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Meeting Report

Cycling to death, in the Tyrolean Alps

FJ Bock[1], K Brinkmann[2], YW Chan[3] and LL Fava*,[1]

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EMBO workshop, Cycling to death 23–27 January 2013, Obergurgl, Austria

In January 2013, over 100 researchers from 18 different countries gathered in Obergurgl, a remote ski resort in the beautiful Tyrolean Alps in Austria. The inspiring idea of this EMBO-funded workshop was to bring together two scientific communities: researchers in the fields of cell cycle and cell death. These two mature fields have been considered distinct for many years and researchers active in one often tend to neglect aspects of the other. It is well established though that cell cycle defects can trigger cell death and it is becoming increasingly clear that some cell death paradigms can only (or preferentially) be found within certain cell cycle 'windows'. Furthermore, defects in both pathways are hallmarks of cancer, amply justifying the concept of an interdisciplinary discussion platform. The quality of the talks and posters, the relaxed and informal atmosphere, enhanced by the beautiful weather and the stunning location, guaranteed the effectiveness of the interchange. Here we summarize a selection of the key contributions in the various fields and apologize to the participants neglected due to space constrains.

DNA damage response and cell cycle

Yosef Shiloh (Tel Aviv) reported the results obtained in a large collaborative effort to define in great detail the cellular responses to ionizing radiation (IR). A combination of RNA-seq and ChIP-seq allowed fine dissection of the transcriptional response, which was largely ATM-dependent. A strong, transcription-mediated component was found to accompany central branches of the DNA damage response (DDR), which were known to be mediated by protein post-translational modifications. This is particularly true for DNA damage-induced cell death. **Libor Macurek** (Prague) discussed how mutations in Wip1, a phosphatase with oncogenic properties, could affect the DDR of cells carrying wild-type (wt) p53. Early termination of Wip1 in exon 6, found both in cancer cell lines and in patient samples, results in a catalytically functional protein with increased stability, which counteracts p53 and impairs G1 arrest upon DNA damage. Interestingly, Wip1 mutations were found in peripheral blood of cancer patients, suggesting that they may predispose them to cancer. **Rene Medema** (Amsterdam) described the results of a phosphatome-wide RNAi screen aimed to identify regulators of the G1 checkpoint triggered by IR. Strikingly, depletion of the phosphatase PP4 imposes a persistent p53-dependent G1 arrest via increased p21 transcription. This effect depends on defective dephosphorylation of the transcriptional cofactor KAP1 that is normally catalyzed by PP4 and is required for restoring p21 repression. **Anton Gartner** (Dundee) described the use of *C. elegans* isogenic DNA-repair deficient strains combined with deep sequencing of the worm's genomes to determine the rate of accumulating germline mutations over time. Interestingly, the use of genotoxic chemicals triggered rearrangements comparable to those found in human cancer, thus defining a toolbox for studying genotoxicity.

Mitotic division

The Spindle Assembly Checkpoint (SAC) is central to generate a cell cycle delay upon mitotic defects. **Jonathon Pines** (Cambridge) described the use of live-cell imaging and biochemical methods to assess the activity of the SAC quantitatively. The data indicate that the SAC is not activated/inactivated as an all or none switch as previously appreciated, but rather senses stimuli of various strength in a graded fashion, leading to differing extents of signaling originating at kinetochores. This results in variable inhibition of the key downstream target, APC/C^{Cdc20}. **William Earnshaw** (Edinburgh) presented the use of engineered centromeres on synthetic chromosomes to explore the epigenetic 'landscape' of the vertebrate kinetochores. Whereas kinetochores appeared plastic in their response to changes of chromatin modifications, increasing or decreasing levels of transcription of alphoid-DNA unexpectedly resulted in their functional disruption. Supporting the notion that centromeric DNA is actively transcribed, RNA polymerase II could be detected at kinetochores during mitosis. **Arne Lindqvist** (Stockholm) employed an automated analysis of immunofluorescence data (obtained on homogeneous cell populations grown on micropatterns) to infer the cell cycle position of fixed cells avoiding cell contact-dependent signaling events that may affect cell cycle dynamics. This information was related to molecular markers for activation of the

[1]Division of Developmental Immunology, BIOCENTER, Medical University Innsbruck, Innsbruck, Austria; [2]Medical Faculty, Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany and [3]London Research Institute, Clare Hall Laboratories, Cancer Research UK, South Mimms, UK
*Corresponding author: LL Fava, Division of Developmental Immunology, Medical University of Innsbruck, Biocenter, Innsbruck 6020, Austria. Tel: +0043 5129 0037 0369; E-mail: luca.fava@i-med.ac.at

mitotic entry network (such as Plk1 activation and CyclinB1 phosphorylation) that could be detected in the nucleus as early as 5 h before mitotic entry. This suggests that activation of the mitotic entry network occurs first in the nucleus.

