Cell Death and Differentiation (2014) 21, 1340–1342

www.nature.com/cdd

Meeting Report

The 6th International p63/p73 Workshop: the C(ancer) and D(evelopmental) roles of p63 and p73

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Cell Death and Differentiation (2014) **21,** 1340–1342; doi:10.1038/cdd.2013.192; published online 17 January 2014

The 6th p63/p73 Workshop, Kazusa Akademia Park, Chiba, Japan, 15–18 September 2013

The 6th International p63/p73 Workshop was a coming home of sorts, back to the country where p63 had been discovered, Japan. It was held in Chiba at the Kazusa Akademia Park, from 15 to 18 September 2013, with 34 oral and 32 poster presentations. A pre-conference programme preluded the workshop with 11-oral presentations by young Japanese fellows. In general, this year's workshop continued to highlight the complex and diverse roles of the p63 and p73 proteins, and also touched upon the therapeutic possibilities in pathological conditions where these proteins are altered. Major work presented at the workshop are highlighted under specific themes, as follows.

Non-cancer-related Functions of p63/p73

Many presentations, as in the last workshop, were focused on understanding the role of p63 and p73 in various developmental processes. As summarised in Table 1, majority of these were on TAp63/DNp63, compared with TAp73. An emerging theme from these presentations was that both p63 and p73 have common critical roles in the regulation of metabolism, thereby having an effect on senescence and aging, as well as neurogenesis and cognition. In the former case, TAp73 appears to have an anti-senescence role, as a loss of TAp73 led to decreased O_2 flux and consumption, most likely due to mitochondrial dysfunction, suggesting a role in metabolism. Consistently, serine biosynthesis was reported to be negatively regulated by TAp73, and on the contrary, positively by DNp73. The net response therefore was that absence of TAp73 led to aging and senescence, which were correlated with elevated p16 and p19 levels. Although the role of p63 in senescence has been previously reported, TAp63$^{-/-}$ mice were shown to develop glucose intolerance and develop insulin resistance, involving TAp63-mediated regulation of AMPK, Sirt1 and so on, thereby regulating the fatty acid synthesis and the decreased fatty acid oxidation. DNp63-mediated regulation of hexokinase 2 was also reported to control mitochondrial basal respiration and intracellular ROS. Altogether, these presentations alluded to a collective role of p63 and p73 in regulating various aspects of metabolism.

Similarly, the roles of both p63 and p73 in various aspects of neurogenesis were presented. Absence of TAp73 led to neuronal defects, such as neurite outgrowth and innervations, eventually being manifested as defects in burrowing behaviour. Moreover, TAp73 absence also resulted in the depletion of neural stem cell (NSC) pool due to TAp73-mediated regulation of Hey 2, therefore suggesting a non-survival role for it in the maintenance of NSC pool to prevent premature neurogenesis. On the flip side, absence of DNp63 also led to decreased NSC, but rather due to a p53→puma-dependent cell death mechanism, suggesting a survival role for DNp63 in maintaining the NSC pool, and thus, adult neurogenesis and NSC-dependent cognitive functions. Similar to the effects of metabolism, the interplay of both p63 and p73 proteins regulate various aspects of neurogenesis.

Besides the common functions, these proteins were shown to have specific roles in other developmental aspects. For instance, p63 was shown to be involved specifically in several processes, such as keratinocyte differentiation and lactation. In this context, continued interest in the field was evident from several presentations on the role of p63 in keratinocyte differentiation. p63 was shown to be required for the establishment of lineage-specific chromatin organisation during epidermal development and differentiation, through the regulation of targets such as the genome organiser Satb1 and the polycomb component Cbx4. Moreover, mechanistic analysis for the failure of mammary gland development in

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Table 1 Non-cancer-related functions of p63/p73 reported in the workshop are summarised

TAp63/DNp63	TAp73
Anti-senescence/aging	Anti-senescence/aging (serine metabolism)
Energy metabolism/blood glucose	Neurogenesis/cognition
Prevent oxidative stress	Sperm cell adhesion/maturation (male fertility)
Neurogenesis/cognition	Cellular reprogramming
Cardiogenesis	—
Lactation	—
Oocyte death	—
Adult stem cell maintenance in skin/ keratinocyte differentiation/epidermal commitment	

Processes in red are common to both p63 and p73, as opposed to specific roles of the proteins in other processes.

embryonic mice lacking p63 using conditional p63 deficiency in basal epithelium cells of the mammary gland revealed the lack of milk production. Detailed data were presented to dissect the mechanistic basis of this defect, which revealed a cell-non-autonomous role for p63 expressed in basal cells, in regulating the milk production in adjacent luminal cells. NRG1 was identified as a target of p63 in basal cells, which was secreted to the luminal cells in which a plethora of cascades such as the Stat5a/b pathway were activated, thus resulting in milk production.

Keeping with the gender-based functions, TAp63's role was firmly established in oocyte death upon exposure to a variety of DNA-damaging chemotherapeutic agents, thereby leading to infertility, as oocyte-specific TAp63 ablation rescued oocyte death upon stimulation. Mechanistically, the Abl-kinase was shown to be upstream of p63, with Bax suggested to be involved in executing the apoptotic process. Male fertility, on the flip side was shown to be regulated by TAp73. Absence of TAp73 was reported to result in a gradual male sterility as the mice aged, in all genetic backgrounds of mice tested. This was due to near empty tubules caused by elevated apoptosis in the testis. Degeneration of Sertoli cell pouches and defects in cell–cell junctions were noted, thereby disrupting the blood–testis barrier and the integrity of the epithelium.

