by poor prognosis and survival rate. Its genetic hallmark is the translocation t(11;14) which leads to the overexpression of cyclin D1 (CCND1) gene which becomes juxtaposed to the immunoglobulin heavy chain (IGH) gene on the newly formed der14 chromosome. This recurrent feature is however not sufficient to promote the development of the disease as expression of CCND1 under different known IgH enhancers in transgenic mice is not sufficient for tumor development. Additional alterations are necessary to develop a malignant phenotype. When a translocation occurs, it can induce overall nuclear reorganization, epigenetic changes and altered gene expression that may contribute to oncogenesis. Here we investigated changes in nuclear positioning of gene loci and their transcription after the t(11;14) focusing our attention on the events occurring on the der11 chromosome. Methods. 3D-immunoFISH and image analysis software were used to analyze gene loci position in nuclear space. To analyze changes of transcriptional level of genes located on the der11, quantitative RT-PCR, bioinformatic analysis and data mining were performed. ChIP was carried out to analyze specific interactions between nucleolin and the genome in MCL. Results. We demonstrated that the expression of many genes located close to the translocation breakpoint was deregulated in MCL compared to other lymphomas and to B-cells from healthy donors. Most of these genes were located on the der11 after the t(11;14). We found that the der11 is relocated in close proximity to the nucleolus. Here the nucleolin, that is part of the transcriptional factor LR-1 can deregulate gene expression by direct binding to promoters. We found that the LR-1 consensus sequence and the nucleolin binding sites are significantly enriched in the regions covered by the deregulated genes compared to the rest of chromosme11 and to cells without the t(11;14). Conclusions. We identified new epigenetic events that contribute to MCL development following t(11;14).

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G-2. Histone deacetylase inhibitor obstructs non-homologous end joining DNA repair in oncogene-transformed but not in normal cells

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Histone deacetylase inhibitors (HDACi) suppress the growth of tumor cells due to the induction of cell cycle arrest, senescence or apoptosis (1-2). There are a few data concerning the reduction of DNA repair capability by HDACi in tumor cells (3-4). Previously we established that HDACi-induced G1/S cell cycle arrest of E1A+Ras-transformed mouse embryonic fibroblasts was accompanied with an accumulation of the marker of damaged DNA - γH2AX foci (5). Among all DNA damages, the double-strand breaks (DSB) are the most crucial for a cell. Since non-homologous end joining (NHEJ) is a principal repair mechanism of DSB whatever of the cell cycle phase, we focused our research on this repair mechanism. The aim of this study is to compare the DNA repair efficiency in transformed and normal cells after HDACi treatment.

Methods: To model the NHEJ repair mechanism we used the host-cell reactivation assay. A luciferase reporter vector pGL3-luc was damaged with endonuclease and etoposide, and then introduced into E1A+Ras-transformed and normal (NIH3T3) mouse fibroblasts, using lipofectamine transfection. Transfected cells were incubated in the presence or absence of HDACi sodium butyrate and afterwards, the efficiency of DNA repair was evaluated by measurement of the luciferase activity. Results: Double-strand breaks, which were introduced by a damaging agent or an endonuclease into the plasmid DNA, decreased luciferase transcription. However, it reclaimed afterwards owing to DNA repair. We have shown that the recovery of DNA has occurred less efficiently in the presence of sodium butyrate in transformed cells, while in normal fibroblasts sodium butyrate did not affect the NHEJ-repair efficiency. Further, the HDACi suppressed the expression of repair proteins (Ku80, Ku70, Mre11) in transformed, but not in normal cells. Conclusions: We have thus demonstrated a fundamental difference between the influence of HDACi on DNA repair via NHEJ-mechanism in oncogene-transformed and in normal cells. DNA repair capability of E1A+Ras-transformed cells was reduced by HDACi, but in normal cells it stays unaffected. This effect is very useful for anticancer chemotherapy since HDACi could be applied in combination with DNA damage agents.

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References: 1. Wang Y. et al., Cancer Lett. 2017; 400: 47-60; 2. Sun G. *et al.*, Journal of cancer 2015; 6(10): 996-1004; 3. Rossiello F. *et al.*, Curr Opin Genet Dev. 2014;26: 89-95; 4. Munshi, A. *et al.* Clin. Cancer Res. 2005; 11: 4912–22; 5. Abramova, M. V. *et al.* Cancer Biol Ther. 2011; 12(12): 1069-77.

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G-3. Nuclear protein phosphatase Wip1 regulate sensitivity of human colorectal cancer cells to DNA damaging anti-cancer agents

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To evaluate impact of nuclear wild-type p53 induced phosphatase Wip1 on sensitivity of human colorectal cancer cell line DLD1 to the DNA damaging drugs: 5-fluouracil and oxaliplatin. Methods: Human colorectal cell line DLD1 with wild-type p53 status was used for all experiments. Stable cell line with overexpression of Wip1 (DLD1-Wip1ON) was derived using lentiviral transduction of DLD1 cells with viral particles containing human Wip1 cDNA under control of EF1a promoter. Wip1 overexpression was validated by immunoblotting. Wip1 gene knockout was performed using CRISPR/Cas9 approach. Briefly, plasmids encoding three gRNAs targeting 2nd, 3rd and 5th exon of Wip1 gene were co-transfected with three donor plasmids to produce the knock-in of selection cassette via homologous recombination. Proper cassette integration and disruption of Wip1 coding sequence were validated by PCR. Cellular toxicity was estimated by MTT essay, senescence – by SA-b-gal staining, cycle – by 7-AAD staining. Results: We showed that overexpression of Wip1 led to phosphatase accumulation in the nucleus and affected DNA damage response