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Meeting Report

Stimulating p53 down-under: a report from the 1st Australian p53 Workshop

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1st Australian p53 Workshop, Peter MacCallum Cancer Centre, Melbourne, 19–21 November 2012

Burgeoning interest in the tumour suppressor p53 in the Australasian region provoked the birth of the first Australian p53 Workshop, held at the Peter MacCallum Cancer Centre in Melbourne, 19–21 November 2012 and attended by over 130 international and national delegates. The Workshop was organized by Ygal Haupt, Andreas Strasser, Sue Haupt, Antony Braithwaite and Paul Neilsen: 33 oral presentations and 23 posters communicated exciting new p53 findings.

Introduction

Realization of the vital capacity of p53 to ward off malignancy, and the severe consequences of its failure, has drawn unparalleled attention from research scientists to clinicians. However, despite more than 30 years of research and $>60\,000$ papers attempting to understand how it works, fundamental questions remain and tailored p53 therapeutics are still largely at a pre-clinical stage of development. The first Australian p53 Workshop provided the forum for discussing some of the newly uncovered and exciting properties of this remarkable protein (Figure 1).

p53, DNA Methylation and interferon

Defining targets that are transcriptionally activated by stress-primed p53 has largely eclipsed the study of the targets it represses. However, a revolutionary study presented by Andrei Gudkov (Buffalo, NY, USA) has indicated that the transcriptional repression function of p53 is fundamental to genomic stability. Concerted cooperation between p53 transcriptional repression and DNA methylation was found to be critical for silencing non-coding DNA repeats. Unsilenced DNA repeats generate dsRNA elements that mimic viral infection and consequently trigger an interferon (IFN)-mediated apoptotic response. This phenomenon (coined as 'transcription of repeats activates interferon' (TRAIN)) was defined in mouse cancer models and it reveals a new tumour-suppressive role for the IFN response and may provide an explanation for its frequent inactivation in cancers and the observation that defects in the IFN response and loss of p53 cooperate in lymphoma development in genetically modified mice.

Cancer promotion by mutant p53 and p53 isoforms

Mutations in p53 are frequent in human cancers and can confer gain-of-function (GOF) activities that promote tumorigenesis. The mutant $p53^{R172H}$ knock-in (KI) mouse generated in the laboratory of Guillermina Lozano (Houston, TX, USA) models the human hot spot mutant $p53^{R175H}$, and in these mice she demonstrated that the GOF migration and invasion properties of metastatic tumours was promoted by the upregulation of phospholipase Pla2g16. Moreover, in this model, Sue Haupt (Melbourne, Victoria, Australia) showed that GOF was compounded by a loss of PML, resulting in a gender-dependent reduced animal survival associated with a more aggressive tumour spectrum, with an increased incidence of sarcomas and higher levels of mutant p53 protein accumulation. Intriguingly, Tomoo Iwakuma (Kansas City, MO, USA) reported that mutant p53 regulation of the stem-like properties of osteosarcoma cells contributed to tumour progression. In contrast, Carl Walkley (Melbourne, Victoria, Australia) showed that complete loss of p53 in the osteoblast lineage of mice resulted in different osteosarcoma phenotypes compared to shRNA-directed suppression of p53, thus allowing the modelling of different osteosarcoma subtypes.

