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Meeting Report

Mutant p53 protein, master regulator of human malignancies: a report on the fifth Mutant p53 Workshop

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Fifth Mutant p53 Workshop, Chigi Palace in Ariccia, Italy, May 2011

Researchers from diverse international backgrounds gathered in May 2011 for the Fifth Mutant p53 Workshop, which took place in the magnificent Chigi Palace in Ariccia, Italy. Some of the highlights are discussed below.

Reprogramming differentiated cells into a state resembling stem cells (induced pluripotent stem cells) is of great interest. Varda Rotter (Rehovot, Israel) showed that wild type (wt) p53 regulates a variety of mesenchymal differentiation programs. Decreased wtp53 levels enhance efficient reprogramming in cells transduced with a combination of Oct4, Sox2 and KLF4. Yet, mouse fibroblasts with a mutant p53 (mutp53) allele (p53R172H, analogous to human hotspot mutation p53R175H) can be efficiently reprogrammed with just Oct4 and Sox2. However, such induced pluripotent stem cells form aggressive tumors in mice, implying that mutp53 endows them with cancer-initiating potential.

Most transcription factors change conformation upon DNA binding. Thanos Halazonetis (Geneva, Switzerland) generated a mutp53 that was stable in solution, yet retained binding to p53 response elements (p53RE). Upon binding DNA, this mutant underwent a conformational switch, which slowed down substantially the off-rate for dissociation of p53 from the p53RE, underscoring the importance of the off-rate in determining how tightly p53 binds to a specific sequence. Alan Fersht (Cambridge, UK) described electron microscopy studies, revealing that the DNA binding domain (DBD) can adopt at least four different conformations. Fersht also demonstrated that p53 acetylation on Lys120 changes the balance between binding to non-specific DNA and to p53RE. He proposed a model whereby p53 slides along DNA with its C-terminus acting like a train or a monorail, and the DBD hopping on and off until a p53RE is encountered. Zippora Shakked (Rehovot, Israel) presented high-resolution crystal structures of the DBD of wtp53, several tumor-associated p53 mutants and rescued proteins incorporating second site suppressor mutations. Comparative analysis of these proteins in their free and DNA-bound states provides a structural basis for understanding the mutational loss of wtp53 function. This may eventually enable restoration of p53 function by small molecules. Related to this issue, Frederic Rousseau (Brussels, Belgium) reported that a conserved sequence in the hydrophobic core of the DBD becomes exposed in the mutated protein, promoting co-aggregation of mutp53 with wtp53, as well as with p63 and p73.

Marianne Farnebo (Stockholm, Sweden) described a new gene – Wrap53 (for WD40-encoding RNA antisense to p53) – at the p53 locus. The Wrap53 transcript initiates within exon 1 of *TP53* and is transcribed in the antisense direction relative to *TP53*. Down-modulation of Wrap53 decreases p53 levels, highlighting a novel p53-regulatory mechanism.

David Lane (Singapore) used zebrafish to study the p53 pathway *in vivo*. Inherently unstable zebrafish mutp53 is stabilized by stress signals; this stabilization persists for extended periods because mutp53 cannot activate Mdm2, which targets p53 for degradation. Furthermore, mutp53 is elevated in some very early and overtly normal clones within human epithelia, suggesting that its accumulation does not require frank malignancy. Thus, mutp53 is similarly regulated in both human and zebrafish tissues.

Mutp53 proteins exert gain-of-function (GOF) by modulating gene expression. Giovanni Blandino (Rome, Italy) showed that mutp53 modulates the expression of microRNA-128-2 by binding to the putative promoter of its host gene, *ARPP21*. miR-128-2 expression in lung cancer cells inhibits apoptosis and confers increased resistance to chemotherapy agents.

