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REVIEW

Tissue-specific roles of p73 in development and homeostasis

Alice Nemajerova* and Ute M. Moll*

ABSTRACT

p73 (TP73) belongs to the p53 family of transcription factors. Its gene locus encodes two opposing types of isoforms, the transcriptionally active TAp73 class and the dominant-negative DNp73 class, which both play critical roles in development and homeostasis in an astonishingly diverse array of biological systems within specific tissues. While p73 has functions in cancer, this Review focuses on the non-oncogenic activities of p73. In the central and peripheral nervous system, both isoforms cooperate in complex ways to regulate neural stem cell survival, self-renewal and terminal differentiation. In airways, oviduct and to a lesser extent in brain ependyma, TAp73 is the master transcriptional regulator of multiciliogenesis, enabling fluid and germ cell transport across tissue surfaces. In male and female reproduction, TAp73 regulates gene networks that control cell–cell adhesion programs within germinal epithelium to enable germ cell maturation. Finally, p73 participates in the control of angiogenesis in development and cancer. While many open questions remain, we discuss here key findings that provide insight into the complex functions of this gene at the organismal, cellular and molecular level.

KEY WORDS: p73, Trp73, TP73

Introduction

p73 belongs to the p53 family of transcription factors (Kaghad et al., 1997). The *TP73* locus (*Trp73* in mice) encodes two classes of isoforms (Fig. 1). The P1 promoter yields TAp73 variants that contain the N-terminal transactivation (TA) domain (Yang et al., 2000). The P2 promoter yields N-terminally truncated variants (deltaNp73, DNp73) that act as dominant-negative inhibitors of p53 (*TP53*), TAp63 (*TP63*) and TAp73 via hetero-oligomerization and promoter competition. Both p73 types can undergo additional C-terminal exon splicing, generating β, γ and δ variants. TAp73 and DNp73 are typically co-expressed at a skewed proportion in a tissue-specific manner (Grespi et al., 2012).

In contrast to p53, p73 is almost never mutated in cancer. Although significantly weaker than p53, TAp73 is a tumor suppressor that acts in part through induction of cell cycle arrest and apoptosis and through regulation of genomic stability (Alexandrova and Moll, 2012; Nemajerova et al., 2009, 2010; Talos et al., 2007). Aged TAp73-deficient mice develop spontaneous lymphoma and lung cancer with increased frequency and have enhanced sensitivity to chemical carcinogenesis (Amelio et al., 2015; Stantic et al., 2015; Tomasini et al., 2008). In response to severe DNA damage, TAp73, like p53, elicits cell death or senescence. Conversely, the anti-apoptotic DNp73 can promote cancer. DNp73 is overexpressed in several human tumors (Deyoung and Ellisen, 2007; Moll and Slade, 2004; Zaika et al., 2002), and overexpression correlates with poor prognosis and advanced stage in breast and colon cancer (Buhlmann and Putzer, 2008; Domínguez et al., 2006). Human small cell lung cancer is characterized by recurrent somatic rearrangements in *TP73*, creating an N-terminally truncated oncogenic version (George et al., 2015).

Mice with global p73 knockout (p73KO) that miss both isoform classes exhibit a mysterious and diverse non-cancerous phenotype that led the way towards uncovering an astonishing breadth of cellular and molecular functions of the isoforms, especially for TAp73 and its context-dependent transcriptional programs (Fig. 1). This Review discusses the gamut of physiological roles of p73 in specific tissues such as brain, ependyma, airways, ovarian follicles, testis, vasculature, and in metabolism.

Roles of p73 isoforms in the central and peripheral nervous system

Global p73KO mice, but not isoform-specific TAp73KO or DNp73KO mice, exhibit severe defects in CNS neurogenesis, highlighting the fundamental role of p73 in brain development and homeostasis. p73KO mice show cortical and olfactory bulb hypoplasia with cortical thinning and severe progressive *ex vacuo* hydrocephalus (100% penetrance), hippocampal dysgenesis and pheromone sensory defects (Meyer et al., 2004; Pozniak et al., 2002; Yang et al., 2000). The infrapyramidal blade of the hippocampal dentate gyrus (DG) is missing or truncated.

In embryonic day 18 (E18) mouse brains, p73α mRNA is highly expressed in the marginal zone, cortical hem, hippocampus, hypothalamus and thalamic eminence, choroid plexus and vomeronasal organ (Yang et al., 2000). TAp73 and DNp73 isoforms overlap in anatomic distribution except in the marginal zone, which expresses only DNp73 (Yang et al., 2000). However, controversy remains regarding which of the two isoforms dominates in the developing and adult brain.

TAp73KO mice (but not DNp73KO mice) exhibit the same hippocampal dysgenesis as global p73KO mice, but lack a forebrain phenotype (normal cortex and ventricles) (Fujitani et al., 2010; Tomasini et al., 2008). This is surprising, given that TAp73 is expressed and active not only in hippocampal DG, but also in cortical subventricular zone (SVZ) neurogenesis (Fujitani et al., 2010). Conversely, the severe cortex phenotype of global p73KO mice, which is not phenocopied by either the DNp73KO or TAp73KO mice, strongly suggests that both isoforms cooperate in the forebrain, but not in the hippocampus where only TAp73 is critical.

Marginal zone Cajal–Retzius neurons (CR), which cover the developing neocortex and extend to the molecular layer of the dentate gyrus, are believed to control cortical neurogenesis. CRs strongly co-express DNp73, reelin (a secreted glycoprotein essential for neuronal migration) and calretinin (another CR marker) (Yang et al., 2000). The cortical hem in the dorsomedial telencephalon, which is the embryonic hippocampal organizer and regulates

Department of Pathology, School of Medicine, Stony Brook University, Stony Brook, NY 11794-8691, USA.

*Authors for correspondence (alice.nemajerova@stonybrook.edu; ute.moll@stonybrook.edu)

A.N., 0000-0002-2235-0613; U.M.M., 0000-0003-1908-7516

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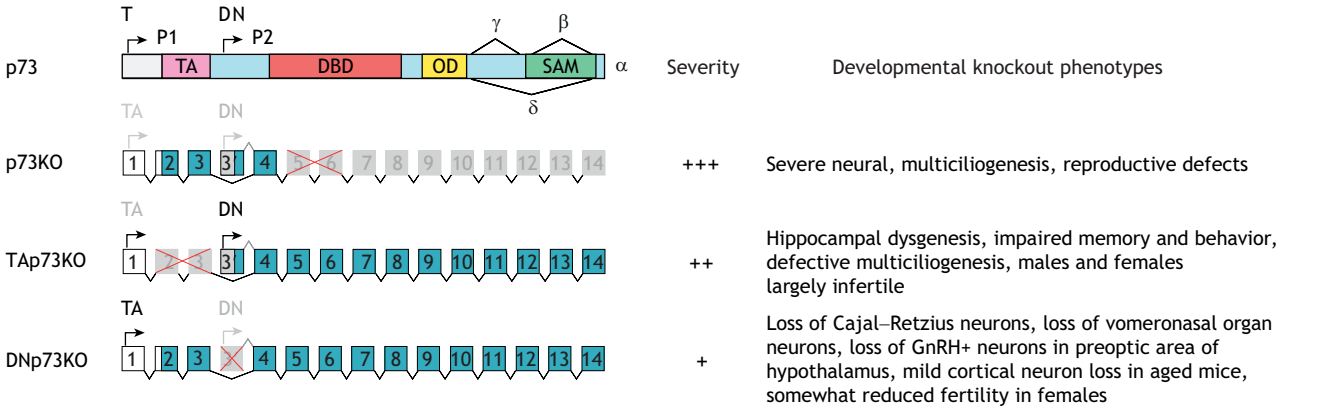