Mitotic defects result in aberrant ploidy, which has a profound impact on cell physiology and is often associated with cancer. **Marion Libouban** (Rome, Sotillo group) described the impact of *in vivo* Mad2 overexpression–leading to chromosome missegregation and aneuploidy on lung adenocarcinomas driven by KRAS. Strikingly, Mad2 accelerated tumorigenesis in a p53 wt background, whereas it confers a disadvantage to tumors in $p53^{+/-}$ mice. These unexpected findings fueled intense debate on the role of aneuploidy in tumorigenesis.

Cell death

Sharad Kumar (Adelaide) talked about the developmental cell death regulation in *Drosophila* in response to the hormone ecdysone. Whereas cell death occurring during the removal of the salivary gland was dependent on Dronc (the *Drosophila* Caspase 9 homolog), Ark1 (Apaf1) and caspase activity, midgut removal required autophagy to mediate cell death. Thus, in different tissues the same cell death trigger can result in execution of distinct pathways such as apoptosis or autophagy. Furthermore, temporal ecdysone-mediated cell death was epigenetically controlled through histone methylation. **John Silke** (Melbourne) presented the crystal structure of MLKL, a mediator of necroptotic cell death, and showed that MLKL harbors a pseudoactive site, unable to catalyze phosphotransfer. Furthermore, RIP3-dependent phosphorylation of MLKL is an essential step during necroptotic signaling and mutations in MLKL mimicking constitutive phosphorylation cause RIP3-independent necroptotic signaling. As predicted from recent work, $MLKL^{-/-}$ mice are resistant to the induction of necroptosis. **Martin Leverkus (**Mannheim) demonstrated that cFLIP is critical to protect cells from autocrine TNFα-induced apoptotic cell death *in vitro* and *in vivo*. Mice lacking cFLIP in the epidermis died at embryonic day 10, while epidermal-specific inducible cFLIP deletion in adult mice caused a severe skin inflammatory phenotype, associated with an early hyperproliferation and later destruction of the epidermis. Moreover, deletion of cFLIP from primary keratinocytes resulted in the induction of TNFα and spontaneous cell death due to autocrine TNFα-induced apoptotic signaling.

Cancer biology

Frederic de Sauvage (San Francisco) presented data concerning the treatment of basal cell carcinoma, which harbor increased Hedgehog activation, with GDC-0449/Vismodegib, an inhibitor of Smoothened. The strong response seen in tumors where the Hedgehog pathway is mutated is thought to be due to decreased proliferation rather than excessive tumor cell death.

Guillermina Lozano (Houston) provided insights into the regulation and function of mutant p53 (carrying the structural mutation R172H, often found in cancer) using knockin mouse models. $p53^{R172H}$ mice are able to rescue the embryonic lethality triggered by MDM2 deficiency, due to the loss of wt function of this mutation. On the other hand, $p53^{R172H}$ mice are sensitized to develop metastatic tumors in the absence of MDM2.

Andreas Strasser (Melbourne) presented a possible explanation for the conundrum that p53-deficient cells still undergo apoptosis after genotoxic stress. These cells upregulate the BH3-only proteins Bim and Bmf, and tumor cells from mice with combined deficiency of p53 and Bim as well as Bmf knockdown were almost completely protected from genotoxic cell death. Bim deficiency also led to earlier onset of thymic lymphomas in $p53^{+/-}$ mice, alleviating the pressure to lose the second p53 allele. In addition, preliminary data on developmental defects in BAX/BAK/BOK-triple deficient embryos were also reported.

Cycling to death

Helen Piwnica-Worms (St. Louis) discussed a novel function for 14-3-3σ in DDR. Besides its role in counteracting CyclinB1–CDK1 accumulation in the nucleus, 14-3-3σ restrains CDK1 activation via Chk1. Chk1 function was compromised in cells lacking 14-3-3σ due to decreased transcription of S-phase checkpoint mediator protein Claspin, leading to increased basal DNA damage in unchallenged cells. **Samuel Sidi** (New York) presented a mechanism leading to Caspase-2 activation upon IR combined with inhibition of Chk1. In human cancer cells, Nucleophosmin (NPM1) bound to the Caspase-2 activator PIDD in response to IR and prevented its phosphorylation by ATM. As NPM1 binding to PIDD was abolished by Chk1 inhibition, NPM1 might provide the inhibitory activity restraining Caspase-2 activation upon DNA damage that is released upon concomitant inhibition of Chk1.

Marcos Malumbres (Madrid) used genetic inhibition of the APC/C to study cell death upon mitotic arrest. Whereas necroptotic regulators appeared dispensable, pan-caspase inhibition and inhibition of MOMP delayed death. Additionally, evidence was presented suggesting that pathways other than apoptosis may be involved in cell death upon prolonged mitotic arrest.

Traditionally, the meeting, now in its fourth iteration, ends with a ski race and we could not summarize it better than John Silke: 'among the two groups there were no clear winners; based on the evidence, the cyclers are no skiers, while the cell death researchers risked more than their cells. The real winner was the meeting itself, the cross-disciplinary focus was a great innovation and the two groups did their utmost to outdo each other with their sociability and their science. It is always hard to predict the long-term impact of such an event but we would hazard a guess, based on the enthusiasm within the meeting itself, that we will see many more. Make sure to book your flight early and bring your skis!'

Conflict of Interest

The authors declare no conflict of interest.

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