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Sumitra Deb (Richmond, VA, USA) reported that the transcriptional activity of mutp53 depends on the integrity of its transactivation domain. Biological effects of mutp53 were discussed by Carol Prives (New York). Using three-dimensional cultures, she showed that mutp53 depletion in aggressive breast cancer-derived cells reverts malignant-appearing cells into more benign cells, which form acinus-like structures. Gene expression analysis highlighted molecular pathways necessary for the effects of mutp53 on breast tissue architecture. Evidence linking mutp53 to EGF receptor (EGFR) family proteins, critical players in breast tumorigenesis, was presented by Karen Vousden (Glasgow, UK). She reported that mutp53 activates EGFR/integrin signaling, promoting invasion and causing loss of cell movement directionality. Giulia Fontemaggi (Rome, Italy) reported that Id4 (inhibitor of differentiation 4), product of a mutp53 target gene, binds mRNAs encoding pro-angiogenic cytokines and modulates their amounts. Furthermore, Id4 binds EGFR mRNA; Id4 depletion causes downregulation of EGFR protein, whereas mutp53 overexpression increases EGFR mRNA translation. Gianluca Bossi (Rome, Italy) reported that mutp53 (R273H) negatively regulates IL-1 Receptor Antagonist (IL-1Ra) expression; depletion of p53R273H elicited a significant increase in IL-1Ra in the culture medium of cancer cells. The involvement of mutp53 in regulation of gene expression was also discussed by Elena Martynova (Milan, Italy). Using ChIP-Seq followed by expression profiling, she found that mutp53 is associated with DNA *in vivo* in keratinocytes and its DNA binding pattern overlaps only mildly with that of p63. Genrich Tolstonog (Hamburg, Germany) also discussed mutp53 binding to DNA. Using a microarray followed by ChIP-chip analysis, he obtained evidence that in glioblastoma cells mutp53 frequently interacts with G/C-rich DNA around transcriptional start sites, residing within active chromatin and associated with phosphorylated RNA pol II.

Post-translational modifications are key regulators of wtp53 activity and this also holds good for mutp53 GOF. Giannino Del Sal (Trieste, Italy) showed that prolyl isomerase Pin1 enhances mutp53 biochemical activities, fully unleashing its GOF properties. This occurs through inhibition of the anti-metastatic transcriptional activity of p63 and induction of a specific transcriptional program associated with poor clinical outcome in breast cancer. Similarly, Silvia Di Agostino (Rome, Italy) reported that Polo-like kinase 2 (PLK2)-mediated phosphorylation of mutp53 enhances its GOF activity, reflected by increased proliferation and chemoresistance of cancer cells. A novel GOF mechanism was described by Hilla Solomon (Rehovot, Israel) who reported that conformational mutations within the Zn^{+2} binding region of p53 (e.g. p53R175H, p53H179R) promote binding to the BTG2 protein attenuating its function and augmenting the oncogenic activity of mutant H-Ras. In contrast, DNA contact mutations (p53R248Q, p53R273H) trigger a strong functional interaction with NF-κB, resulting in prominent enhancement of a cancer progression gene signature. Ge Zhou (TX, USA) reported that AMPK (AMP-dependent kinase) is regulated by mutp53: through direct interaction with AMPK, mutp53 inhibits its activation in head and neck squamous cell carcinoma. Reduced mutp53 levels elicit AMPK activation, attenuating cell growth, and protein and lipid synthesis.

