a diverse array of functions, they must act on numerous pathways to carry out these functions. Furthermore, there is an equally large number of pathways that regulate p63 expression. We will focus here on some of the key signaling pathways that regulate, and are regulated by, the TAp63 and ΔNp63 isoforms to demonstrate the vast array of functions p63 isoforms can carry out.

### Regulation of p63

p63 isoforms are critical to a number of aspects of cell biology, and below we discuss some of the many signaling events that can regulate p63 expression (see poster).

#### DNA damage

A major regulator of p63 is DNA damage. The response to DNA damage appears to be isoform and cell type specific (Moll and Slade, 2004; Yoh and Prywes, 2015). TAp63 isoforms are expressed in response to DNA damage or other stresses in epithelial tissue, neurons and the germline (Su et al., 2013). TAp63 is primarily found as a closed dimer with low activity due to the C-terminal inhibitory domain and N-terminal TA domain blocking the oligomerization domain (Deutsch et al., 2011). This differs from ΔNp63 isoforms typically found as tetramers (Russo et al., 2018). However, in response to DNA damage, TAp63α is phosphorylated by CHK2 (also known as CHEK2) and casein kinase 1 (CK1), leading first to the formation of an open dimer and then an active tetramer (Deutsch et al., 2011). Furthermore, in oocytes, TAp63 mediates apoptosis in response to DNA damage by inducing Puma and Noxa (also known as BBC3 and PMAIP1, respectively) (Kerr et al., 2012). Conversely, GSK-3β reduces TAp63 expression to protect oocytes from premature death (Wen et al., 2019). In head and neck squamous cell carcinoma (HNSCC), DNA damage triggered by chemotherapy decreases ΔNp63-mediated transcriptional repression by blocking p63-responsive elements or sequestering TAp63 into less-active heterotetramers (Melino, 2011). A different report found that, in response to cisplatin treatment, c-Abi phosphorylates the Y55, Y137 and Y308 residues of ΔNp63α; this stabilizes it by inducing its binding to Yes-associated protein (YAP; also known as YAP1), consequently regulating cell viability in HNSCC cells (Yuan et al., 2010). Additionally, c-Abi was shown to phosphorylate TAp63α on Y149, Y171 and Y289 residues, resulting in increased protein stability of TAp63α (Gonfloni et al., 2009).

The ataxia-telangiectasia mutated (ATM) kinase is also critical to the p63 response to DNA damage. ATM reduces ΔNp63α and increases p53 after irradiation (Huang et al., 2008). This impairs the proliferative capacity of epithelial stem cells with regard to p53-mediated repair of DNA damage. Furthermore, DNA damage leads to phosphorylation of TAp63γ by inhibitor of nuclear factor-κB kinase-α (IKKα; also known as IKBKα) in response to ionizing radiation, resulting in its stabilization (MacPartlin et al., 2008).

Additionally, two mechanisms related to the NF-κB pathway have been found to mediate DNA-damage-induced ΔNp63 degradation. IKKβ is capable of binding to ΔNp63, phosphorylating it to induce degradation (Chatterjee et al., 2010). The p65 subunit of NF-κB can also bind directly to ΔNp63 in cisplatin-treated cells, leading to degradation of ΔNp63 (Sen et al., 2010).

Degradation of ΔNp63 in response to DNA damage is carried out by the proteasome. The scaffold protein syntenin-binding protein 4 (STXB4P) binds to ΔNp63 and stabilizes it (Otaka et al., 2017). In response to DNA damage, homeodomain-interacting protein kinase 2 (HIPK2) phosphorylates ΔNp63 on T397. STXB4P is downregulated, resulting in the destabilization of ΔNp63 through receptor of activated kinase 1 (RACK1), which facilitates the binding of ΔN63 to ubiquitin ligases (Lazzari et al., 2011). Multiple ubiquitin ligases that bind p63 have been identified, including

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**Box 1. Structure of p63 isoforms**