Fig. 1. The p73 gene and its knockout phenotypes. The p73 locus encodes two classes of isoforms. The P1 promoter yields TAp73 variants containing the transactivation (TA) domain. The P2 promoter yields N-terminally truncated variants (deltaN, DNp73). DNA-binding domain (DBD), oligomerization domain (OD) and sterile alpha motif domain (SAM) are shared. C-terminal splice variants generate alternate β, γ and δ isoforms of the TA and DN classes. Global (p73KO) and isoform-specific knockout mice (TAp73KO and DNp73KO) were generated by deleting the indicated exons. They exhibit distinct developmental phenotypes that also differ in severity as discussed in the main text.

neocortex size and patterning (Caronia-Brown et al., 2014), also contains many cells that are positive for DNp73 and reelin (Meyer et al., 2002). Loss of p73 in p73KO postnatal day 3 (P3) brains leads to loss of CR neurons in the cortical marginal zone and hippocampal molecular layer. In contrast, other sites of reelin expression (cerebellum, cortical layer V and olfactory bulb) are devoid of p73 expression and appear normal in p73KO brains (Yang et al., 2000). Thus, DNp73 appears to induce reelin and calretinin specifically in marginal zone CRs.

p73 regulation of neural stem cell survival, self-renewal and differentiation in neurogenesis

Neurogenesis is the process by which newborn neurons are created from neural stem cells (NSC). In mouse development, this starts around E11 and is completed at birth, but continues in two select regions of the adult CNS, the subgranular zone (SGZ) of the hippocampal DG and the SVZ of the forebrain. Cortical hypoplasia, hydrocephalus and hippocampal dysgenesis in p73KO mice are already present at birth and correlate with prenatal proliferative defects in SVZ neurogenesis *in vivo* (Talos et al., 2010). In embryonic brains, both TAp73 and DNp73 are expressed (Yang et al., 2000), although TAp73 is predominant (Agostini et al., 2010; Gonzalez-Cano et al., 2010; Talos et al., 2010). In both embryonic and adult neurogenesis, p73 is required for maintaining the neurogenic pool by promoting self-renewal and proliferation and inhibiting premature senescence of neural stem and/or progenitor cells. This was shown by neurosphere assays that compared p73KO and wild-type (WT) NSCs from E14 and P0 whole brain, SVZ and SGZ (Talos et al., 2010), and by reduced thickness of nestin-positive SVZ at E17.5 *in vivo* (Agostini et al., 2010). This p73 function is independent of p53 as shown by p73p53 double KO NSCs (Gonzalez-Cano et al., 2010). Notably, p73KO NSCs show premature exhaustion and depletion due to S-phase defects and increased senescence (but not increased apoptosis) (Talos et al., 2010), which is associated with severe downregulation of Sox2, Sox3, Notch and Notch signaling pathway components that are critical for NSC maintenance (Agostini et al., 2010; Talos et al., 2010). In support of a p73–Sox2 axis, brain-specific Sox2KO mice closely phenocopy p73KO mice, in that they also exhibit cortical hypoplasia, hydrocephalus and progressive loss of the lower DG blade, and neurospheres undergoing reduction in NSC numbers (Favaro et al., 2009). p73 is also required for the generation of mature neurons. Although p73KO NSCs from E14 and P0 mice are multipotent and differentiate (albeit prematurely) into neurons, astrocytes and oligodendrocytes, the derived neurons and oligodendrocytes show defects in number and quality (while astrocytes appear unaltered) (Gonzalez-Cano et al., 2010; Talos et al., 2010) (Fig. 2). Is p73 also required during commitment from neuroectoderm to neural stem cell fate? The answer is no, as p73 is dispensable for fate commitment since induced pluripotent stem cells (iPSC) from p73KO mice are able to differentiate normally into NSCs. However, p73 is essential for NSC maintenance and for blocking premature differentiation (Alexandrova et al., 2013).

In adult neurogenesis, loss of p73 also severely impairs proliferation and maintenance of NSC and progenitor cells. This was shown in the neurogenic compartment of the hippocampal DG by BrdU-labeled traceable p73KO;nestin-GFP mice (Talos et al., 2010) or nestin plus GFAP staining in p73KO brains (Agostini et al., 2010). Similar proliferative and maintenance defects are seen in adult olfactory neurogenesis from SVZ precursors of the forebrain as well as in hippocampal neurogenesis in TAp73KO mice (Agostini et al., 2010; Fujitani et al., 2010). TAp73 promotes the long-term maintenance of NSCs through its transcriptional target Hey2, which promotes neural precursor maintenance by preventing their premature differentiation (Fujitani et al., 2010).

TAp73 promotes terminal neuronal differentiation

TAp73 promotes terminal neuronal differentiation through its downstream targets p75 neurotrophin receptor ($p75^{NTR}$, also known as Ngfr) and microRNA miR34a (Agostini et al., 2011a; Niklison-Chirou et al., 2013) (Fig. 2). Neural progenitors exit from the cell cycle to become immature postmitotic neurons, which then undergo terminal differentiation, including migration, axonal and dendritic outgrowth and branching, synapse formation and connectivity to the existing neuronal circuitry. Extrinsic signaling molecules such as neurotrophins and nerve growth factor (NGF) are critical for axonal growth and dendritic arborization in cortical neurons (Chao and Bothwell, 2002). $p75^{NTR}$ transmits signals from neurotrophins and NGF in processes that range from promoting central neurite outgrowth, peripheral sensory axonal outgrowth and neuronal survival (Chen et al., 2009). Accordingly, $p75^{NTR}$-deficient mice largely phenocopy the hippocampal defect of

