around the latter – all together revealing a radial-concentric order. The lamin B1-positive nuclear contour becomes irregular and lamin forms folds in nuclear interior reaching the nucleoli. Actin ring around the nucleus becomes thicker, while its extending cytoplasmic fibers less tense. Full suppression of both syntheses by high dosage/prolong AcD or a-amanitin brings to disorganization of the radial-concentric nuclear order. Conclusions: The links of the perinucleolar and lamin-associated heterochromatin with inner nuclear compartments are involved in topological coordination between the ribogenesis and mRNA maturation, where the radial tension of the actin cytoskeleton exerted via concentric elasticity of the nuclear lamin is part of this regulation.

doi: http://dx.doi.org/10.7124/bc.0009F8

S-4. Exploring the features of Burkitt's lymphoma-associated t(8;14) translocations generated via a CRISPR/Cas9-based system

A. A. Shmakova[1,2], D. Germini[1,2], Y. S. Vassetzky[1,2,3]

[1] UMR 8126, CNRS, Univ. Paris-Sud, Institut Gustave Roussy, Villejuif, France; [2] LIA 1066 LFR2O French-Russian Joint Cancer Research Laboratory, Villejuif, France; [3] Koltzov Institute of Developmental Biology, Moscow, Russia
anya.shmakova@list.ru

Burkitt lymphoma (BL), an aggressive B-cell lymphoma, is most frequently associated with a chromosomal translocation t(8;14) that relocates the MYC oncogene near the IGH gene locus. Exploring translocation formation mechanisms and approaches to diminish the risk of their formation is a promising aim. We aimed to investigate the kinetics of t(8;14) accumulation in a cell culture and to assess how targeting different cellular pathways affects translocation frequency. Methods: CRISPR/Cas9 was used to generate double-strand breaks (DSBs) in the IGH and MYC gene loci in a human B-cell line. We evaluated the t(8;14) level 48 hours, two, three, four, five and six weeks after DSBs induction by qPCR [1]. We also analyzed the level of t(8;14) 48 hours after the simultaneous DSBs induction and addition of chemical inhibitors to cell medium. We used NU7026 (DNA-PKcs inhibitor, canonical NHEJ), L67 (Lig1/Lig3 inhibitor, alternative NHEJ), sodium azide (electron-transport chain inhibitor). Results: We found that acquiring t(8;14)does not provide any selective advantage in the overall cell population as the level of t(8;14) progressively declined after DSBs induction. This can be due to increased expression of MYC in cells with t(8;14), which is known to induce apoptosis via the p53 pathway [2]. When testing the effect of different drugs on translocation efficiency in our *in vitro* system, we found that sodium azide decreased the level of t(8;14), which can be explained by energy depletion, as DNA repair is an ATP-dependent pathway [3]. We also observed that L67 significantly decreased the level of t(8;14), while NU7026 significantly increased the level of t(8;14). Conclusions: We revealed that *in vitro* IGH-MYC translocation provides no selective advantage as the translocation is lost during long cell culturing. We also found that energy depletion and alternative NHEJ inhibition can decrease the level of t(8;14) translocations. We

can thus conclude that translocations in our model are mainly due to alternative NHEJ.

The work was supported by grants from Plan Cancer (ENVIBURKITT), ANRS and LNCC to YV. AS was supported by a fellowship form the Boehringer Ingelheim Fonds.

References 1. Germini D, Bou Saada Y, Tsfasman T *et al.* A One-Step PCR-Based Assay to Evaluate the Efficiency and Precision of Genomic DNA-Editing Tools. Mol Ther Methods Clin Dev. 2017;5: 43–50. 2. Phesse TJ, Myant KB, Cole AM *et al.* Endogenous c-Myc is essential for p53-induced apoptosis in response to DNA damage *in vivo*. Cell Death Differ. 2014;21: 956–966. 3. Lu J, Sharma LK, Bai Y. Implications of mitochondrial DNA mutations and mitochondrial dysfunction in tumorigenesis. Cell Res. 2009;19: 802–815.

doi: http://dx.doi.org/10.7124/bc.0009F9

S-5. MDM2 ubiquitin-ligase down-regulate energy metabolism of cancer cells

Oleg Shuvalov[1], Alexey Petukhov[1,2], Olga Fedorova[1], Alexandra Daks[1], Baidyuk Ekaterina[1], Alena Kizenko[1] and Nickolai Barlev[1]

[1] Institute of Cytology RAS; Saint-Petersburg, Russia; [2] Institute of Hematology, Almazov Federal North-West Medical Research Centre, Saint-Petersburg, Russia

oleg8988@mail.ru

MDM2 is an E3 ubiquitin ligase that is controlled by p53, one of the most important tumor suppressors. At the same time, MDM2 targets p53 for ubiquitin-dependent degradation. This mechanism, which protects normal cells from excessive p53-induced death, is frequently deregulated in different types of tumors. MDM2 also has p53-independent oncogenic functions. Besides p53, MDM2 ubiquitinylates a number of proteins, including the catalytic subunit of telomerase (hTERT), transcription factors Snail and Slug, *etc.* Thus, MDM2 possesses both tumor promoting and tumor suppressing functions, depending on a particular cellular context. To uncover additional targets of MDM2, we carried out a proteomic study in which GST-MDM2 was used as a bait to pull-down interacting proteins, which were then identified by mass-spectrometry (LC-MS/MS). Whole cell extracts derived from U2OS (human osteosarcoma) cells, MCF7 (breast carcinoma, luminal subtype) were used as a source of proteins that potentially bind MDM2. According to the data obtained, we consistently observed a significant number of various metabolic enzymes among the proteins interacting with MDM2. Importantly, they are the key enzymes of glycolysis, and are frequently deregulated in different cancers. We have verified interactions of MDM2 with several enzymes identified and investigated the influence of MDM2 on their expression, ubiquitination and stability. Moreover, we have shown that MDM2 affects the metabolic state of cancer cells, as well as their susceptibility to several pharmacological inhibitors of corresponding metabolic pathways. Taken together, these data revealed a novel role for MDM2 ubiquitin ligase in the regulation of cancer-related metabolic pathways. The common set of metabolic alterations is considered now as one of the "hallmarks of cancer" and the corresponding enzymes are considered as promising targets for anticancer therapeutics. These notions emphasize the importance of MDM2 for cancer metabolism and warrants further investigations.

This work was supported by Grant from the Russian Government # 14.W03.31.0029