The p63 gene consists of 15 exons spanning ~270 kb (Murray-Zmijewski et al., 2006; Mills et al., 1999) (see poster) and maps to chromosome 3q27 (Murray-Zmijewski et al., 2006). It encodes two main classes of isoforms that are generated by alternative promoters. TAp63 transcripts are produced from the promoter upstream of exon 1 (Yang et al., 1998). TAp63-encoding transcripts contain three TA-specific exons (exons 1, 2 and 3) that encode an N-terminal transactivation domain, homologous to that of p53 (Murray-Zmijewski et al., 2006). An alternate promoter within the intron downstream of exon 3 results in transcripts encoding truncated ΔNp63 isoforms that lack the N-terminal transactivation domain (Sethi et al., 2015). However ΔNp63 isoforms retain the ability to induce genes via a region in exon 3 (Helton et al., 2006). The ability of ΔNp63 to activate target genes depends on the first 26 residues and the first 14 residues for ΔNp63α and ΔNp63γ, respectively (Bergholz and Xiao, 2012; Helton et al., 2006). Additionally, the PXXP and PPXY motif are required for optimal transactivation. Therefore, the 14 residues unique to the ΔNp63 isoforms and the adjacent region, comprise an activation domain for ΔNp63 isoforms (Helton et al., 2006) (see poster). A further truncated form of p63 can be produced, and is denoted the ΔNp63 isoform. This isoform lacks the first 26 amino acids of ΔNp63 isoforms due to an alternative translational start site in exon 4 (Rinne et al., 2008).

Alternative splicing occurring at the 3' end of the p63 mRNA generates multiple C-terminal variants (β, γ, δ and ε) for both TAp63 and ΔNp63 (King et al., 2013; Mangiulli et al., 2008). At the C-terminus, the longest isoform is α, which contains exons 11–14. At the protein level, the α isoform contains a sterile alpha-motif (SAM) involved in protein–protein interactions and a transactivation inhibitory domain (TID) (Soares and Zhou, 2018). The β and δ isoforms lack exon 13, and γ does not have exons 11–14 but has a γ-specific exon 10 (Ghioni et al., 2002). The ε isoform is generated by a premature stop codon located in exon 10 (Mangiulli et al., 2009). All isoforms contain the DNA-binding domain and oligomerization domain (OD). TAp63 and ΔNp63 isoforms are typically differentially expressed. TAp63 variants are prevalent in the heart, testis, kidney, thymus, brain and cerebellum, while ΔNp63 transcripts are highly detected in epithelia, kidney, spleen and thymus, but not the heart, liver, testis or brain (Di Como et al., 2002; Kawasaki et al., 2006; Nylander et al., 2002) (see poster).
neuronal precursor cell-expressed developmentally downregulated 4 (NEDD4), itchy E3 ubiquitin protein ligase (Itch), WW domain containing E3 ubiquitin protein ligase 1 (WWP1), F-box and WD-40 domain-containing protein 7 (FBW7, also known as FBXW7), putative E3 ubiquitin-protein ligase RING1B, and p53-induced RING-H2 protein (PIRH2; also known as RCHY1) (Li and Xiao, 2014). Pirh2 is also a transcriptional target of ΔNp63, establishing an auto-regulatory loop, and is required for epithelial differentiation (Jung et al., 2013). Binding of these E3 ligases in turn targets ΔNp63 for proteasomal degradation (Li and Xiao, 2014). Furthermore, feedback loops occur frequently as part of p63 regulation, as discussed further below.