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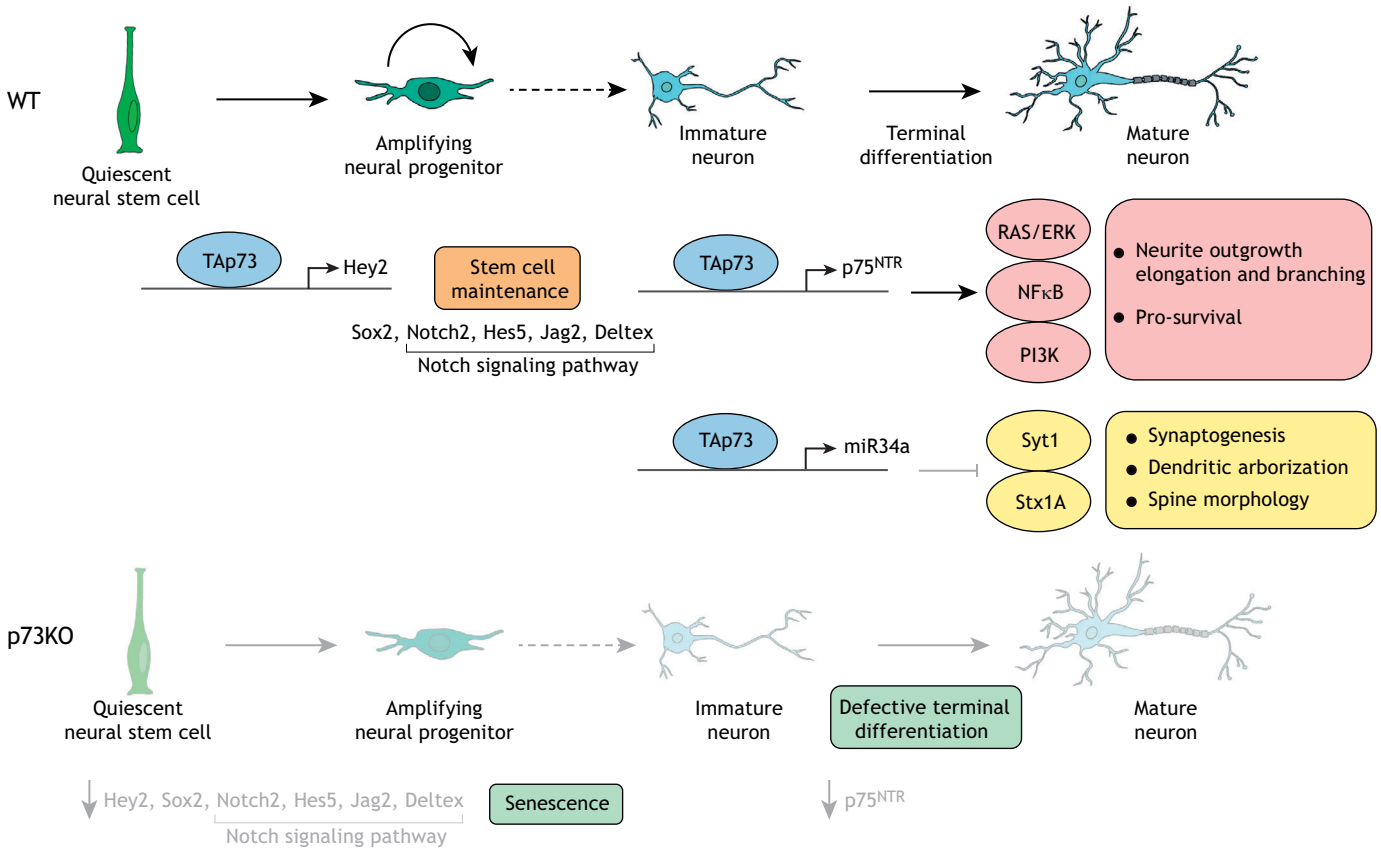

Fig. 2. TAp73 plays multiple critical roles in the development of neuronal cells. TAp73 is required for embryonic and adult neural stem cell (NSC) maintenance through its role in the induction of Sox and Notch pathways. In particular, Hey2 is a direct transcriptional target of TAp73 (top). TAp73 also regulates terminal neuronal differentiation through direct induction of $p75^{NTR}$ and miR34a, which in turn signal via RAS–NFκB–PI3K and Syt1–Stx1A pathways, respectively. Loss of TAp73 results in marked reduction of NSCs (bottom). Furthermore, differentiation of mutant NSCs into immature neurons and oligodendrocytes is defective.

TAp73KO mice (Catts et al., 2008; Colditz et al., 2010). $p75^{NTR}$ is a direct transcriptional target of TAp73 and its downregulation contributes to the neurological abnormalities associated with TAp73 loss. TAp73KO mice show strongly reduced levels of $p75^{NTR}$ in cortex and hippocampus, accompanied by behavioral and electrophysiological abnormalities. Also, the peripheral nervous system of TAp73KO mice shows a thermal sensitivity defect due to decreased sensory nerve innervation (Niklison-Chirou et al., 2013). Moreover, in response to NGF, TAp73KO cortical neurons show reduced dendritic arborization and network connectivity *in vitro* (Niklison-Chirou et al., 2013).

Neuronal differentiation by TAp73 is also mediated through regulation of synaptogenic protein targets via miR34a. TAp73 directly drives miR34a expression and, accordingly, miR34a levels are reduced by 50–60% in p73KO cortex and hippocampus (Agostini et al., 2011a). miR34a, which is highly expressed in brain, modulates the expression of synaptotagmin-1 (Syt1) and syntaxin-1A (Stx1A) via seed sequences within their 3′UTRs. This was shown by miR34a overexpression or inhibition in primary neuronal cultures, leading to reduced or increased neurite arborization, respectively (Agostini et al., 2011b).

Pro-survival role of DNp73 in discrete neuron types

DNp73 promotes survival in discrete neuronal populations *in vivo*. Embryonic and young DNp73KO mice show increased apoptosis of neurons in select regions comprising the preoptic area, vomeronasal neurons, GnRH-positive cells and Cajal–Retzius neurons (Tissir et al., 2009). In addition, the ependymal cell-covered choroid plexus is atrophic. However, overall DNp73KO mice exhibit a surprisingly subtle and discrete CNS phenotype. They are healthy and do not exhibit hydrocephalus, cortical loss or neurological abnormalities (Tissir et al., 2009). Older DNp73KO mice from an independent second strain show a similarly subtle phenotype with only mild neuronal loss by 10 months of age and mild cortical thinning by 26 months, again not showing hydrocephalus (Wilhelm et al., 2010). The fact that the cell-death phenotype is only mild might suggest that DNp73 is not as important for neuronal survival *in vivo* as inferred by earlier studies *in vitro* where adenoviral delivery of excess DNp73α and DNp73β rescued mature cortical and sympathetic neurons from apoptosis that was induced by NGF withdrawal or p53 overexpression (Pozniak et al., 2000, 2002).

