

Role of p63 in epidermal development

Gerry Melino

Medical Research Council, Toxicology Unit, Leicester, UK. University Tor Vergata, Rome, Italy.
Email: gm89@le.ac.uk

Here show a major role for p63 in the development of both for cornified (skin) and non cornified (thymus) epithelia formation. Therefore it is also pivotal for normal education of T-cells in the thymus.

Development of the epidermis, the outer layer of the skin formed of keratinocytes, requires the activity of the transcription factor p63, since p63^{-/-} mice are born without epidermis and consequently die at birth. The TP63 gene contains two distinct promoters, which drive the expression of two proteins, one with an amino-terminal trans-activation domain (TAp63) and one without (Δ Np63), although their relative contribution to epidermal formation and homeostasis is not known. To address this issues, we reintroduced TAp63 α and/or Δ Np63 α into p63^{-/-} mice by in vivo genetic complementation. While extremely rare patches of differentiated keratinocytes were detected in p63^{-/-} and p63^{-/-};TA mice, there were significant signs of epidermal basal layer formation in p63^{-/-}; Δ N mice. When both TAp63 α / Δ Np63 α were re-introduced in p63^{-/-} mice, a still greater number of patches of differentiated skin appeared. In addition, microarray analysis indicates that Δ Np63 α induces the expression of basal layer markers, while TAp63 α drives the expression of upper layer markers. Indeed, Δ Np63 α directly transactivates the keratin 14 promoter; we also show that TAp63 α action is mediated at the molecular level by direct transactivation of IKK α . The action of p63 is not restricted to the epidermis, as the p63^{-/-} mice have an abnormally small thymus with very low numbers of thymocytes, although the expression of K5/K8 differentiation markers appears normal in thymic epithelial cells. In p63^{-/-}; Δ N mice, and even more in p63^{-/-}; Δ N;TA, but not in p63^{-/-};TA, the thymus was bigger, with a significantly increased cellularity in comparison to p63^{-/-}. The data presented are consistent with a role for Δ Np63 α in controlling the expansion of epithelial cells from progenitor precursors in epidermis and thymus, to allow TAp63 α , acting subsequently and synergistically to Δ Np63 α , to control epithelial differentiation via IKK α .

p63 regulate apoptosis during DNA damage, thus supporting the p53/p73 pathway. The activity of p63 depends on its protein levels, and evidence suggests that post-transcriptional regulation plays a major role in p63 response. However, the molecular mechanisms underlying the regulation of p63 protein stability remain largely unknown. Here we report that p63 stability is directly regulated by the ubiquitin/proteasome pathway and p63 is degraded through specific mechanisms, different from that of p53. Upon DNA damage, p63 is degraded through a NEDD4-like-mediated mechanism and PML protein modulates p63 half-life by inhibiting its degradation in a nuclear body (NB)-dependent way. As a result, PML significantly increases the ability of p63 to transactivate promoters of the *bax* and *p21* genes and potentiates p63-dependent apoptosis and tumor suppressive activity. In turn, p63 pro-apoptotic function is markedly impaired in *PML*^{-/-} cells. Thus, our findings demonstrate that PML plays a crucial role in modulating p63 function. A C-terminal PY motif of p63 binds to a specific E3 ligase of the NEDD4-like family, resulting in ubiquitination and degradation of p63. Consistent with the defect described in KO mice, TAp63/ Δ Np63 differently regulate epithelial development; TAp63/ Δ Np63 are both crucial also for the development of the thymus.

Involvement of p73, a p53-family member, in neurodegeneration, metabolism and senescence

Melino G,

University Tor Vergata, Rome, Italy; Medical Research Council, Toxicology Unit, Leicester, UK

p63 and p73 have been identified as the ancestral members of the p53 family. Despite the high sequence and structural similarity, the mouse knockouts revealed a crucial role in neural development for p73 and in epidermal formation for p63. We identified several transcriptional targets, the mechanisms of regulation of cell death, and the p63 isoform involved in epithelial development. Both genes are involved in female infertility and maternal reproduction as well as in cancer formation, although with distinct mechanisms. TAp73 knockout mice (TW Mak G&D 2008) show high tumor incidence with hippocampal dysgenesis. Conversely, Δ Np73 knockout mice (TW Mak G&D 2010) show a very low incidence of cancer, with sign of moderate neurodegeneration with a significant loss of cellularity in the cortex. This indicate a tumor suppressor role for TAp73 and an oncogenic role for Δ Np73.

p73 steady state protein levels are kept low under normal physiological conditions through degradation by the 26S proteasome, mediated by the HECT-containing E3 ubiquitin ligase ITCH, for which we are developing an inhibitor. We have also described additional mechanisms of degradation: (1) the orphan F-box protein FBXO45 ; (2) the ring finger domain ubiquitin ligase PIR2 (p73-induced Ring Finger 2), and (3) the antizyme ubiquitin-independent, proteasome-dependent pathway, both specific for the Δ Np73 isoforms.

Here, we describe the involvement of p73 in neuronal development. TAp73 knockout mice (TW Mak G&D 2008) show hippocampal dysgenesis. Conversely, Δ Np73 knockout mice (TW Mak G&D 2010) show sign of moderate neurodegeneration with a significant loss of cellularity in the cortex. TAp73 is able to drive the expression of miR-34a, acting on specific binding sites present on the miR-34a promoter. In agreement with these *in vitro* data, miR-34a transcript expression is significantly reduced *in vivo* both in the cortex and hippocampus of p73^{-/-} mice. In keeping, we show a role for miR-34a, in parallel to TAp73 expression, during *in vitro* differentiation of ES cells. Expression of miR-34a increases during *in vitro* neuronal terminal differentiation, of ex vivo primary cortical neuronal cultures, in parallel with the expression of TAp73. Moreover, we also detect an increase *ex vivo* of miR-34a steady state expression during postnatal development of the brain and cerebellum, when synaptogenesis occurs. We further confirm a role for miR-34a in synaptogenesis, as overexpression or silencing of miR-34a results in an inverse expression of a number of synaptic genes, via their 3'-UTR. In particular, miR-34a overexpression decreases synaptotagmin I and syntaxin-1A expression, and the endogenous levels of miR-34a are able to regulate only synaptotagmin I expression. Our findings show that p73 drives the expression of miR-34a during terminal, synaptic differentiation.

Finally, we describe the involvement of p73 in senescence and metabolism. TAp73-null mice show a significant premature spontaneous aging phenotype at 12 months of age: alopecia, epidermal thinning, reduced subcutaneous fat, increased visceral fat TAp73, osteoporosis with scoliosis. This indicate a significant phenotype related to obesity and ageing. Both *in vivo* and *in vivo* TAp73-null mice show unbalanced redox defences. TAp73 is able to drive the expression of glutaminase type 2 (GLS2), acting on specific binding sites present on its promoter. In agreement with these *in vitro* data, TAp73-null cells show clear metabolic defects in the glutamine pathway affecting GSH and redox balance. In keeping, we show a role for TAp73 in the regulation of metabolic pathways.