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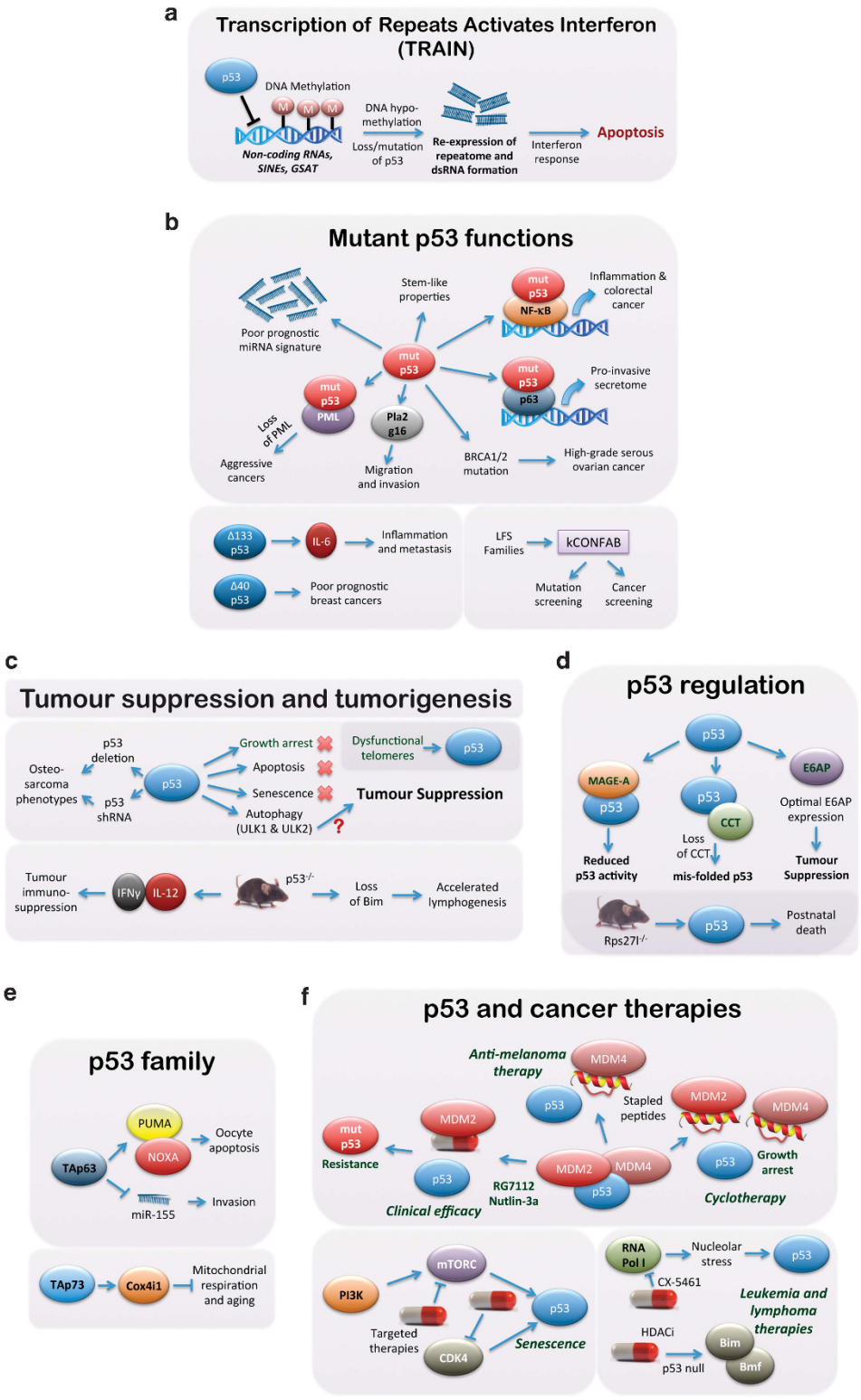

Figure 1 A summary of the exciting new discoveries presented at the first Australian p53 workshop. (**a**) TRAIN describes the fundamental role for p53 transcriptional repression of the 'repeatome'. (**b**) Several novel pathways utilized by mutant p53 or oncogenic p53 isoforms were discovered. (**c**) The workshop highlighted the ongoing progress towards a clear understanding of the critical downstream p53 pathways that impart its tumour suppression. (**d**) The key roles of MAGE-A, CCT, E6AP and RPS27L in their regulation of p53 were presented. (**e**) Novel functions for TAp63 and TAp73 were presented in non-cancer-associated pathways such as fertility and mitochondrial respiration and aging. (**f**) The exciting progress towards the translation of p53-based therapies into the clinic was evident through the diverse range of topics presented at the workshop. Abbreviations: kConFab, Kathleen Cuningham Foundation Consortium for research into Familial Breast cancer; LFS, Li Fraumeni Syndrome.