Dual role of p73 in ependymal cells

The brain ventricular system is composed of four interconnected cavities where cerebrospinal fluid (CSF) is produced by modified ependymal cells (ECs) covering the choroid plexus (CP) and reabsorbed by ECs lining the lateral ventricular walls. p73, in particular TAp73, plays an important role in EC maturation, ciliogenesis and planar cell polarity (Hernandez-Acosta et al., 2011; Medina-Bolívar et al., 2014; Yang et al., 2000). ECs are multiciliated with cilia asymmetrically limited to the anterior part of the apical surface, beating synchronously for directional CSF

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flow (Ohata and Alvarez-Buylla, 2016). ECs also display a rotational and translational planar cell polarity (PCP) that coordinates cilia beating (Mirzadeh et al., 2010). Notably, ECs are also important for maintaining the neurogenic niche of the postnatal SVZ. ECs are formed perinatally from radial glial cells that transform into central stem cells (B-cells), which give rise to surrounding ECs. These structures are neuro-regenerative units (so-called pinwheels; see Fig. 3) on the lateral surface of the ventricles (Mirzadeh et al., 2008).

p73KO ECs show defects in maturation and ciliogenesis. They form impaired pinwheel structures that affect the neurogenic SVZ capacity and have defective PCP, resulting in basal-body anchoring defects (Fig. 3). Thus, p73 is required for ependymal maturation and neurogenic SVZ cytoarchitecture (Fujitani et al., 2017; Gonzalez-Cano et al., 2016). TAp73KO ECs exhibit the same PCP and basal-body anchoring defects, identifying TAp73 as the regulator of PCP (Fuertes-Alvarez et al., 2018). Mechanistically, TAp73 regulates translational PCP (asymmetrical polarization of cilia clusters at the anterior apical surface) by directly inducing transcription of Mlck kinase (also known as Mylk), which modulates non-muscle myosin-II activity, thereby affecting the dynamics of actin and microtubule-based transport and Golgi organization. TAp73 is also required for the asymmetric membrane localization of PCP core proteins Vangl2, Prickle2 and Fzd3 (Fuertes-Alvarez et al., 2018). Interestingly, ciliary axonemes in TAp73KO ECs appear less disheveled than in p73KO counterparts. The latter suggests a compensatory role for DNp73 in TAp73KO ependymal cells, even though DNp73KO brains have normal ECs and lack PCP defects (Fuertes-Alvarez et al., 2018). But does the ciliogenic defect of p73KO ECs causally contribute to the severe hydrocephalus of p73KO brains? The answer is still out, because no direct CSF flow and pressure measurements exist.

In sum, p73 plays a complex, fundamental role in brain development and homeostasis. In embryonic and adult neurogenesis, p73 is required for maintaining the neurogenic pool by promoting self-renewal and proliferation, and by inhibiting premature senescence of NSCs or their progenitors. Mechanistically, TAp73 induces the Notch and Sox2 pathways. TAp73 also promotes terminal neuronal differentiation via its direct downstream targets $p75^{NTR}$ and miR34a. DNp73 has a pro-survival role in discrete neuron types including Cajal–Retzius neurons and the choroid plexus. In ECs, TAp73 has a dual role by controlling ciliogenesis and planar cell polarity.

TAp73: master regulator of multiciliogenesis

In addition to the defects described above, p73KO mice also exhibit striking airway infections, female and male infertility, and runting. TAp73 was found to function as a master transcriptional regulator governing motile multiciliogenesis, as the common unifying mechanism underlying these disparate p73KO phenotypes (Fujitani et al., 2017; Gonzalez-Cano et al., 2016; Marshall et al., 2016; Nemajerova et al., 2016).

Cilia are microtubule-based surface organelles with essential functions. Most vertebrate cells form a single, immotile primary cilium that signals mechano-chemical stimuli via the Hedgehog pathway (Bangs and Anderson, 2017). However, distinct epithelia – those lining airways, brain ependyma, the female oviduct and the testicular efferent duct – undergo multiciliogenesis by amplifying their centrioles to nucleate hundreds of motile cilia per cell that beat vigorously and synchronously in a whip-like motion to generate directional fluid flow across tissue surfaces and move (viscous) fluids. Thus, multiciliated cells (MCCs) are vital for respiration, neurogenesis and fertility. The motile cilium, composed of an axonemal 9+2 microtubular shaft and anchored by a basal body at the apical membrane, is a highly complex nanomachine with over 600 different proteins required for assembly, structure and function (Brooks and Wallingford, 2014). Dysfunctional ciliogenesis underlies diverse human diseases, including hydrocephalus, primary ciliary dyskinesia, Bardet–Biedl syndrome, asthma, chronic obstructive pulmonary disease (COPD), anosmia and sterility (Choksi et al., 2014; Nigg and Raff, 2009).

MCCs are critical for the mucosal barrier and for constantly cleaning inhaled pollutants, pathogens, allergens and dust from the

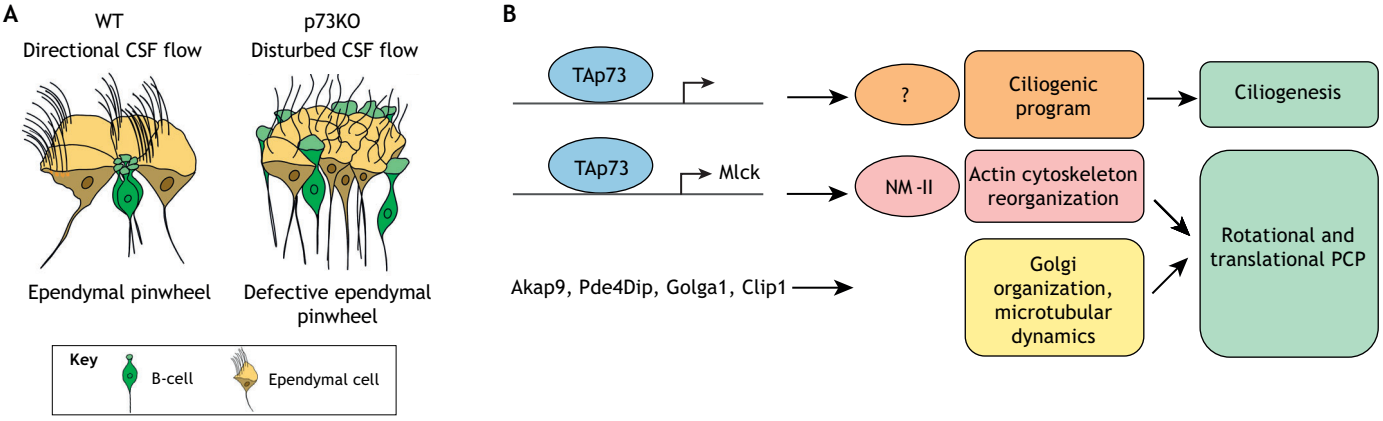

Fig. 3. p73 regulates ependymal cell development, ciliogenesis and planar cell polarity. (A) Wild-type (WT) ependymal cells (ECs) form perinatally from GFAP-positive radial glial cells, which transform into a central stem cell (B-cell, remaining GFAP-positive) and surrounding, multi- and bi-ciliated ECs (becoming GFAP-negative). Together they form neuro-regenerative units along the ventricular walls, called pinwheels. p73-deficient ECs fail to organize into pinwheels, thereby disrupting subventricular zone niche architecture and function, and exhibiting defective ciliogenesis with disturbed cerebrospinal fluid (CSF) flow. Wild-type cells have cilia asymmetrically distributed at the anterior apical surface, and all cilia are polarized with the same orientation. p73KO cells have lost their polarization, with basal bodies distributed throughout the apical surface. (B) Other as-yet-undescribed direct TAp73 targets in ciliogenesis might also play a role in this process (indicated by the question mark). p73 is also essential for the polarized junctional assembly of PCP core proteins and the asymmetric localization of their downstream signaling effectors. Mechanistically, TAp73 regulates translational PCP (asymmetrical polarization of cilia clusters at the anterior apical surface) and actin dynamics through the direct transcriptional induction of myosin light-chain kinase (Mlck), which modulates non-muscle myosin-II (NM-II) activity, thereby affecting actin and microtubular transport dynamics, as well as Golgi organization.