Cell surface receptors
The tyrosine kinase receptor epidermal growth factor receptor (EGFR) can induce ΔNp63 expression. EGFR activation of phosphatidylinositol 3-kinase (PI3K) signaling has been shown to upregulate ΔNp63α in keratinocytes (Barbieri et al., 2003); in cancer, EGFR activation of ΔNp63α is mediated by STAT3 (Ripamonti et al., 2013). These pathways are likely linked through mammalian target of rapamycin (mTOR). PI3K activation of mTOR has been shown to lead to mTOR-dependent activation of the STAT3-p63-Jagged pathway (Ma et al., 2010). In addition to EGFR-mediated activation of ΔNp63α, the interaction of α6β4 integrin with specific proteins stimulates ΔNp63α expression, such as interaction with transglutaminase 2 (TG2; also known as TGM2) (Fisher et al., 2016). In normal epithelial tissue, α6β4 integrin is associated with stable adhesion and does not activate FAK–SRC signaling (Duperret and Ridky, 2013; Epifano and Perez-Moreno, 2012). However, in squamous cell carcinoma, α6β4 integrin associates with the actin cytoskeleton and forms new signaling complexes that allow for the activation of FAK–SRC and PI3K–PD1 signaling (Duperret and Ridky, 2013; Epifano and Perez-Moreno, 2012; Kim and Gumbiner, 2015). FAK and SRC are non-receptor tyrosine kinases that form a complex and phosphorylate various adapter proteins that promote cell motility, cell cycle progression and survival. PI3K is responsible for generating phosphoinositol lipids that regulate cell growth, proliferation, survival and cytoskeletal changes. PI3K activation results in pyruvate dehydrogenase kinase 1 (PDK1) activation, which is responsible for phosphorylating AKT family proteins, among other targets, such as large tumor suppressor kinase 1 (LATS1), a key kinase in the Hippo signaling cascade (Fisher et al., 2016; Kim and Gumbiner, 2015). LATS1 phosphorylates and inactivates YAP (Hao et al., 2008), so when LATS1 is inactivated by PDK1, YAP is free to enter the nucleus where it binds to ΔNp63α and stabilizes its expression by preventing its proteasomal degradation (Fisher et al., 2016).

In addition to TG2, neuropilin-1 (NRP-1), a transmembrane protein that acts as a co-receptor for numerous extracellular ligands, forms a complex with the scaffolding protein GAIP C-terminus interacting protein 1 (GIPC1) and α6β4 integrin to activate a downstream kinase cascade that also leads to ΔNp63α stabilization (Grun et al., 2018). NRP-1 interacts with vascular endothelial growth factor A (VEGF-A); this leads to an interaction of VEGF-A with its receptor VEGFR, resulting in enhanced angiogenesis (Klagsbrun et al., 2002). Consistent with this role, NRP-1 is often overexpressed in cancer, where it increases blood vessel formation and tumor growth (Grun et al., 2016; Jimenez-Hernandez et al., 2018; Lin et al., 2018). In squamous cell carcinoma, NRP-1 has been shown to interact with α6β4 integrin and GIPC1 independently of binding to VEGF-A. This interaction results in a suppression of Hippo signaling, relocation of YAP to the nucleus, and ultimately increased ΔNp63α expression (Grun et al., 2018).

Finally, Notch, a key regulator of maintenance, cell fate specification and differentiation, has been shown to regulate p63 in a cell-type-specific manner (Dotto, 2009; Siebel and Lendahl, 2017). In keratinocytes, mammary epithelial and ectodermal progenitor cells, Notch activation reduces p63 expression in a manner dependent on interferon-regulatory factor 3 (IRF3) and IRF7, transcriptional regulators associated with regulation of the type 1 interferon system (Nguyen et al., 2006).

Wnt/β-catenin pathway
Wnt/β-catenin signaling is a highly conserved pathway involved in the regulation of cellular proliferation, differentiation, migration, apoptosis and stem cell renewal. p63 is directly regulated by the Wnt/β-catenin pathway through binding of lymphoid enhancer binding factor 1 along with β-catenin between the TAp63 and ΔNp63 promoters (Ferretti et al., 2011). Additionally, there is a β-catenin-responsive element within the proximal ΔNp63 promoter (Chu et al., 2008). Wnt/β-catenin has also been shown to activate the transcriptional co-factor limb-bud and heart (LBH) (Rieger et al., 2010). LBH increases transcription of ΔNp63α in mammary epithelial cells, while downregulating TAp63α transcription, leading to enhanced replicative potential and stemness (Lindley et al., 2015).