Moshe Oren's (Rehovot, Israel) discussed a role for mutant p53 in the regulation of the NF-κB pathway, and reported that the p53 mutant KI mice were more susceptible to chemically induced chronic colon inflammation, and to subsequent accelerated development of inflammatory bowel disease-associated colorectal cancer (IBD-CRC). In addition to point mutations in p53, certain p53 isoforms also display GOF activities, and a critical association between isoform-provoked inflammation and tumorigenesis was identified by Antony Braithwaite (Dunedin, New Zealand). In the first mouse model of the human Δ133p53 isoform, he showed that the development of metastatic tumours was associated with chronic inflammation, due to elevated expression of pro-inflammatory cytokines induced by this p53 isoform. The importance of p53 isoforms in human patients was also emphasized by Kelly Avery-Kiejda (Newcastle, New South Wales, Australia), who identified the upregulation of the Δ40p53 isoform in breast cancers with poor patient prognosis. Another potential GOF mechanism of mutant p53 was reported by Giovanni Blandino (Rome, Italy) from a study of 116 human head and neck squamous cell carcinomas (HNSCC), where an eight miRNA signature was highly associated with mutations in p53. This serves as a powerful predictor of poor clinical outcomes in HNSCC patients. Furthermore, Sam Mattiske (Adelaide, South Australia, Australia) showed evidence of mutant p53 driving cancer cell invasion through upregulation of the oncogenic miR-155.

A novel ability of mutant p53 to alter the transcriptome to exert its GOF was also described by Paul Neilsen (Adelaide, South Australia, Australia), who demonstrated that p53 mutants utilized p63 as a molecular scaffold to modulate gene expression and promote invasion through the release of a pro-invasive secretome. Studies of the relationship between mutant p53 and other cancer genes were presented by David Bowtell (Melbourne, Victoria, Australia), who reported that p53 mutation and loss of BRCA1 or BRCA2 function in human high-grade serous ovarian cancer is associated with the deficiency in homologous recombination repair of DNA double-stranded breaks, chromosomal instability and widespread copy number changes. An explicit description of the intricate processes involved in identifying and screening human Li Fraumeni families (bearing germline p53 mutations), and collecting valuable biopsies to the kConFab consortium was contributed by Heather Thorne (Melbourne, Victoria, Australia). A practical request was made to researchers to inform the consortium of which patient information that would be pertinent to collect. Gillian Mitchell (Melbourne, Victoria, Australia) discussed current strategies available for cancer screening and prenatal testing in LFS families.

Tumour suppressor mechanisms

p53 has long been thought to prevent tumour formation by inducing apoptosis or cell cycle arrest/senescence in response to different stress conditions. However, this is now being re-examined. Laura Attardi (Stanford, CA, USA) demonstrated that although a substitution mutant in the first transactivation domain (TAD) of p53 failed to induce cell cycle arrest and apoptosis after DNA damage, it retained full tumour suppressor activity *in vivo*. This was supported by a key mouse genetic analysis by Elizabeth Valente (Melbourne, Victoria, Australia) reporting that the *p21*$^{-/-}$*puma*$^{-/-}$*noxa*$^{-/-}$ mice were not tumour prone, unlike the p53-deficient mice. Interestingly, p53 was found to induce autophagy genes (e.g. ULK1, ULK2), suggesting the possibility that autophagy may contribute to tumour suppression by p53 (Laura Attardi). Andreas Strasser (Melbourne, Victoria, Australia) showed that loss of pro-apoptotic *bim,* but not *bmf,* accelerated lymphomagenesis in p53-deficient mice suggesting, that apoptosis is important in this context. Mark Smyth (Melbourne, Victoria, Australia) used p53-deficient mice or an MCA-induced fibrosarcoma model to reveal that tumour immunosuppression is controlled by adaptive immunity and IFNγ/IL-12. Roger Reddel (Sydney, New South Wales, Australia) explained how dysfunctional telomeres with spontaneous telomere-specific DNA damage, increased to a threshold with successive cell divisions, eventually resulting in p53-mediated senescence.

