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Meeting Report

The 4th International p63/p73 workshop: p53's (older) sisters take centre stage

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4th International p63/p73 workshop in Toronto, Canada, 30 August to 1 September 2009

The fourth p63/p73 workshop, supported by Epistem and Cell Death and Differentiation, was recently held in Toronto, Ontario, Canada at Mt. Sinai Hospital, organized by T Mak, F McKeon, C Prives and G Melino. Numerous investigators in the p63/p73 field were in attendance for the nearly 40 oral presentations and numerous posters of exciting new research. There exist diverse biological actions of p63/p73, ranging from stem cell maintenance to apoptosis and development; these roles are further complicated by the existence of many isoforms of each protein. This year's workshop highlighted the multitude of actions exerted by p63 and p73, especially isoform-specific roles, and emphasized the need for more research as the burgeoning field continues to develop.

Structure and Evolution

The p53 family contains three members, with functions ranging from epithelial and neuronal development to regulation of apoptosis. Of the three family members, p63 seems to be the most ancestral, evidenced by its similarity to invertebrate p53-like proteins. The evolution of the p53 family through phylogenetic analysis was analyzed in *C. elegans* in which the sole p53-like protein, CEP-1, induces apoptosis in meiotic germ cells. Parallel CEP-1-dependant and -independent pathways also regulate apoptosis and the promiscuity of Akt in mammals is thought to have derived from regulating both sides of this bifurcation. Throughout evolution, the DNA binding domain of the three p53 family members has been well conserved, but addition and variation of other domains separated the members. Findings presented on structural evolution confirmed that the TID of TAp63α prevents tetramer formation, favoring an inactive dimer. I n addition, homo- and heterotetramers occur in p73, with the most thermodynamically favorable heterotetramer consisting of a p73 dimer and a p63 dimer.

Regulation of p63/p73

Considerable similarity exists between the p53 family members, yet structural differences lead to differential regulation. A study showed that although c-Abl phosphorylates both p63 and p73, the results vary. Phosphorylation promoted stabilization and association with YAP for both proteins, but ΔNp63α promoted cell viability, whereas p73 phosphorylation accentuates the transactivation of proapoptotic genes. Conversely, phosphorylation of Y121 on p73 by an unknown kinase inhibits transcription of proapoptotic genes. Multiple means of preferentially regulating isoforms were also discussed. t was shown that, upon DNA damage, a novel TAp73 transcriptional target, p73-induced ring finger (PIR), preferentially ubiquitylates ΔNp73, promoting TAp73-mediated apoptosis. A unique, non-classical, polyamine-induced antizyme pathway that bound ΔNp73β facilitated its destruction. A genome-wide RNAi screen for inhibitors of p73-mediated apoptosis was also described. Numerous potential therapeutic targets were validated, including DAXX, which seems to inhibit p73 and promote p63-mediated cell survival. Some regulatory mechanisms are highly conserved within the p53 family, from humans to *C. elegans*. The ASPP family of proteins was shown to enhance p53/p63/p73-mediated apoptosis, whereas the inhibitory iASPP potentiated cell growth. In addition to transcriptional control, cytosolic p53 initiates apoptosis after PUMA disrupts the inactive Bcl-xL/p53 to induce premeabilization of the mitochondrial membrane.

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In Vivo Models of p63/p73 Function

Among the findings discussed at the workshop were results regarding new mouse models designed to determine the function of p63/p73 *in vivo*. TAp73-deficient macrophages were shown to be resistant to apoptosis, exhibiting decreased phagocytic ability and an increased lifespan, suggesting that TAp73 isoforms are required for normal macrophage activities.

With regard to p63, major focus was given to isoform-specific p63 mouse models. It was shown that p63 was required for the development of cardiomyocytes and the inhibition of cardiogenesis may be responsible for the lethality observed in p63 null mice. The role of TAp63 in senescence and aging was also presented. Studies of TAp63 null mice revealed that these animals develop defects associated with aging, including increased levels of $p16^{INK4a}$. TAp63 isoform-specific knockout mice also develop severe ulcerated wounds with increased DNA damage in epithelial cells, suggesting that TAp63 has important functions in wound healing, aging and DNA damage maintenance.

