

Meeting Report

The 2nd International Workshop 'Novel Therapeutic Strategies in Cancer' in St-Petersburg

Cell Death and Disease (2013) 4, e475; doi:10.1038/cddis.2012.209; published online 24 January 2013

Workshop on Novel Therapeutic Strategies in Cancer, St-Petersburg, Russia, 11–12 September 2012

Cell death and differentiation during development

The 2nd workshop 'Novel Therapeutic Strategies in Cancer' sponsored by the Russian Government program, supporting the research in Russian universities under the guidance of the world-leading scientists was held in September 2012. Presentations reviewed recent findings of the leading scientists from Russia, Europe, US and China on various aspects of cell death regulation and aging in cancer, cancer metabolism and stem cell research.

The opening keynote lecture by Douglas R Green (Memphis, USA) was focused on elucidating complex mechanisms of cell death in embryonic development via the opposing functions of the pro-apoptotic protease, caspase-8. Caspase-8 is known to promote cell death but also mediates cell survival, preventing the TNF-induced necrosis. The latter function is essential for proper development of mouse embryos after triggering the TNF signaling. The lethality of caspase-8-deficient embryos was rescued by knockout of receptor interacting protein kinase-3, which mediates programmed necrosis, or 'necroptosis'. The knockout experiments in mice showed that caspase-8 prevented necrosis by formation of the proteolytically active complex that contained FLICE-like inhibitory protein long.

Another aspect of cell proliferation control during the aging of brain was discussed in the report of Grigori Enikolopov (Cold Spring Harbor, USA; Moscow, Russia). The process of neuronal self-renewal takes place in hippocampus of the adult brain and is ensued by differentiation of neural stem cells into astrocytes. Aging is associated with a continuous decline in the number of new neurons. He presented recent findings on the mechanisms, which control division and differentiation of neural stem cells during aging. It turned out that the main mechanism driving the age-related decrease in adult hippocampal neurogenesis was the disappearance of neural stem cells via their conversion into mature astrocytes. Therefore, age-related deficit may be compensated by increasing the number of stem cells or number of neurons produced.

Cell cycle control in stem cells and tumors

Irina Neganova (Newcastle, UK) discussed the role of CDK1 and CDK2 in control of division and apoptosis in human ES

cells (hESC). The data shown by her indicated that CDK1 and CDK2 exerted different functions in hESC. Importantly, ESC of human and mouse origin differed in their requirements for Cdk2; being critically important for the maintenance of pluripotency in human cells. Another interesting feature of hESC was that the knockdown of CDK1 resulted in S/G2 arrest and promoted apoptosis, whereas the knockdown of CDK2 elicited the G1 phase arrest, DNA damage checkpoint activation and differentiation of hESC.

Alexander Ishov (Gainesville, USA) described a novel p53-independent function of ubiquitin-specific processing Protease-7 (USP7). The study showed that UPS7 together with Daxx stabilized the mitotic E3 ubiquitin ligase, Checkpoint with Forkhead and RING finger (CHFR). Importantly, one of the substrates of CHFR is mitotic kinase, Aurora A. Therefore, cells with depleted USP7 also exhibited attenuated levels of CHFR, resulting in the accumulation of cyclin B and Aurora A and hence, multi-polar mitosis. High levels of USP7 induced resistance of tumor cells to taxane-based chemotherapy. The latter was attenuated by the pharmacological inhibition of Aurora A. Thus, USP7 represents a potential prognostic factor for taxane response in patients, and Aurora A inhibitors may improve taxane therapy in case of the USP7-dependent resistance.

Valery Pospelov (St. Petersburg, Russia) presented the data illustrating different effects of autophagy on proliferating and senescent cells. Treatment of transformed rat fibroblasts with HDAC inhibitors induced their size growth (hypertrophy) and cellular senescence. Interestingly, these senescent cells also exhibited activated DNA damage response (DDR). Rapamycin, an inhibitor of mTORC1, which was shown to decelerate cellular senescence, also decreased DDR. In addition, the senescent cells underwent autophagy, which protected cells from hypertrophy. However, treatment of proliferating cells with a pp242, another inhibitor of mTORC1/mTORC2 complexes, alone or in combination with HDAC inhibitors led to the autophagy-mediated cell death.

Nickolai Barlev (Leicester, UK; St. Petersburg, Russia) discussed functions of lysine methyltransferase Set7/9 in cell cycle arrest after DNA damage. The results he presented argue that Set7/9 is critical for transition of tumor cells through G1/S phase upon doxorubicin-induced genotoxic stress.

Set7/9 was required for expression of a number of cell cycle-dependent DNA repair genes and regulated both the activity and stability of the Cdk2/Cyclin E complex. Therefore, pharmacological inhibition of Set7/9 should cause attenuation of cyclin E/Cdk2 levels, and could hence represent a novel approach for anti-cancer therapy.

p53 and its family in oncogenesis and development

Loss of the p53 function is one of primary causes of cellular transformation. Xin Lu (Oxford, UK) discussed reactivation of p53 as a strategy to induce apoptosis in melanoma cells. Mutations in p53 occur less frequently in this type of cancer compared with others, however it is often inactivated. In line with this, p53 agonists such as nutlin have shown promise as potential therapies. Another appealing target for p53 reactivation is iASPP, an evolutionarily conserved inhibitor of p53. Importantly, it is thought to be a key inhibitor of p53 in highly malignant human melanoma cells. Targeting iASPP may therefore provide an opportunity to restore apoptotic function in malignant melanoma cells, and may have a role in future clinical mono- and combination therapies.