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airways (conveyor-belt-like mucociliary clearance) (Fliegauf et al., 2007; Hogan et al., 2014). p73KO and TAp73KO mice show severe respiratory symptoms due to cilia dysfunction, causing severe chronic respiratory tract infections, pneumonia and secondary emphysema resembling COPD (Nemajerova et al., 2016). Our lab found that wild-type MCCs, which strongly express nuclear p73, have long, lush cilia, whereas p73KO and TAp73KO MCCs exhibit a severe reduction in cilia number and length with complete loss of mucociliary transport (Nemajerova et al., 2016). The early ciliogenic stages I and II (undergoing centriolar amplification) are only mildly affected, but marked defects progressively worsen in later stages III and IV (basal body docking and axonemal extension). p73KO basal bodies, while relatively intact in numbers, show severe docking defects and fail to properly align at the apical membrane, and therefore are unable to extend axonemes. In support, we find that TAp73 itself is induced by Mcidas (also known as Multicilin), a transcriptional coregulator of E2F4 required for triggering MCC differentiation. We conclude that p73 mainly functions after MCC fate specification to orchestrate basal-body docking, axonemal extension and motility (Nemajerova et al., 2016).

Mechanistically, genomic analysis together with the complete ciliary rescue of p73KO MCCs by lentiviral TAp73 in organotypic airway cultures identify TAp73 as the evolutionarily conserved key transcriptional integrator of multiciliogenesis. TAp73 directly binds to and activates promoter and/or enhancer regions of the essential regulators FoxJ1, Rfx2, Rfx3 and miR34bc, plus nearly 50 structural and functional ciliary genes, eight of which are associated with human primary ciliary dyskinesia (Fig. 4) (Nemajerova et al., 2016).

These key findings were confirmed in an independent p73KO mouse strain (Marshall et al., 2016). Here, RNAseq and ChIPseq analyses demonstrated that p73 binds to 105 cilia-associated genes, including Foxj1. However, in addition to a role in later MCC differentiation, this study also proposes p73 to have a role in MCC fate specification in a subset of basal cells that they found to co-express p73 and p63 (Marshall et al., 2016).

Based on these findings, it is clear that TAp73 occupies a position at the top of the transcriptional network hierarchy governing the multiciliogenesis program.

Roles of TAp73 in male and female reproduction

TAp63 and TAp73 are crucial guardians of the germ line in development and adult life, safe-guarding against DNA damage by eliminating unstable damaged germ cells via apoptosis. TAp63 and TAp73 exhibit high homology in their transactivation (TA), DNA-binding, and oligomerization domains (Dotsch et al., 2010). A common p63/p73-like ancestor, nvp63, exists in the sea anemone *Nematostella vectensis*, where it acts to protect against DNA-damaged gametes by driving their apoptosis, thereby ensuring genetic stability and healthy offspring (Pankow and Bamberger, 2007). In mammals, TAp63, but not p53, is the main guardian of the female germ line. Upon γ-irradiation, TAp63KO mice fail to remove damaged oocytes within primordial follicles, whereas wild-type and p53KO mice reduce the primordial follicle pool by 90% (Suh et al., 2006). Interestingly, the preantral and Graafian follicles are spared from TAp63-induced apoptosis, because p63 expression is restricted to oocytes of primordial follicles (Santos Guasch et al., 2018). Likewise, in the male germ line of hominids, a unique

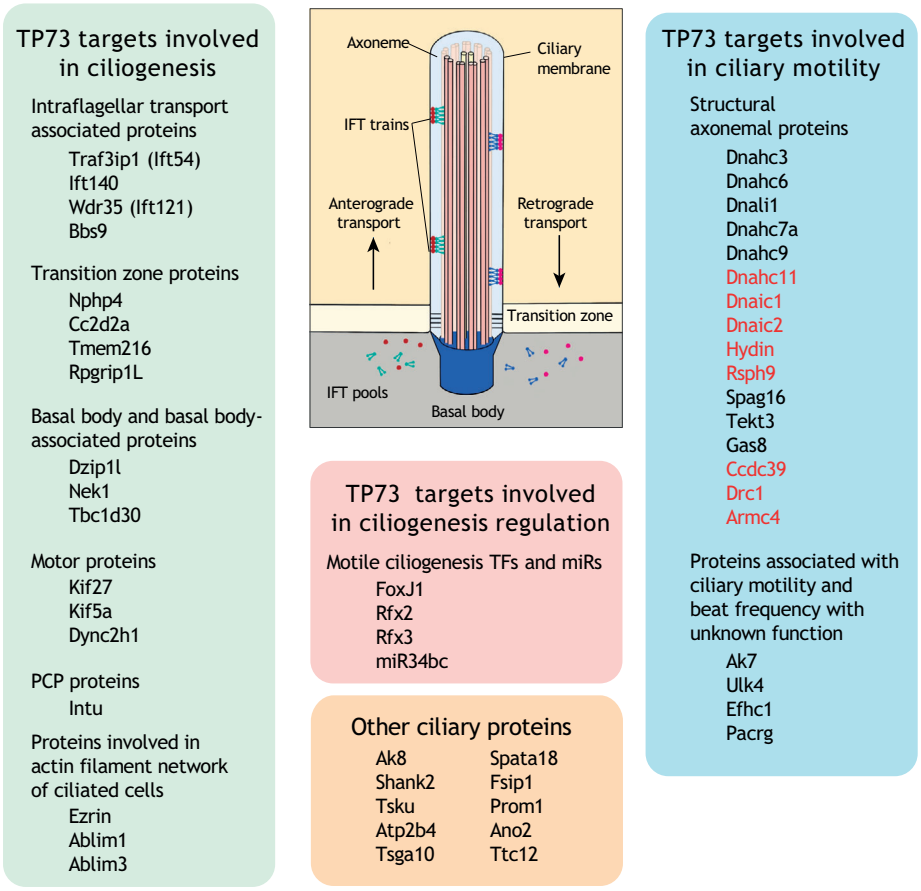

Fig. 4. TAp73 is the master transcriptional regulator of motile multiciliogenesis. Distinctive epithelia that line airways, brain ependyma, the female oviduct and the testicular efferent duct undergo motile multiciliogenesis by amplifying their centrioles to convert into basal bodies and then extend hundreds of motile cilia on their apical cell surface. Cilia beat vigorously and synchronously to generate directional fluid flow across tissue surfaces to move fluids. TAp73 occupies a position at the top of the gene network hierarchy of this program. It directly binds to promoter/enhancer regions of genes and activates the key ciliogenic regulators FoxJ1, Rfx2, Rfx3 and miR34bc, as well as nearly 50 structural and functional ciliary genes, eight of which are associated with known hereditary human primary ciliary dyskinesia syndromes (Hydin, Dnaic1, Drc1, Armc4, Dnahc11, Dnaic2, Ccdc39 and Rsph9, in red). The individual factors are grouped by functional class. Many of the 'other ciliary proteins' listed are also important for sperm development.