Mouse models continue to provide valuable insights into the *in vivo* mutp53 functions. Guillermina Lozano (TX, USA) found that mutp53 is inherently unstable, but is stabilized by genotoxic agents or reactive oxygen species. Notably, feeding mice with the antioxidant *N*-acetylcysteine prevents mutp53 stabilization. Surprisingly, Lozano found that tumors harboring mutp53 are more sensitive to doxorubicin than their wtp53 counterparts. Analysis after doxorubicin exposure revealed that wt tumors underwent senescence, presumably sparing them from drug-induced cell death, whereas mutp53 tumors actually underwent massive apoptosis. Hein Te Riele (Amsterdam, The Netherlands) introduced mutp53 alleles in mouse embryonic stem cells by oligonucleotide-directed gene modification. He reported that RasG12V expression in mutp53 (p53R245Q and p53C173F) mouse embryonic fibroblasts increased the levels of mutp53 and stimulated its nuclear localization. Thus, oncogenic signals can augment mutp53 GOF. Shunbin Xiong (TX, USA) discussed the ability of mutp53 to promote metastasis of osteosarcoma cells in a $p53^{R172H/+}$ mouse model. Microarray analysis identified a set of genes expressed differentially between osteosarcomas of $p53^{R172H/+}$ and $p53^{+/-}$ mice, implicating the adipocyte phospholipase A2 as a master regulator of tumor progression and invasion. Stefano Piccolo (Padua, Italy) showed that mutp53 promotes metastasis by opposing p63. The cytokine TGFβ allows the exploitation of this metastatic program by cooperating with mutp53 to promote the formation of a stable ternary complex between Smads, mutp53 and p63, disabling p63's transcriptional capacity. Wolfgang Deppert (Hamburg, Germany) reported that WAP-T mice, in which SV40 large T is specifically expressed in the mammary gland, develop low metastasizing invasive mammary carcinomas ($<10\%$). However, the metastatic capacity is markedly increased on a mutp53 (p53R245W or p53R270H) background. Gene expression analysis revealed consistent Ceacam1 downregulation in WAP-T/mutp53 mice; remarkably, deletion of Ceacam1 in WaP-T mice strongly increased metastasis ($>60\%$). Yuan Zhu (Ann Arbor, MI, USA) described a series of brain tumor models associated with different p53 genotypes: p53-null, p53R172H, and an in-frame p53 mutation lacking exons 5 and 6 (p53ΔE5,6), revealing a critical role of neural stem cells and transit-amplifying progenitor cells in gliomagenesis. Remarkably, mutp53 represses p53-independent apoptosis in the developing brain. Moshe Oren (Rehovot, Israel) reported that mutp53 augments NF-κB activity. In cultured cancer cells mutp53 significantly extended the duration of NF-κB activation in response to TNFα, rendering the response more 'chronic'. In agreement, in a mouse model of inflammation-associated cancer, mutp53 enabled sustained inflammation, resulting in accelerated emergence of invasive tumors. Curtis Harris (Bethesda, MD, USA) showed that chronic inflammation, such as in ulcerative colitis, is associated with cytokine secretion and elevated levels of nitric oxide. While in normal cells this leads to DNA damage, in mutp53-expressing colon lesions of ulcerative colitis patients it elicits more vigorous nitric oxide secretion, causing massive DNA damage and facilitating malignancy. Ygal Haupt (Melbourne, Australia) combined *in vitro* and *in vivo* models to demonstrate physical and functional links between mutp53 and PML. He reported that PML enhances the GOF effects of mutp53, whereas PML loss