Hedgehog signaling
The Hedgehog signaling pathway is an important, evolutionarily conserved pathway that transmits signals from the cell membrane to the nucleus. Hedgehog is involved in embryonic development and is critical for the formation of organismal polarity, as well as wound healing and maintenance of somatic stem cells and pluripotent cells. An important downstream target of Hedgehog signaling is p63 (Takebe et al., 2015). The hedgehog ligand Indian hedgehog (IHH) activates the transcription factor Gli3, which in the absence of IHH, is in a repressive form denoted Gli3R (Wang et al., 2000). The balance between the levels of Gli3 and Gli3R affects the level of p63; IHH-mediated induction of active Gli3 upregulates TAp63 expression, while reducing the usage of the ΔNp63 promoter. Additionally, shRNA-mediated disruption of Gli3 expression is sufficient to alter p63 promoter usage. This signaling likely acts on the mammary gland in a stage-specific manner, and can initiate elaboration of mammary progenitors and enhance clonogenicity of mammary stem cells to maintain mammary epithelial stasis (Li et al., 2008).

NF-κB
In addition to the above-mentioned role of NF-κB in regulating p63 in response to DNA damage, NF-κB-mediated repression of p63 also has a role in epithelial cell differentiation. Overexpression of the p65 subunit (also known as RelA) in epithelial cells leads to the induction of epithelial-mesenchymal transition (EMT) and reduced p63. This is likely due to NF-κB-mediated enhancement of zinc finger E-box binding homeobox (ZEB1) expression. ZEB1 is a transcription factor critical to the induction of EMT, that represses the ΔNp63 promoter (Chua et al., 2007). Additionally, NF-κB activates the TAp63 promoter, suggesting that NF-κB can induce a shift to TAp63-driven differentiation (Wu et al., 2010).

Major transcriptional targets of p63
p63 has been shown to be critical for many aspects of life. A major reason for this is the multitude of signaling cascades that p63
isoforms are able to regulate. Below we discuss some of these and their cellular effects.

Chromatin-modifying proteins

The polycomb group proteins (PcG) are a family of proteins involved in regulating differentiation via transcriptional repression and can form two major polycomb repressive complexes (PRCs), PRC1 or PRC2. The PcG family member chromobox protein homolog 4 (CBX4) is a transcriptional repressor required for preserving cell cycle dormancy of quiescent stem cells (Luís et al., 2011). Cbx4 is a direct downstream target of p63 and mediates its effects on epidermal cell proliferation, repressing non-epidermal lineage genes during epidermal differentiation via its canonical PRC1 function, as well as by regulating basal keratinocyte proliferation and differentiation via its SUMO E3 ligase activity (Cohen and Ezhkova, 2016; Mardaryev et al., 2016).

Another chromatin-modifying protein that is regulated by p63 is lymphoid-specific helicase (LSH; also known as HELLS) (Keyes et al., 2011), which is implicated in embryonic development and cellular senescence (Muegge, 2005). The LSH promoter contains consensus p63-binding sites, and ΔNp63α has been shown to be the predominant isoform binding the LSH promoter. ΔNp63α-driven Lsh expression is required for senescence bypass, and is induced during tumor initiation in keratinocytes and progression to squamous cell carcinoma (Keyes et al., 2011).

Additionally, p63 directly regulates the expression of the ATP-dependent chromatin remodeler BRG1 (also known as SMARCA4) in epidermal progenitor cells (Botchkarev, 2015). During epidermal morphogenesis, BRG1 binds to the epidermal differentiation complex (EDC), a keratinocyte lineage-specific gene cluster comprising over 50 genes encoding proteins involved in terminal differentiation and cornification of keratinocytes (Mardaryev et al., 2014). BRG1 binds to distinct regions of the EDC and is required for its relocation towards the nuclear interior (Mardaryev et al., 2014); this has a critical role in proper formation of stratified epithelia.

Another key chromatin-modifying protein target of p63 is Satb1. Satb1 is a global chromatin organizer and transcription factor that plays an important role in integrating higher-order chromatin architecture with gene regulation (Ding et al., 2018). Satb1 contributes to epidermal morphogenesis by establishing a tissue-specific chromatin organization and gene expression pattern in epidermal progenitor cells. p63 binds to a proximal regulatory region of the Satb1 gene, and ablation of p63 results in reduced Satb1 expression levels in the epidermis (Fessing et al., 2011). This results in failure to properly activate EDC-associated genes, impairing epidermal morphogenesis (Fessing et al., 2011).