p53 regulation

The regulation of p53 is critical for its tumour suppressor function and involves crosstalk amongst a complex network of signalling pathways and regulatory proteins. David Meek (Dundee, UK) explored a role for malignancy-associated MAGE-A proteins in the negative regulation of p53. MAGE-A proteins suppressed p53-dependent transcription and growth arrest, and modulated MDM2-mediated ubiquitination of p53. Ygal Haupt (Melbourne, Victoria, Australia) showed that reduced E6AP expression in B-cell lymphoma restricts tumour growth, whereas its complete loss in MEFs bypass senescence by downregulation of p53 levels, suggesting that correct E6AP expression levels are the key to suppress cancer development. Antonio Garcia Trinidad (Glasgow, UK) provided novel insight into the control of the tertiary structure of p53, which was shown to be controlled by the chaperonin CCT. Disruption of the p53–CCT interaction led to the misfolding of p53 into a mutant p53 conformation and acquisition of an invasive capacity similar to those of GOF mutants. A critical role for another regulator of p53, RPS27L, was presented by Yi Sun (Ann Arbor, MI, USA). Deletion of Rps27l led to a p53-dependent postnatal death associated with increased apoptosis of cells in the thymus and bone marrow.

p53 family members

p63 and p73 are ancestral relatives of p53 that have been shown to be integral regulators of development. Gerry Melino (Leicester, UK) reported that TAp73 protected against aging by regulating mitochondrial activity and the prevention of ROS accumulation. He showed that TAp73 controlled mitochondrial respiration through transactivation of the mitochondrial complex IV subunit cytochrome *c* oxidase subunit 4 (Cox4i1). Furthermore, he proposed a new role for TAp73 in metabolism via regulation of enzymes in the glutamine and serine biosynthesis pathways. Clare Scott (Melbourne, Victoria, Australia) showed that TAp63 is essential for the

DNA-damage-induced apoptosis in the oocyte that is dependent on PUMA and NOXA.

p53 and cancer therapy

The potential of 're-awakening' the p53 pathway as a therapeutic approach is currently under intensive investigation. David Lane (Singapore) reported several novel 'stapled' peptides that are highly specific inhibitors of MDM2 and MDM4. These peptides are potent p53 activators, yet unlike Nutlin-3a, they induce p53-dependent growth arrest. Linda Mileshkin (Melbourne, Victoria, Australia) discussed the efficacy of the first MDM2 antagonist, RG7112, (a Nutlin-3a derivative) in the treatment of patients with well-differentiated or de-differentiated liposarcoma. In contrast, Daniel Speidel (Sydney, New South Wales, Australia) highlighted the risks of using Nutlin-3a by the frequent selection for p53 mutation as a mechanism for resistance to Nutlin-3a. Grant McArthur (Melbourne, Victoria, Australia) used preclinical models of oncogene-driven lymphoma and melanoma to demonstrate that pharmacological inhibition of mTORC1 or CDK4 led to the activation of p53 and cellular senescence. Rick Pearson (Melbourne, Victoria, Australia) provided evidence that activation of PI3K/AKT is capable of inducing p53/mTORC1-dependent senescence in primary human fibroblasts, characterized by a secretory phenotype, whereas inhibition of mTORC1 suppressed c-Myc-driven B-lymphomagenesis through p53-dependent senescence. Ross Hannan (Melbourne, Victoria, Australia) reported that accelerated ribosomal RNA gene (rDNA) transcription and nucleolar integrity are necessary for oncogenic transformation. He showed that an inhibitor of Pol I transcription, CX-5461, caused cancer-specific activation of p53 and induction of apoptosis that resulted in improved survival in mouse models of leukaemia and lymphoma. Ricky Johnstone (Melbourne, Victoria, Australia) showed that histone deacetylase inhibitors killed tumour cells through induction of *bim* and *bmf* in the absence of p53, providing an alternative to conventional agents that are less effective when p53 is mutated. Clare Fedele (Melbourne, Victoria, Australia) reported that the p53 pathway is inactivated in the majority of cutaneous melanomas as a result of abnormally upregulated expression of MDM4, identifying it as a promising target for antimelanoma therapy.

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