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isoform called GTAp63 induces apoptosis in response to genotoxic stress (Beyer et al., 2011). GTAp63, driven by the long terminal repeat of a nearby upstream endogenous retrovirus (ERV9) that integrated into our genome about 13 million years ago, is the sole p63 species in human testis and highly expressed in germ cell precursors. This strict quality control for gametes possibly enables the many decades long fertility periods of male hominids (Beyer et al., 2011).

Male and female TAp73KO mice are largely infertile (Holembowski et al., 2014; Tomasini et al., 2008). Although TAp63 is the main executioner of acutely damaged gametes, TAp73 also protects genomic integrity and fertility, albeit in a different manner. The fertility defect in female TAp73KO mice has several causes: in addition to the lack of cilia lining the oviduct, which causes impaired egg transport, these mice also have a mildly reduced number of primordial follicles, fewer ovulated oocytes (oocyte trapping) and poor oocyte quality, i.e. increased spindle abnormalities causing multinucleated blastomeres (Tomasini et al., 2008). A direct role for TAp73 in the spindle assembly checkpoint (SAC), which regulates both mitosis and meiosis, was proposed as the common mechanism underlying the observed aneuploidy of p73KO somatic cells (Talos et al., 2007) and the spindle defects of TAp73KO oocytes (Tomasini et al., 2008). Indeed, TAp73 interacts directly with components of the SAC complex, such as Bub1, Bub3 and BubR1, the inhibitor of anaphase-promoting complex protein Cdc20, and regulates their proper localization. TAp73 also regulates the binding of BubR1 to Cdc20 (Tomasini et al., 2009). For unknown reasons, the fertility of DNp73KO mice is strain-dependent. While male and female DNp73KO mice on the BL6 background are fertile (Wilhelm et al., 2010), DNp73KO mice on the 129SvJ background have reduced fertility, particularly females (Tissir et al., 2009).

In the male germline, TAp73 functions as an essential adhesion and maturation factor in the seminiferous epithelium of the testis, thereby orchestrating spermiogenesis (Holembowski et al., 2014) (Fig. 5). Sperm production occurs in Sertoli nurse cells. These tall somatic cells stretch from the basement membrane up into the lumen, with each Sertoli cell enveloping 30 to 50 immature germ cells in deep cytoplasmic pockets, which provide physical support, nutrients and paracrine signals for the developing sperm (Griswold, 1998). During their differentiation, germ cells migrate upwards into the lumen by constantly detaching and reattaching to these pockets via dynamic junctional restructuring (Mruk and Cheng, 2004). During their journey, they also pass the blood–testis barrier (BTB), which consists of tight-, gap-, adherens- and desmosome-like junctions between neighboring Sertoli cells that physically separate the basal stem cell niche from the apical differentiation compartment. The BTB protects developing germ cells (which express a unique protein profile within the body) from autoimmune reactions and exogenous toxins (Xia et al., 2005). p73KO and TAp73KO mice, but not DNp73KO mice, display ‘near-empty seminiferous tubules’ due to massive premature loss of immature germ cells, mainly from the apical compartment (Holembowski et al., 2014; Inoue et al., 2014). This phenotype is caused by defective cell–cell adhesion of germ cells to the nursing pouches, with secondary degeneration of Sertoli cells and BTB. TAp73, which is exclusively produced in germ cells, controls a balanced transcriptional program of adhesion and migration genes that includes proteases, inhibitors of proteases and serine peptidases, receptors and integrins. These are required for germ–Sertoli cell adhesion and dynamic junctional germ–Sertoli restructuring, enabling germ cells adhesion, detachment and reattachment to the next higher nursing pouch. TAp73α directly binds to gene loci of adhesion- and migration-associated genes and regulates their transcription, for instance Serpina3n, Tnfrsf1b, Tnfrsf12a, Itga5 and Timp1. In support, retrograde injection of a mix of lentiviral Serpina3n, Serping1, Tnfrsf12a, Itga5 and Timp1 into the efferent ductules of wild-type testis, to mimic deregulated expression of

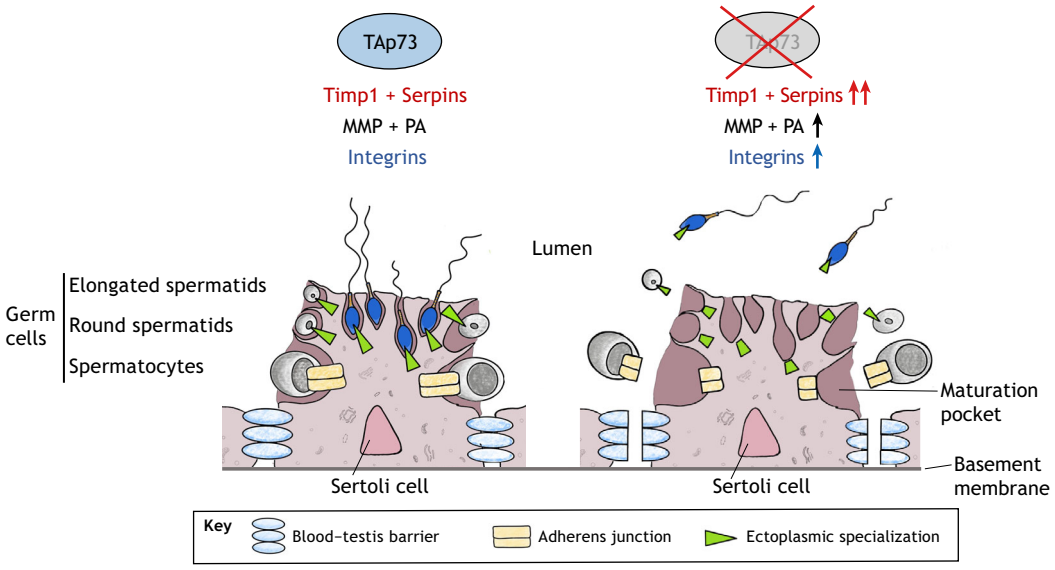

Fig. 5. Role of TAp73 in germ cell maturation. Germ cells (gray cells) mature in pockets of somatic Sertoli cells (large pink cell). This requires the cyclical disassembly and reassembly of blood testis barrier (BTB) and Sertoli–germ cell junctions to allow the upward migration of germ cells on their maturational journey to the tubular lumen. TAp73 is exclusively produced in germ cells and controls a balanced transcriptional program that mediates adhesion to and disadhesion from Sertoli cell pockets, which includes peptidase inhibitors (Timps, Serpins), proteases (MMP, matrix metalloproteinases; PA, plasminogen activator-type serine proteases), receptors and integrins, thereby ensuring germ cell maturation. Upon TAp73 loss, there is broad dysregulation of these adhesion and migration effectors. This leads to junctional defects (adherens junctions, ectoplasmic specialization) and defective BTB with secondary degeneration of Sertoli cells. The end result is a massive loss of immature germ cells (spermatocytes, round and elongated spermatids) and mature spermatozoa, causing impaired fertility.