Another important aspect of p53 activity was highlighted in the presentation of Karen Vousden (Glasgow, UK). She described the role of p53 in cell survival and metabolic adaptation. Particularly, p53 expression was shown to help cells to survive serine starvation. Serine starvation induces *de novo* serine synthesis by upregulating the expression of enzymes in the serine synthesis pathway, causing the diversion of glycolytic intermediates and disruption of glycolysis. Interestingly, p53 was not required for the activation of the serine synthesis pathway, but allowed cells to undergo this metabolic adaptation. A p53-inducible protein TIGAR protects cells from cell death acting as a fructose-2,6-bisphosphatase. The TIGAR activity promotes NADPH production, protecting the cell from ROS-associated apoptosis and autophagy. To investigate possible functions of TIGAR in malignant transformation a TIGAR null mouse was generated and is currently being actively studied.

Gerry Melino (Rome, Italy; Leicester, UK) discussed the involvement of the p53 family member, p73, in metabolism and senescence. Unlike p53, the function of p73 is restricted to specific tissues. Also, p73 was shown to be required for the development of neural system. He presented the new data on the Tap73-null mice. These mice showed significant phenotype of premature aging and obesity. In addition, redox balance and glutamine pathway were affected due to the attenuation of glutaminase type 2 expression. The latter was found to be a direct transcriptional target of Tap73. The phenotype of Tap73-null mice clearly differed from the one of deltaNp73 knockouts (an alternatively spliced isoform of TP73, lacking the amino terminus). These data provide firm genetic evidence for a distinct role of the Tap73 protein in metabolism.

Two reports discussed the regulation and functioning of TP63, another member of the p53 family. TP63 gene is primarily expressed in epithelium, and is critical for epithelial development. Similar to p73, there are several isoforms of TP63 known to date. The TAp63 α isoform, lacking some of its carboxyl terminus, serves as a quality control factor in the

female germ line. This suggests that TAp63 α 's activity is under tight control of an inhibitory mechanism. Volker Doetsch (Frankfurt, Germany) presented their structure- function data, suggesting that inhibition was achieved by blocking the tetramerization domain via the transactivation inhibitory domain. DNA damage-induced phosphorylation of TAp63 α disrupted the repression and triggered tetramer formation, resulting in a ~20-fold higher DNA-binding affinity.

Paola Tucci (Calabria, Italy) described the effect of p63 on epithelial to mesenchymal transition (EMT). The study showed that p63 (both TAp63 and Δ Np63 isoforms) regulated expression of miR-205 in prostate cancer cells. The latter, in turn, targeted several markers of EMT, including ZEB1 and vimentin. Importantly, p53 mutants inhibited expression of both p63 and miR-205, and hence, cell migration. In accordance with these *in vitro* data, Δ Np63 or miR-205 significantly inhibited the incidence of lung metastasis *in vivo* in a mouse tail vein model. Similarly, one or both components of the p63/miR-205 axis were absent in metastases or colonised lymph nodes in a set of 218 human prostate cancer samples. Collectively, the data suggest that p63/miR-205 may be a useful clinical predictor of metastatic behaviour in prostate cancer.

Tumor microenvironment

It is commonly accepted that a certain environment is required for initiation and progression of tumor growth. Yufang Shi (Shanghai, China; New Jersey, USA) talked about the recruitment of mesenchymal stem cells (MSC) into growing tumors. MSCs form a substantial part of tumor cells environment. MSCs isolated from spontaneous mouse lymphomas (L-MSC) enhanced tumor growth in comparison with bone marrow MSCs. Depletion of monocytes/macrophages, but not neutrophils, completely abolished tumor promotion of L-MSCs. Furthermore, L-MSCs expressed high levels of CCR2 ligands, which apparently are important for monocyte/macrophage accumulation. Importantly, L-MSC-mediated tumor promotion was largely abolished in CCR2 -/- mice. Therefore, these findings demonstrate that in an inflammatory environment, tumor-resident MSCs promote tumor growth by recruiting monocytes/macrophages.

Evgenia Stepanova (Moscow, Russia) reviewed the efficacy of various anti-angiogenic compounds from plants. These compounds were highly cytotoxic, induced apoptosis of tumor cells, and inhibited NF- κ B activity as well as angiogenesis *in vitro*. Many tumors acquire microcirculation in an alternative, angiogenesis-independent manner via so-called 'vasculogenic mimicry' (VM). It was found that the presence of VM in patients with renal carcinoma correlated with the increased risk of metastases and therefore poor survival outcome.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements. This work was supported by funding from RFBS (10-04-01234), grants from Federal Programme for Scientific training in Innovative Russia (Grant 8280), Russian Government Programme for the Recruitment of the leading

scientists into the Russian Institutions of Higher Education (11.G34.31.0069), and grant from MCB from the Russian Academy of sciences.

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