Cell surface proteins

As mentioned above, multiple cell surface markers regulate p63 expression. Many of these markers are in turn transcriptionally activated by p63, creating feedback loops. For instance, ΔNp63α expression increases both the mRNA and protein levels of EGFR, as well as increasing EGFR activity, as measured by determining levels of phospho-EGFR (Y1086) (Holcakova et al., 2017). Furthermore, silencing of ΔNp63α in epithelial cells reduces both the total and phospho-EGFR levels, inhibiting the ability of EGF to activate EGFR (Holcakova et al., 2017). Additionally, direct transcriptional activation of the NRG1 gene—a member of the EGF family—by p63 occurs selectively in epithelial basal cells. Here, activation of NRG1 is required for activation of luminal ERBB4 and/or STAT5A expression, which leads to luminal progenitor cell maturation in breast epithelia (Forster et al., 2014).

Another group of cell surface markers regulated by p63 are integrins. TAp63γ and ΔNp63α induce expression of genes encoding integrins, as shown by their downregulation upon expression of p63-directed shRNAs (Carroll et al., 2006; Yang et al., 2011). In particular, genes encoding integrins α6, β4 and α3 are transcriptionally regulated by ΔNp63α (Carroll et al., 2006). α6β4 integrin is an essential component of hemidesmosomes, which provide stable adhesion to basal epithelial cells and the underlying basement membrane. Additionally, the PERP gene, encoding a key desmosomal protein important for desmosome stabilization, is also transactivated by ΔNp63α (Beaudry et al., 2009). Thus, ΔNp63α-induced expression of these adhesion markers increases cellular adhesion to the exogenous extracellular matrix (ECM) and also confers resistance to anoikis—a form of anchorage-dependent cell death—in breast epithelial cells (Carroll et al., 2006). This makes ΔNp63α critical for maintaining epithelial integrity, adhesion and survival.

Another cell surface marker gene that is a transcriptional target of p63 is the vitamin D receptor (VDR). ΔNp63α and TAp63γ can bind the VDR promoter, and silencing of p63 reduces VDR expression (Kommagani et al., 2006). Furthermore, a naturally occurring p63 missense mutant of TAp63γ (R279H) acts in a dominant-negative manner to inhibit TAp63γ-mediated upregulation of VDR (Kommagani et al., 2006).

Finally, ΔNp63α isoforms also induce the expression of the gene encoding the cell surface antigen CD44, which defines cancer stem cells in breast, colorectal and squamous cell carcinomas (Wang et al., 2018). Regulation of CD44 abundance by ΔNp63 could be achieved either directly or through indirect activation of the Hedgehog signaling pathway (Compagnone et al., 2017; Di Franco et al., 2016; Gatti et al., 2018). By sustaining the production of CD44, ΔNp63 contributes to the maintenance of cancer stem cell self-renewal (Gatti et al., 2018).

Proteins involved in cell metabolism

p63 isoforms are important regulators of energy metabolism. This is particularly true in squamous cell carcinomas, where the major glucose transporter GLUT1 is highly overexpressed (Hsieh et al., 2019). This high GLUT1 expression is driven by cooperation between ΔNp63 and SOX2. However, the precise mechanism in which ΔNp63 and SOX2 interact to transactivate GLUT1 is yet to be elucidated (Hsieh et al., 2019). Additionally, hexokinase 2 (HK2) is a direct ΔNp63α target gene in human keratinocytes. HK2 regulates mitochondrial ROS generation and catalyzes the first step of glycolysis, producing glucose-6-phosphate (Viticchie et al., 2015). Furthermore, ΔNp63α transcriptionally regulates glutathione biogenesis, utilization and regeneration, and The Cancer Genome Atlas (TCGA) database shows that p63 amplification or overexpression upregulates the glutathione metabolism pathway in tumors (Wang et al., 2017).