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TAp73-controlled adhesion proteins, phenocopies the massive dis-cohesion and apoptosis found in TAp73KO testis (Holembowski et al., 2014). These findings were corroborated by an independent study (Inoue et al., 2014).

In remarkable evolutionary convergence, p73 is also required for ovarian folliculogenesis (Santos Guasch et al., 2018). Mechanistically, p73 regulates the cell–cell adhesion network in granulosa cells, which line the primary, secondary, antral and ovulating ovarian follicles (Fig. 6). In mouse ovaries, p73 is expressed in granulosa cells. Human ovaries express predominantly TAp73α and TAp73β (Santos Guasch et al., 2018). p73KO ovaries exhibit a 35–60% decrease in the ratio of primary versus antral follicles compared to wild-type (but no difference in primordial follicles), and a >90% decrease in the number of corpora lutea. RNAseq analysis of microdissected granulosa cells from wild-type and p73KO antral follicles, and from TAp73β-overexpressing cultured granulosa cells, identified multiple gene ontology categories related to motility and adhesion, including genes required to form the follicular focimatrix, a basal lamina between granulosa cells that promotes follicle maturation. p73 binds to focimatrix gene loci, such as *LAMA5*, *NID1*, *NID2* and *PXN* (Santos Guasch et al., 2018). This adhesion defect adds to the infertility of female TAp73KO mice.

Taken together, testis and ovary are unique organs with a strict division of labor among the p53 family members to protect the male and female germline – TAp63 (and to a minor extent p53) safeguard germline fidelity, whereas TAp73 ensures fertility by enabling germ cell maturation.

Role of p73 in angiogenesis

DNp73 controls developmental angiogenesis

p73 plays an important role in endothelial biology and angiogenesis. p73 is critical for endothelial cell differentiation, vasculogenesis (establishment of a mature vascular plexus) and angiogenesis

Fig. 6. Role of TAp73 in follicle development. TAp73 is required for ovarian follicle development and corpus luteum formation. TAp73 is expressed in granulosa cells and regulates a p73-dependent adhesion gene network from cell-to-cell and from cell-to-surrounding basal lamina (focimatrix) that ensures follicle maturation. While primordial follicle numbers are intact in p73KO ovaries, there is attenuated follicle development with a marked decrease in the number of maturing follicles and a >90% decrease in the number of corpora lutea, i.e. residual follicles post-ovum release, which produce progesterone.

(branching of new vessels by sprouting from preexisting vasculature) (Fernandez-Alonso et al., 2015). In the postnatal day 5 (P5) retina, a classic vascularization model, p73 loss results in moderate but significant defects in vascular development with a disorganized central plexus and disoriented tip cells, causing a reduction in vessel density and branching. Furthermore, frequent empty sleeves of vascular basement membrane suggest vessel regression. In this system, loss of p73 also affected the resident astrocytic network (the retinal microglia which provides guidance for organized endothelial cell migration by laying down a VEGF-A gradient). In addition, p73KO retinas showed a chaotic astrocytic reticulation and clumping with reduced VEGF-A expression. Moreover, the pro-angiogenic TGFβ axis was attenuated, with a major decrease in the levels of angiogenic factors such as TGF-β1, TGF-βR1, ALK1 and ID1 (Fernandez-Alonso et al., 2015). Therefore, p73 is clearly required for retinal vascular organization *in vivo*. It promotes endothelial cell migration, vessel sprouting and tubulogenesis by regulating the proangiogenic milieu that is mediated by VEGF and TGF-β activity.

These findings were corroborated *in vitro* in mouse embryonic stem cells (mESCs) and induced pluripotent stem cells (iPSCs), which are used to model early vasculogenesis in embryoid bodies (Fernandez-Alonso et al., 2015). Notably, the DNp73 isoform is the predominant variant in mESCs and its expression is tenfold higher than that of TAp73. The functional inhibition of p73 in mESCs (by using the pan-dominant-negative p73 DD mutant), as well as p73 knockout in iPSCs, impaired the formation of vascular structures and endothelial sprouting, and hindered endothelial differentiation. Likewise, specific knockdown of DNp73, but not of TAp73, in human umbilical vein endothelial (HUVEC) cells impaired their migration and tubulogenesis, and greatly reduced the expression of the angiogenic target genes of VEGF-A and TGF-β (Fernandez-Alonso et al., 2015).

DNp73 controls tumor angiogenesis

In keeping with the pro-angiogenic role of DNp73 in vascular development, DNp73 also promotes tumor angiogenesis. DNp73 is frequently overexpressed in human cancers, thereby shifting the balance between the opposing p73 isoforms towards DNp73 (Zaika et al., 2002). Indeed, in a syngeneic transplantation model of B16-F10 mouse melanoma cells, constitutive DNp73 expression led to higher vessel density, an increased mitotic index and elevated VEGF-A expression in subcutaneous tumors than in vector-control cells (Fernandez-Alonso et al., 2015). Conversely, selective loss of DNp73 in subcutaneous nude mouse tumors of E1A/Ras-transformed DNp73KO vs wild-type mouse embryonic fibroblasts (MEF) caused a 50% reduction in blood vessel formation. In agreement, in hypoxia, loss of DNp73 is accompanied by loss of HIF1α and proangiogenic and/or proinflammatory genes (Stantic et al., 2015). Moreover, in a transgenic zebrafish model [Tg(fli:EGFP)] where EGFP is expressed throughout the vasculature, thereby enabling visualization of tumor cell interaction with endothelial cells, DNp73KO MEF$^{E1A/Ras}$ xenografts showed a 50% reduction in angiogenic sprouting. Similarly, spontaneous lymphomas from DNp73KO EμMyc transgenic mice had a 30% reduction in blood vessels compared to wild type. In agreement, upregulation of DNp73 in human breast cancer is associated with poor prognosis and tumor angiogenesis, as reported in a study of 1048 breast cancer tumors from The Cancer Genome Atlas. Here, high DNp73 mRNA levels correlated with increased angiogenesis and hypoxia signatures in gene set enrichment analysis (Stantic et al., 2015).