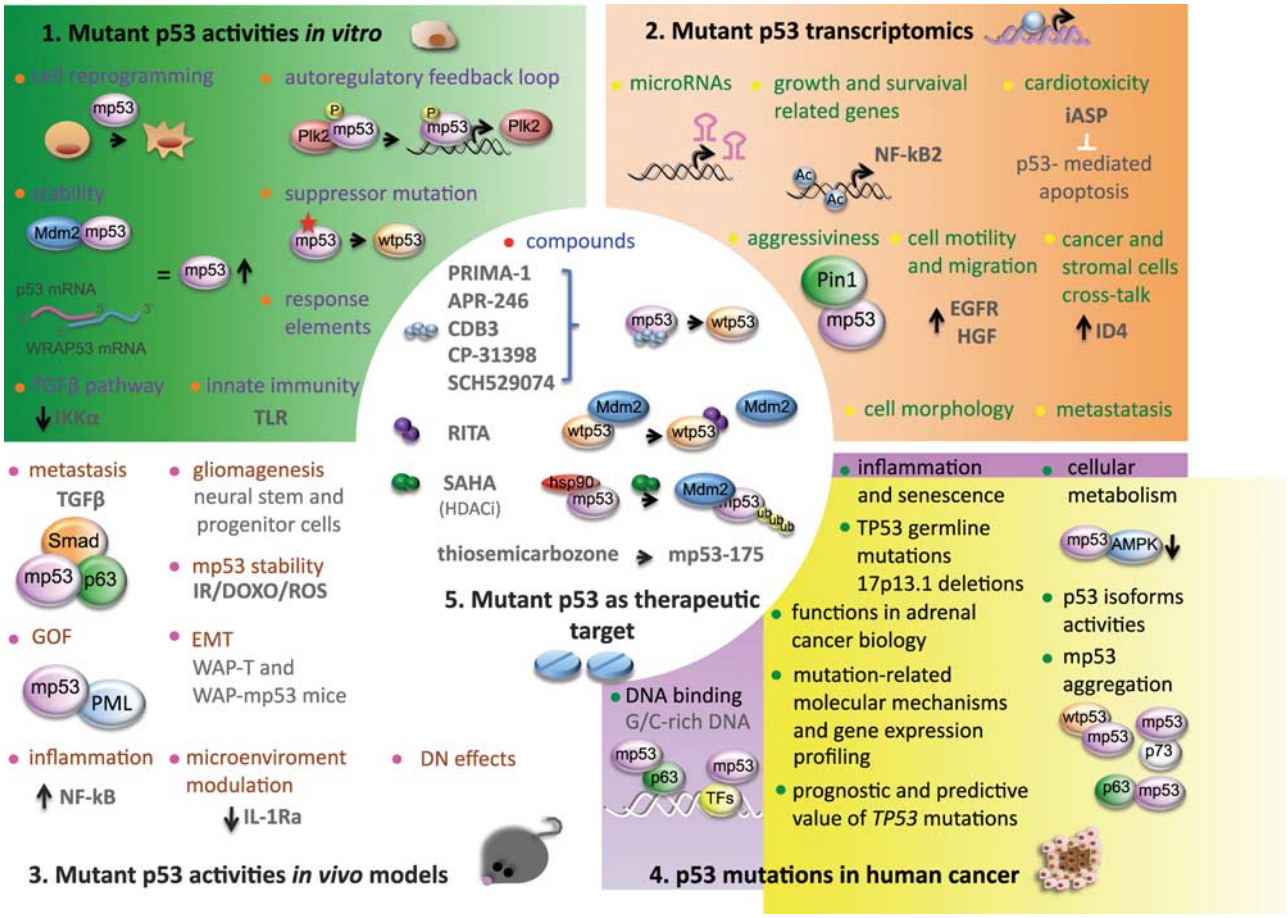

Figure 1 Concepts and findings discussed in the Mutant p53 Workshop. The main areas covered were mutp53 activities *in vitro* (**1**), mutp53 transcriptomics (**2**), *in vivo* models to study the contribution of mutp53 to tumor development (**3**), the clinical significance of p53 mutations in cancer patients (**4**) and the potential of mutp53 as a target for novel anti-cancer therapies (**5**)

alters the tumor spectrum of p53R172H knock-in mice. Kanaga Sabapathy (Singapore) discussed knock-in mouse strains expressing varying levels of p53R246S and reported that this mutant exerts a dominant negative effect over wtp53 *in vivo*, in a cell type-specific and mutp53 dose-dependent manner.

Germline mutations in the *TP53* gene underlie most cases of the Li-Fraumeni cancer predisposition syndrome (LFS). As discussed by Pierre Hainaut (Lyon, France), individuals with germline *TP53* mutations demonstrate a biphasic disease risk. The 'childhood phase' displays a tendency to develop cancer types that are rare in the general population, whereas 'adult phase' cancers are predominated by more 'common' cancer types, typically with early onset. The risk of childhood *versus* adult cancer in such individuals depends on the particular *TP53* mutation as well as on modifiers, including polymorphisms in *TP53* and in genes encoding p53 regulators such as Mdm2.