Proteins involved in regulation of muscle cells

TAp63 isoforms play important roles in regulating skeletal and cardiac muscle. During myoblast differentiation, TAp63γ regulates numerous proteins involved in skeletal muscle contractility, including the components of the dystrophin-glycoprotein complex (Cav3), titin complex (Myh1, Myh2, Neb, Tnn2 and Tnn), fast-twitch muscle fibers (Atp2a2, Myh1 and Myh2) and slow-twitch muscle fibers (Mb, Myh1 and Tnn1) (Cefalú et al., 2015). These genes are critical to basic muscle function and growth. In addition to skeletal muscle, TAp63 is also important in cardiomyocytes (Rouleau et al., 2011). GATA transcription factors are a family of zinc finger proteins that bind the consensus DNA sequence GATA
(Romano and Miccio, 2020). GATA4 and GATA6 are required for cardiogenesis, and in embryonic stem cells, TAp63 regulates GATA4 and GATA6. p63-null mice display defects in cardiac development including, alterations in cardiac gene expression, myofibrillogenesis and beating activity, which can be rescued with GATA6 expression in TAp63-deficient cells (Rouleau et al., 2011).

\[ \text{IKK}\alpha \]

The NF-κB family of transcription factors is a key regulator of immune responses, inflammation and cancer. NF-κB is quickly activated in response to various stimuli, allowing the rapid activation of target genes that encode cytokines, membrane proteins and transcription factors (Mitchell et al., 2016). Inhibitor of NF-κB kinase subunit α (IKKα) functions in the activation of NF-κB in response to a subset of tumor necrosis factor (TNF) family members. Moreover, IKKα is involved in keratinocyte differentiation, but this function is independent of its kinase activity (Israel, 2010). TAp63 is capable of driving the expression of the IKKα-encoding gene (CHUK) both directly and indirectly (Candi et al., 2006). Direct regulation of CHUK by TAp63 is through one of the three p53-consensus motifs within the CHUK promoter. In addition, TAp63 also regulates CHUK through the induction of ETS1, and GATA3, which subsequently induce CHUK expression (Candi et al., 2006).

\[ \text{p21} \]

p21 (also known as CDKN1A) is a cyclin-dependent kinase (CDK) inhibitor that has an important role in cell cycle progression. p21 can arrest cell cycle progression in G1/S and G2/M transitions by inhibiting the CDK4–CDK6–cyclin-D and CDK2–cyclin-E complexes (Karimian et al., 2016). All isoforms of TAp63 transactivate the p21-encoding gene, with TAp63β showing the highest levels of p21 induction (Helton et al., 2008). The transcriptional activation of p21 by TAp63 requires both the AD and proline-rich domains of TAp63. Specifically, the PXXP motif of the proline-rich domain at residues 124–127 are critical (Helton et al., 2008). Therefore, TAp63 plays a critical role in regulating senescence and proliferation via regulation of p21 (Guo et al., 2009).

\[ \text{Hedgehog signaling} \]

Much like integrin signaling, the Hedgehog pathway is involved in the regulation of p63, and, in turn, is also regulated by p63 itself. p63-mediated regulation of Hedgehog signaling contributes to stem cell maintenance through the ability of p63 to bind to the promoters of several genes encoding Hedgehog signaling pathway components (Chari et al., 2013; Melino et al., 2015; Sari et al., 2018). For instance, Ihh is directly transcriptionally regulated, either positively or negatively by TAp63 and ΔNp63, respectively, and Ihh is a driver of mammary progenitor maturation (Sari et al., 2018). p63 also transactivates sonic hedgehog (SHH) and Gli2 in mammary cancer progenitor cells by directly binding to their promoters (Memmi et al., 2015). These components of the Hedgehog signaling pathway are thus important mediators of the p63-driven stem cell phenotype.

\[ \text{Wnt} \]