In contrast, the role of TAp73 in tumor angiogenesis remains controversial. On the one hand, in keeping with its known tumor suppressor role, TAp73 has been found to suppress tumor angiogenesis by repressing proangiogenic and proinflammatory cytokines, as well as HIF1α (Amelio et al., 2015; Stantic et al., 2015). Selective loss of TAp73 in the MEF$^{E1A/Ras}$ subcutaneous tumor model results in larger and more vascularized tumors and leaky blood vessels due to reduced endothelial cell–cell contacts. Furthermore, TAp73KO MEF$^{E1A/Ras}$ tumors exhibit highly stabilized HIF1α, as well as an upregulation of many proangiogenic genes such as *Ccl2*, *Cxcl1*, *Cxcl2*, *IL6*, *Ereg*, *Vegfc* (all HIF1α targets), whereas *Bai1* (also known as *Adgrb1*), the precursor of anti-angiogenic vasculostatins, is downregulated (Stantic et al., 2015). The same tumor-promoting proangiogenic effect of TAp73 loss was observed in TAp73KO mice in a skin carcinogenesis model, and in various xenograft tumor models with stable TAp73 knockdown (Amelio et al., 2015). Mechanistically, ectopic expression of TAp73 in SaOs2 osteosarcoma cells degraded HIF1α. Because an endogenous p73-HIF1α complex could be immunoprecipitated in H1299 lung cancer cells, the authors proposed an anti-angiogenic mechanism mediated by TAp73 that involves MDM2-dependent ubiquitination and proteasomal degradation of HIF1α (Amelio et al., 2015). In support, their gene-set enrichment analysis found a negative enrichment of ‘HIF1α target genes’ and ‘angiogenesis pathway’ categories in tumors with detectable TAp73 expression, compared to p73 loss or DNp73 expression, in a human lung adenocarcinoma dataset of 226 biopsies (Amelio et al., 2015).

On the other hand, another study did not support an angio-suppressive function of TAp73, but instead discovered that TAp73 positively regulates tumor angiogenesis, which might explain the surprisingly high occurrence of non-mutated, TAp73-overexpressing human tumors (Dulloo et al., 2015b). The authors found that hypoxia stabilizes TAp73 by HIF1α-mediated suppression of the Siah1 E3 ligase, which normally targets TAp73 for proteasomal degradation (Dulloo et al., 2015b). Furthermore, hypoxic signals lead to the TAp73-mediated activation of a proangiogenic transcriptome, promoting tumorigenesis. Consequently, subcutaneous allografts of TAp73KO MEF$^{E1A/Ras}$ are less vascular and reduced in size, while TAp73 overexpression in H1299 xenografts leads to increased vasculature (Dulloo et al., 2015b). These authors also found a tumor angiogenesis-promoting function for DNp73 (Dulloo et al., 2015a). As is the case for TAp73, hypoxia stabilizes DNp73 through HIF1α-mediated suppression of the Siah1 E3 ligase. Interestingly, DNp73β is capable of binding to the VEGF-A promoter and inducing its expression to the same extent as TAp73β. While this is a TA-domain-independent activity by DNp73, its underlying mechanism remains to be defined (Dulloo et al., 2015a). Thus, hypoxia might be a specific context in which these normally antagonistic isoforms take on similar functions in angiogenesis. At any rate, the opposing effects of TAp73 that have been observed in tumor angiogenesis are puzzling, and obviously more work is needed to better understand whether these antithetical actions are indeed context-dependent, or part of a hypoxia continuum in cancer (Sabapathy, 2015).

Metabolic and immune functions of TAp73

TAp73 also exerts metabolic functions. Ectopic expression of TAp73 in SaOs2 cells increases their glycolytic rate, amino acid uptake and phospholipid biosynthesis, suggesting that TAp73 can promote the Warburg effect and anabolic metabolism (Agostini et al., 2014; Amelio et al., 2014a, 2014b). Such a putative anabolic

function, if it occurs *in vivo*, for instance, under hypoxia, would oppose the tumor suppressor role of p73. Therefore, it has been speculated to instead represent an adaptive antioxidant defense to regenerate the glutathione (GSH) stores that are hijacked by cancer. Indeed, TAp73 can induce glutaminase-2, which converts glutamine to glutamate (Amelio et al., 2014b). Glutamate and glycin are substrates for GSH synthesis (Amelio et al., 2014b). Moreover, TAp73 can promote NADPH production (and thus GSH regeneration) via the pentose phosphate pathway by inducing the rate-limiting enzyme glucose-6-phosphate dehydrogenase (G6PD) (Du et al., 2013). TAp73 has been shown to protect against organismal and cellular aging by promoting mitochondrial respiration and preventing ROS accumulation. Older (18 months) TAp73KO mice show accelerated aging (kyphosis, body weight and fat loss) with decreased cellular ATP levels and O_2 consumption and increased ROS levels, oxidative stress and senescence (Rufini et al., 2012). Mechanistically, the mitochondrial complex IV subunit Cox4i1 has been demonstrated to be a direct TAp73 target (Rufini et al., 2012).

TAp73 also plays an as-yet-unexplored role in immunity. p73KO mice show severe chronic upper and lower airway infections, which are largely caused by a severely defective mucociliary clearance (Marshall et al., 2016; Nemajerova et al., 2016; Yang et al., 2000). However, whether an additional innate immune defect in these mice aggravates this phenotype remains an open question. TAp73 plays a role in macrophage polarization, and TAp73KO mice are hypersensitive to LPS-induced septic shock. Their macrophage M1 phenotype (high levels of pro-inflammatory cytokines) is prolonged at the expense of the M2 phenotype (anti-inflammatory, tumor-promoting), thus suppressing the resolution of the inflammatory response (Tomasini et al., 2013). It also remains to be explored whether there is an indirect immune axis that involves TAp73-induced FoxJ1, which, aside from its role in multiciliogenesis, has been shown to modulate the immune response. In fact, FoxJ1 dampens T-cell activation and autoimmunity (Lin et al., 2004).

Conclusions and outlook

The discovery of p73 as an ancient homolog of the tumor suppressor p53 back in 1997 (Kaghad et al., 1997) initially spawned expectations of a narrow role in DNA damage response, genomic stability and tumor suppression. This expectation was supported by the observation of dominant-negative DNp73 overexpression in human epithelial carcinomas and TAp73 deletions in hematological malignancies, which was experimentally confirmed in cell and mouse cancer models. In contrast to p53 and p63, which have known inherited disease alleles, so far no human disease has been linked to an inherited hypomorph or loss-of-function mutant p73 allele, possibly because any such p73 mutation might produce severe CNS defects that are embryonic lethal. However, as discussed above, global and isoform-specific knockout mice have revealed an unexpected wealth of tissue-specific functions of the p73 locus in development, differentiation and homeostasis. Additional, more subtle, p73 functions might still be discovered. As caveat, the p73 protein is only expressed at very low levels in all normal mouse and human tissues, and currently available antibodies are suboptimal. This makes its direct detection difficult, and determining the isoforms present at their respective ratio is nearly impossible. For future research, a way forward will be p73 isoform-specific reporter mice, similar to those already existing for DNp73 (Tissir et al., 2009). Another open question is when and how the TA- and DN-isoforms of p73 cooperate. Cooperativity, for example in the CNS (except in the hippocampal DG), is strongly supported by the fact that both TAp73 and DNp73 are co-expressed in most (but not all) p73-positive regions, and that each isoform-specific KO mouse has a weaker CNS phenotype than the global KO. With this in mind, stay tuned, as there remains much more to learn.

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Competing interests

The authors declare no competing or financial interests.

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