Constructing mouse models to analyze epidermal development is crucial for understanding the contribution of p63 to epithelial stratification. Reexpression of the ΔNp63β isoform resulted in epidermal expansion. The role of p63 in epidermal development through its downstream targets was discussed. It was shown that, although IKKα is a target of p63, IKKα knockout mice do not exhibit the skin fragility seen in AEC patients, who also show reduced IKKα. Emphasizing the role of TAp63 and DNA damages in the epidermis were studies revealing that p63 is expressed in the dermal sheath of hair follicles. Interestingly, precursor cells in the dermal sheath lacking TAp63 showed increased self-renewal and proliferation, and also prevalent genomic instability. TAp63 is induced in oocytes after recombination and guards against DNA damage. Cotreatment of cisplatin with imatinib suppressed TAp63 induction, preventing chemotherapy-induced depletion of oocytes. In addition, TAp63 null mice possess more oocytes than do wild type, but these present with accumulated DNA damage, suggesting TAp63 provides a postpachytene checkpoint for guarding the female germline.

Cancer and Stem Cells

Contrary to initial observations with whole-gene knockouts, growing evidence has accumulated demonstrating that specific p63 and p73 isoforms can function as tumor suppressors. p73 was shown to not be essential for lymphatic tumor suppression, although it prevents dissemination. The ultimate goal of cancer research is to discover ways to effectively target cancer cells. A talk focused on cancer research involving the altered metabolism and redox state of cancer cells. Several genes involved in metabolism are regulated by p63, and it was suggested that designing drugs targeting enzymes involved in NADPH production would be a more effective treatment method.

Stem cells participate in both normal development and in the progression of certain disease states. Understanding the mechanisms of stem cell self-renewal may contribute to a better understanding of cancer. Much research has been carried out to elucidate the role of p63 in stem cell maintenance. A study presented on Bulge Stem Cells of the hair follicle highlighted that these cells exhibit an increased rate of DNA repair, preventing apoptosis after irradiation. Within the cornea, p63 is required for the commitment of corneal cells in the mouse, but is dispensable in a human embryonic stem cell system. This discrepancy between species highlights the need for further research.

It is not only p63 that has an integral role in stem cell maintenance but p73 also functions in the renewal of neural stem cells. TAp73 knockout mice showed a reduced stem cell population as the result of increased neurogenesis, highlighting the requirement of TAp73 in maintaining neural stem cells.

Development

It has been 10 years since the first p63/p73 knockout mice were generated, and the understanding of how both proteins work toward development continues today. Proper development of many tissues requires functional cell junctions. It was shown that the desmosomal component, PERP, is a p63 target, suggesting that p63 contributes to successful cell junctions. In addition, mice deficient for another p63 target, caspase-14, show enhanced water loss and increased UVB sensitivity. An upstream examination of epidermal development demonstrated that loss of metalloproteinase ADAM17 elevates p63 levels via the Notch signaling pathway.

Mutations within p63 produce well-known developmental defects. The ectodermal dysplasia p63 Q634X mutation results in a lowered transcriptional activity of ΔNp63α, and an opposite transcriptional pattern in TAp63α. It was shown that mice harboring the AEC p63 point mutation L514F showed characteristics similar to AEC patients, and also showed reduced stem cells in the basal layer.

Novel Targets

The ability of p63 and p73 to transcriptionally regulate genes is an ever-growing area of research. As such, many lectures were given on transcriptional targets of p63 and p73. Novel p63 targets, CEBPδ and SATB2, were shown to have opposing effects in keratinocytes. SATB2 promotes chemoresistance by augmenting ΔNp63α repression of TAp73, whereas CEBPδ prevents mitosis in the upper layers of the epidermis. TAp63 was induced by TLP and led to a repression of N-Myc in order to regulate apoptosis. All p53 members induce another novel target, RNPC1, on stress. RPNC1 represses the family members in addition to preventing p21 mRNA degradation. The p53 family, however, does not equally regulate all targets. The vitamin D receptor is only induced by p63 and p73 to prevent cell invasion and enhance osteoblastic differentiation, respectively. Only p53 controls fatty acid oxidation by differentially regulating the expression of Lpin1 in a glucose- and tissue-specific manner, whereas mTOR was shown to be a negative regulator of p73 through an expansive ChIP-on-chip analysis. Various transcriptional targets were discussed and provided insight into the regulation of apoptosis by p63/p73/p53, and their involvement in

metabolic pathways is suggestive of the large functions of the family.

Concluding Remarks

Much work has to still be carried out in the p63/p73 field, but the research presented at this workshop illuminated key areas that are ripe for discovery, particularly the roles between p63 and p73 isoforms. The workshop facilitated a friendly and casual meeting of minds, between veterans of the field and budding scientists. Discussions during coffee breaks and after presentations will likely push the boundaries of the p63/p73 field and will surely make way for exciting discoveries at the next p63/p73 workshop.

Conflict of interest

The authors declare no conflict of interest.

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