The canonical Wnt cascade has emerged as another critical regulator of p63, particularly in epithelial stem cells. In the absence of a Wnt ligand, β-catenin is constitutively phosphorylated, and degraded by the proteasome (Zhan et al., 2017). When Wnt ligands are present, a member of the Frizzled (FZD) receptor family and its co-receptor low-density-lipoprotein-related protein (LRP) are engaged, inhibiting β-catenin phosphorylation (Zhan et al., 2017). This results in β-catenin accumulation in the nucleus, where it interacts with the LEF/TCF family of DNA-binding proteins to bind to its target promoters (Zhan et al., 2017). In particular, the FZD7 receptor and WNT5B ligand are under the direct transcriptional control of ΔNp63 (Chakrabarti et al., 2014). ΔNp63 depletion in mammary progenitors results in impaired self-renewal, and FZD7 expression can rescue the impaired self-renewal of both normal and malignant mammary progenitors following ΔNp63 depletion, suggesting that FZD7 is a key downstream regulator of ΔNp63-driven stemness (Chakrabarti et al., 2014).

\[ \text{Notch} \]

The Notch signaling pathway is induced in response to the engagement of Notch receptors (Notch1–4) by membrane-bound Delta or Jagged ligands, leading to Notch cleavage and release of its intracellular domain (NICD) into the cytoplasm (Siebel and Lendahl, 2017). NICD then translocates into the nucleus, where it converts the DNA-binding protein CBF1 from a repressor into a transcriptional activator (Siebel and Lendahl, 2017).

ΔNp63 affects Notch signaling by regulating the expression of multiple genes encoding Notch pathway components, including Notch1, Notch3, Jag1, Jag2 and Hes1 (Kent et al., 2011; Mezzomo et al., 2017; Ravindran and Devaraj, 2012; Yalcin-Ozuysal et al., 2010). ΔNp63α is directly involved in transactivation of Notch by binding to the Notch1 promoter; this increases mRNA and protein levels of Notch1 and leads to increased accumulation of the Notch1 intracellular domain. This in turn induces the expansion of breast cancer stem cell subpopulations (Du et al., 2010). In the mammary gland, Notch inhibits ΔNp63α to induce luminal commitment. However, ΔNp63α maintains mammary cell fate by counteracting Notch activation (Balboni et al., 2013).

Additionally, reciprocal antagonistic interactions between ΔNp63α and Notch regulate epithelial cells (Koh et al., 2015; Nguyen et al., 2006; Tadeu and Horsley, 2013). In the basal layer of the epidermis, opposing gradients of ΔNp63α and Notch activation govern the balance between self-renewing and transit-amplifying cells (Senoo, 2013). Therefore, the interplay between p63 and Notch are critical to the regulation of stem cell quiescence and progenitor commitment (Melino et al., 2015).

\[ \text{BMP} \]

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor β (TGF-β) superfamily of cytokines. BMP ligands activate signaling by forming complexes with receptor kinases at the cell surface. Typically, BMP signaling induces cellular quiescence and maintenance of embryonic and adult stem cells (Wu et al., 2016). In the mammary epithelium, ΔNp63α activates canonical BMP signaling by inducing the expression of Bmp7, which induces the stem cell phenotype of mammary progenitors (Balboni et al., 2013).

\[ \text{p63 in disease} \]

p63 is involved in a number of disease syndromes. Germline mutations of p63 are found in humans and cause six rare autosomal dominant developmental diseases (see Box 2) (Soares and Zhou, 2018). In addition to mutations in p63 that cause disease, changes in p63 expression are associated with numerous cancers. p63 was initially hypothesized to be a tumor suppressor based on its homology to p53. However, mutation of the p63 gene in cancer is rare, indicating that p63 is not a classical tumor suppressor such as p53 (Gonfloni et al., 2015). Furthermore, spontaneous tumors are
Box 2. Germline mutations of p63

Germline mutations are genetic variations within germ cells, resulting in the mutation being passed on to offspring. Below are known diseases that result from various mutations occurring in the p63 gene within germ cells.