David Malkin (Toronto, ON, Canada) described the search for modifiers (single nucleotide polymorphisms, copy number variations) that affect the clinical course of LFS. Constitutional deletions across 17p13.1, at or near the *TP53* locus, were found to confer distinct cancer or developmental delay/congenital anomaly phenotypes. Can the growing knowledge about mutp53 benefit cancer patients? Malkin provided a positive answer, showing that implementation of a comprehensive surveillance protocol for *TP53* mutation carriers significantly improved the survival of LFS patients. Gerard P Zambetti (Memphis, TN, USA) described the International Pediatric Adrenal Tumor Registry and Tissue Bank, aiming to explore p53 function in adrenal cancer and provide a resource facilitating translational research. Importantly, new data bears promise for imminent improvement of the clinical management of LFS-associated adrenal cancer.

TP53 somatic mutations can have prognostic value in breast cancer. Anne-Lise Børresen-Dale (Oslo, Norway) reported that different functional classes of p53 mutations are associated with different gene expression profiles and differential deregulation of distinct pathways. This may instruct the development of novel targeted therapies based on the particular type of p53 alteration. Magali Olivier (Lyon, France) discussed a retrospective analysis of the prognostic and predictive value of *TP53* mutations in the BIG02-98 randomized phase III trial of adjuvant chemotherapy, in which patients were treated with doxorubicin-based regimens alone or combined with docetaxel. Although not statistically significant, a trend for better response to docetaxel was observed for p53 truncating mutations.

While p53 can be either wt or mutant, the single *TP53* gene can give rise to multiple protein isoforms. JC Bourdon (Dundee, UK), discoverer of many of those isoforms, reported that expression of one particular isoform, p53γ, modifies the prognostic value of p53 mutations in breast cancer.

Drugs restoring tumor suppressor functionality to mutp53 will potentially confront the cancer cell with high wtp53 activity levels, thus providing a large therapeutic window either as monotherapy or in combination with genotoxic chemotherapy. Klas Wiman (Stockholm, Sweden) reported studies with the small molecules PRIMA-1 and PRIMA-1Met (APR-246). These compounds restore wt conformation in cells harboring mutp53, suppress tumor growth *in vivo* and synergize with chemotherapeutic drugs. APR-246 is already in phase I clinical trial in patients with hematological malignancies or prostate cancer. Galina Selivanova (Stockholm, Sweden) reported that RITA, which blocks p53/Mdm2 interaction, also binds mutp53 and partially restores p53 functionality. Consequently, RITA suppresses the growth and promotes the apoptotic death of diverse mutp53-expressing cancer cells. Ute Moll (New York, NY, USA) reported that HSP90 binds mutp53, inhibiting the E3 ligases Mdm2 and CHIP, and contributing to cancer-specific mutp53 stabilization. Pharmacological inhibition of HSP90 with the drug 17AAG disrupts this interaction, liberates mutp53 and reactivates endogenous Mdm2 and CHIP to promote mutp53 degradation.

Individual cancer-associated p53 mutations can differ greatly with regard to their impact on protein properties. Arnold Levine (Princeton, NJ, USA) discussed a compound belonging to the thiosemicarbozone family that selectively targets the p53R175H hotspot mutant, restoring its wt structure and activity. This compound also promotes efficient apoptosis of cancer cells expressing p53R175H and kills p53R172H knock-in mice with evidence of extensive apoptosis, at a dose not toxic for wt mice. Notably, unlike other p53 mutation xenografts, those derived from p53R175H human tumors are selectively inhibited.

Xin Lu (Oxford, UK) reported that iASPP, an anti-apoptotic protein that inhibits both p53-dependent and p53-independentapoptosis, has a protective role against chemotherapy-induced cardiotoxicity. Remarkably, spontaneous mutations in iASPP are associated with cardiocutaneous disorders in mice and in calves.

Altogether, the Workshop documented impressive progress toward elucidating the biochemical and biological activities of mutp53 and its relevance to cancer. The emerging picture of the mutp53 universe is illustrated in Figure 1.

Conflict of Interest

The authors declare no conflict of interest.

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