Furthermore, TAp73 was also shown to be required for the efficient generation of induced-pluripotent cells (iPS). Absence of TAp73 reduced iPS colony numbers, concomitant to increased differentiation, accompanied by a decrease in nanog levels. Multiple lineages were affected, such as vascular structures, and TAp73$^{-/-}$ iPS-derived colonies were more differentiated during teratoma formation, highlighting an opposite role for TAp73 in this process compared with p53.

Cancer-related Functions of p63/p73

Data presented by several investigators pointed to the fact that both TAp73 and TAp63 were capable of inhibiting cell invasion and the epithelial–mesenchymal transition, through the activation of targets such as the vitamin-D receptor to inhibit metastasis. Moreover, evidence was provided for the tumour suppressive role for p63 in late stage squamous cell carcinoma (SCC), as well as in neuroblastoma (NB). In the latter case, TAp63 was shown to inhibit oncogeneic pathways operative in NB, such as the direct downregulation of MycN, and a higher TAp63 expression correlated with better prognosis. On the contrary, DNp63 was shown to be oncogenic in early stage SCC, and its expression was elevated in these cancers. In addition, p63 was demonstrated to have an anti-apoptotic function in inducing chemoresistance in melanomas. Haploinsufficiency of ASPP in mice induces SCC formation, by allowing the translocation of RelA/p65 to the nucleus for p63 gene activation. Keeping in line with the pro-survival role of these proteins, TAp73 was shown to cooperate with several AP-1 factors to promote cellular survival and also had the propensity to promote angiogenesis, findings that are consistent with its overexpression in some cancers. Collectively, the notion that emerges is that both p63 and p73 proteins do maintain their classical tumour suppressive roles in some contexts, but could be pro oncogenic in others.

3Ts: Tools, Targets and Therapeutics

Several presentations provided insights using novel mouse models, and also targets and possible therapeutic utility of some of the findings that have been accumulated to date. A substantial number of mouse models were reported and used to uncover functions of the p63/p73 proteins. For instance, an allelic series of several knock-in mice were generated to recapitulate the EEC syndromes derived due to mutations in TAp63, such as the R318H mutation found in the human case. Analysis of these mice revealed a role of p63 in determining the penetrance of the disease, highlighting a genetic modifier role of p63. Moreover, DNp63a and DNp63b transgenic mice were reported, which developed epidermal hyperplasia associated with chronic inflammation, modelling human atopic dermatitis. Furthermore, mice lacking Rbm38, an RNA-binding protein that was shown to bind to the 3′UTR of p63 and p73 to regulate their mRNA stability, were also reported. Similarly, transgenic mice expressing N-Myc and NCYM, were used to interrogate their roles and dependence on p63 in NB formation.

Besides mouse models, several groups reported genome-wide chromatin-immunoprecipitation studies that provided further insights into p63 and p73 binding sites on the genome. Data from such studies demonstrated two major points: (1) that a significant portion of binding is in the non-coding genomic region and could thus result in a large repertoire of non-coding RNA; and (2) that in the absence of canonical p63/p73 binding sites, these proteins could potentially regulate target-gene activation via tethering with other transcriptions factors such as NF-Y that were shown to be bound to their canonical CCAAT boxes. Thus, it appears that p63 and p73, like all 'old-fashioned' transcription factors, are not confined to their respective responsive elements for the transactivation of target genes. Although in-depth analysis of these are yet to be carried out, one concept that has emerged is that p63/p73 may utilise and ride on other transcription factors to load onto promoters without p53RE sites to promote transactivation. In this context, several targets genes were reported, including

DEPDC1 that regulates metastasis, PDGFRβ that regulates cellular proliferation and BMCC1 that induces apoptosis, amongst others.

Attempts in translating the findings to clinical use have slowly but surely begun, in this relatively young field. For instance, the familial syndrome of EEC, with multiple complexities that arise due to mutations in p63, could potentially be treatable. Evidence was provided for the ability of PRIMA^{Met} to activate the mutated p63, similar to its effects on p53, thereby allowing the reversal of epithelial differentiation and restoring p63-related signalling pathways, thereby offering hope for restoration of visual deficiency in EEC patients. In another case, given that the activation of TAp63 upon exposure to irradiation or cisplatin during cancer therapy would lead to oocyte cell death, thereby leading to female infertility, inhibitors of the p63-activating c-Abl such as Gleevec that are already in use in the clinics were found to prevent oocyte death. Thus, these studies demonstrate the possibilities that lie ahead for harnessing some of the basic findings to be translated to clinical utility.

Concluding Remarks

The workshop clearly demonstrated that the p63/p73 field, which started about 14 years back with reports on the cloning of both these genes is now in a phase where the tools and knowledge are available to allow for growth and expansion of the understanding of the role of these proteins in various patho-physiological processes, not only individually, but in concert, and also for the in-depth analysis of their involvement in the context of complex systems. Importantly, it is apparent that the findings could be translated to clinical use much earlier than one could have envisaged, and promises for a bright future ahead.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements. We would like to thank the Cell Death and Differentiation Conferences and the Chiba Cancer Centre for their support, and the organisers and chairs for putting together a productive and an engaging workshop.