Ectodactyly ectodermal dysplasia–clefiting

Ectodactyly ectodermal dysplasia–clefiting (EEC) syndrome has three major features: ectodactyly (a split or cleft anomaly of the hands and feet), ectodermal dysplasia (developmental defects of hair, teeth, nails, and sweat glands) and clefiting (cleft lip with or without cleft palate). Mutations in codons 204, 227, 279, 280, and 304 of p63 lead to amino acid substitutions and are present in ~75% of EEC patients. These mutations affect the p63 DNA-binding domain (Rinne et al., 2007; van Bokhoven and Brunner, 2002).

Acro-dermato-ungual-lacrimal-tooth malformations

Acro-dermato-ungual-lacrimal-tooth malformations (ADULT) syndrome differs from EEC syndrome owing to the absence of facial clefiting (Brunner et al., 2002a,b).

Limb–mammary syndrome

Limb–mammary syndrome (LMS) consists of split-hand and/or foot malformation, mammary gland and nipple hypoplasia and isolated cleft palate (Rinne et al., 2007; van Bokhoven and Brunner, 2002).

Ankyloblepharon-ectodermal dysplasia-cleft lip/palate

Ankyloblepharon-ectodermal dysplasia-cleft lip/palate (AECD) syndrome consists of partial or complete fusion of eyelids and ectodermal dysplasia-clefiting (Zhang et al., 2019).

Split-hand/foot malformations

Split-hand/foot malformations (SHFM) patients have median clefts of the hands and feet but no other features of EEC syndrome (Ianakiev et al., 2000).

Rapp–Hodgkin syndrome

Rapp–Hodgkin syndrome (RHS) patients suffer from ectodermal dysplasia, cleft lip and palate, deformed ears and genitourinary abnormalities (Holder-Espinasse et al., 2007).

Primary ovarian insufficiency

Primary ovarian insufficiency (POI) patients experience infertility associated with early arrest of menstruation and high follicle stimulating hormone (FSH) before the age of 40 (Veitia, 2020).

Extremely rare in mouse models heterozygous for null p63 alleles (Gonfloni et al., 2015; van Bokhoven et al., 2011; Keyes et al., 2006). However, the human p63 gene maps to chromosome 3q27–28, a region frequently amplified in squamous cell carcinomas and some adenocarcinomas, with copy number of the p63 locus and p63 protein levels being significantly increased in 88% of squamous carcinomas, and in 42% of large cell carcinomas and adenocarcinomas of lung (Massignon et al., 2003). The predominant isoform expressed is ΔNp63α (Botchkarev and Flores, 2014). ΔNp63α expression correlates with a poor response to cisplatin in multiple cancers. This is likely due to the number of pro-survival pathways regulated by ΔNp63α, allowing it to act as an oncogene (Botchkarev and Flores, 2014; Moll and Slade, 2004; Gonfloni et al., 2015). In contrast to ΔNp63α, exogenous expression of TAp63 isoforms, can induce cellular senescence in cultured cells and inhibit tumor formation and metastasis upon xenograft implantation in nude mice, suggesting that TAp63 is a tumor suppressor (Su et al., 2013; Guo et al., 2009).

Conclusions

The p63 gene encodes multiple protein isoforms that serve a vast array of functions encompassing nearly all aspects of cellular signaling. Genetic studies in mice have shed much light on the roles of TAp63 versus ΔNp63 isoforms and their various functions. However, the roles of p63 in multiple aspects of cancer, including tumorigenesis, cancer progression, and metastasis as well as how they impact other diseases are still being discovered. Thus, a better understanding of the functions and interactions of the different p63 isoforms will undoubtedly prove beneficial to the design of effective therapies for cancer and beyond.

Competing interests

The authors declare no competing or financial interests.

Funding

This review was made possible through the support of the Cold Spring Harbor Laboratory and Northwell Health Affiliation, the Office of the Director, National Institutes of Health under award numbers R01CA190997 and R21OD018332 (to A.A.M.) and 5F32CA225134 (to M.L.F.), and the Cancer Center Support Grant 5P30CA045508 and 1F31CA247400 (to S.B). Deposited in PMC for release after 12 months.

Cell science at a glance

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