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CELL VIABILITY AND DNA DAMAGE IN MRC5 AND HeLa CELL LINES AFTER HISTONE H1 KNOCKOUT BY CRISPR-Cas9 GENOME EDITING TECHNOLOGY

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Chromatin research mainly focused on the core histones, whereas the role of H1.5 linker histone is poorly understood. Today CRISPR-Cas9 (clustered, regularly interspaced, short palindromic repeats associated protein 9) genome editing technology provides an opportunity to analyze functions of different genes introducing targeted loss-of function mutations. Here we demonstrate the role of histone H1.5 in cell viability and genome integrity in HeLa and MRC5 cells using trypan blue exclusion test and single-cell gel electrophoresis (comet) assay.

***Keywords*:** CRISPR-Cas9, comet assay, HeLa, HIST1H1B gene, MRC5.

Introduction. The structure of the chromatin in eukaryotic genome is maintained by five types of histones: linker histone H1 and core histones H2A, H2B, H3, and H4 [1]. The H1 family of linker histones is the most divergent class of histone proteins which include eleven different H1 subtypes in humans [2]. H1 family members can be classified as replication-dependent (H1.1–H1.5 and H1t in human) and are mainly expressed during S-phase, and replication-independent (H1.0 and H1X), which are expressed over the complete cell cycle [3]. H1.2 to H1.5 and H1X are ubiquitously expressed, H1.1 is restricted to certain tissues, and H1.0 accumulates in terminally differentiated cells [4]. Th'ng et al. showed that in neuroblastoma cell culture H1.5 is preferentially located in heterochromatic regions [5]. Recent findings indicate that H1.5 binds to families of genes encoding membrane or membrane-related proteins in terminally differentiated cell types. Depletion of H1.5 in fibroblasts leads to increased chromatin accessibility at its target loci [6]. With a different level of compaction, euchromatin and heterochromatin respond differently to the attack by DNA damage-inducing agents [7, 8]. Changes in chromatin structure resulting from the dysfunction of histone, H1 may affect cell survival and the formation of DNA damage. To understand fully the contribution of histone H1.5 in DNA integrity and cells viability, we performed the

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knockout of HIST1H1B gene by CRISPR-Cas9 in MRC5 (human fibroblasts) and HeLa (human adenocarcinoma) cells.

Materials and Methods.

Cell Cultivation. MRC5 and HeLa cells ($2.5 \cdot 10^5$) were cultured in a 3 *mL* of antibiotic-free medium (DMEM) in a 6-well tissue culture plate. Prior to transfection cells confluence was approximately 80%.

HIST1H1B Gene Knockout. HIST1H1B gene knockout was realized using Histone H1 CRISPR/Cas9 KO Plasmid (Santa Cruz Biotechnology) as per the manufacturer's protocols. Histone H1 CRISPR/Cas9 KO Plasmid consists of a pool of 3 plasmids, each encoding the Cas9 nuclease and a target-specific 20nt guide RNA designed for maximum knockout efficiency. The optimal plasmid DNA concentration was determined experimentally. After 24 *h* of cultivation cells were transfected with 1 μg of plasmid DNA and incubated for 72 *h* at 37°*C*. After incubation, successful transfection of CRISPR/Cas9 KO plasmid was visually confirmed by detection of the green fluorescent protein via fluorescent microscope. At the end of incubation cells were harvested, centrifuged and used for analysis by trypan blue exclusion test and comet assay (single cell gel electrophoresis).

Analysis of Cell Viability by Trypan Blue Exclusion Test. Cell viability after HIST1H1B gene knockout was studied using trypan blue exclusion test [9]. HeLa and MRC-5 cells were trypsinized for 5 *min* at 37°*C* and counted using hemocytometer. Statistical analysis was performed using Student's *t*-test.

Analysis of DNA Damage by Comet Assay. For evaluating the levels of DNA damage the alkaline version of DNA Comet assay was performed [10]. At least 200 cells were analyzed from each cell culture. The DNA percent in tail and tail moment were measured as an index of DNA break frequency using a Comet Assay IV imaging system (Perceptive Inst., UK). Data that were not normally distributed were analyzed by the Mann-Whitney *U*-test using statistical program SPSS19.

Results and Discussion. The knockout of HIST1H1B gene in MRC5 and HeLa cells has no significant influence on cell viability, but significantly increase % of DNA in tail and tail moment (see Table) in both analyzed cell cultures compared with control ($p<0.05$). Interestingly, after knockout of HIST1H1B gene the percentage of DNA fragmentation in both cell cultures were approximately equal to 22.3% in MRC5 and 24.4% in HeLa.

Levels of cells viability and DNA damage in MRC5 and HeLa cells before and after transfection with CRISPR/Cas9 plasmid

Variants	Viable cells, %	DNA in tail, % (mean ± S.E.)	Tail moment (mean ± S.E.)
MRC5 (non-transfected control)	89	14.7 ± 1.5	5.4 ± 0.5
MRC5 (transfected)	92	22.3 ± 2.0*	10.3 ± 1.0*
HeLa (non-transfected control)	95	15.0 ± 1.3	4.5 ± 0.4
HeLa (transfected)	94	24.4 ± 2.4*	9.5 ± 1.0*

* $p<0.05$ – significant difference compared to control.

As eukaryotic DNA is packed into a higher-order chromatin structure; the compacted structure of chromatin adds a layer of control on the processes of DNA damage response [11, 12]. These processes include break induction, break detection,

damage signaling, cell-cycle regulation (checkpoint), senescence and apoptosis, repair and post-repair restoration of pre-damage chromatin structure [11, 13]. In our study the knockout of HIST1H1B gene led to increase the levels of DNA damage in MRC5 and HeLa cells indicating its important role for maintenance of DNA integrity, though this effect had no influence on cell viability. Due to the H1 knockout the decondensation of heterochromatin occurs and reduces the level of DNA protection and therefore probably leads to increase the number of DNA breaks. Thus, CRISPR-Cas9 genome editing technology is a promising approach for determining the role of chromatin structure and particularly histone H1 in genome stability and cell viability.

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