INCREASE IN THE LEVEL OF DOXORUBICIN-INDUCED DNA DAMAGE IN HELA CELLS WITH CRISPR/CAS9 MEDIATED HIST1H1B GENE KNOCKOUT

Kuchukyan E. T., Sargsyan A. A., Harutyunyan T. A.*

Department of Genetics and Cytology, Yerevan State University,

1 Alex Manoogian, 0025, Yerevan, Armenia

**Correspondence: kuchukyanelza@gmail.com*

Annotation: Important role in cancer development and progression is related to changes in modifications of histones. Doxorubicin is a class of chemotherapy drug, that declines or stops the development of cancer cells. The aim of this study was to increase the genotoxic potential of doxorubicin via knockout of the H1.5 histone HIST1H1B gene, using CRISPR-Cas9 technology. Due to our results DOX significantly increases the levels of DNA damage in the HeLa cell after the knockout of the HIST1H1B gene using CRISPR-Cas9.

Keywords: doxorubicin; HeLa cells; CRISPR-Cas9; anticancer drug; histone; comet assay.

Аннотация: Важная роль в развитии и прогрессировании рака связана с изменениями модификаций гистонов. Доксорубицин - это класс химиотерапевтических препаратов, которые снижают или останавливают развитие раковых клеток. Целью исследования было повышение генотоксического потенциала доксорубицина путем нокаута гена гистона H1.5 HIST1H1B с использованием технологии

CRISPR-Cas9. Согласно полученным результатам DOX значительно увеличивает уровни повреждения ДНК в клетке HeLa после нокаута гена HIST1H1B с использованием CRISPR-Cas9.

Ключевые слова: доксорубицин; клетки HeLa; CRISPR-Cas9; противоопухолевый препарат; гистон; метод ДНК-комет.

1. Introduction

Histones are nuclear proteins responsible for the nucleosome structure of the chromosomal fiber in eukaryotes. In several studies, the alterations of the linker histone H1 in cancer cells were demonstrated. However, the significance of replication-dependent histone H1.5 encoding gene HIST1H1B for the sensitivity of cancer cells to anticancer drugs remains unclear [1].

Doxorubicin (DOX) is a chemotherapy medication used to treat various types of cancer [2]. DOX induces DNA double-strand breaks by DNA intercalation and inhibition of topoisomerase II. Earlier we investigated doxorubicin-induced micronuclei as well as translocation of mtDNA into the nuclear genome of human lymphocytes. In this study we analysed the impact of the HIST1H1B gene knockout (KO) on spontaneous and DOX-induced DNA damage in HeLa cells using the comet assay.

2. Methods

Cell cultivation and HIST1H1B gene knockout. HeLa cells ($2x10^5$ cells/ml) were cultured in 3 mL of DMEM and transfected with 1 μg of CRISPR-Cas9 KO plasmid for the knockout of HIST1H1B gene (sc-404845-NIC) for 24 h. The selection was performed using 2 μg/ml puromycin (sc-108071) for 72 h.

Treatment with DOX and comet assay. Wild type and KO cells were treated with DOX at concentrations 0.0175, 0.035 and 0.07 μg/ml for 24 h. Levels of DNA damage were analyzed by alkaline DNA comet assay [3]. The tail intensity of the comets (% of DNA in tail) was used as an indicator of DNA damage. Statistical analysis was performed using a Mann-Whitney *U*-test (SPSS19) and p values < 0.05 were considered as statistically significant.

3. Results and Discussion

To investigate the role of HIST1H1B in doxorubicin-induced genotoxicity, we compared the levels of DNA damage measured by comet assay in wild-type (WT) and HIST1H1B KO HeLa (KO) cells (figure 1). In the control group, the levels of DNA damage were higher in the KO cells (26.83 ± 2.85 % of DNA in tail) compared to WT cells (13.98 ± 1.20 % of DNA in tail). DOX significantly increased the levels of DNA damage in WT cells at concentrations 0.035 (17.45 ± 1.19 %) and 0.07 μg/ml (47.71 ± 1.71 %) as well as in KO cells at concentrations 0.0175 (51.08 ± 2.24 %), 0.035 (49.37 ± 1.89 %), and 0.07 (59.78 ± 1.26 %) compared to the corresponding controls. The

results showed a more pronounced genotoxic effect of DOX in knockout cells.

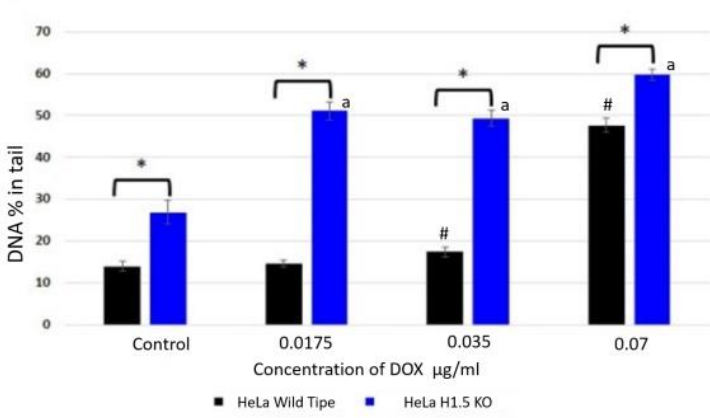

Figure 1. Levels of DNA damage in WT and HIST1H1B gene KO cells treated with DOX. *p <0.05 - significant difference compared to wild-type cells. #p <0.05 - significant difference compared to wild-type cells control. ap <0.05 - significant difference compared to KO cells control.

Conclusion

DOX significantly increases the levels of DNA damage in the HeLa cell line after the knockout of the HIST1H1B gene, induced by CRISPR-Cas9. Further studies should be conducted to evaluate the importance of the HIST1H1B gene targeting for anticancer therapy.

Acknowledgement

Supported by the Science Committee of RA (21AG-1F068).

Conflicts of Interest:

The authors declare no conflict of interest.

References

[1] L. Mariño-Ramírez, M. G. Kann, B. A. Shoemaker, and D. Landsman, “Histone structure and nucleosome stability,” *Expert Rev. Proteomics*, vol. 2, no. 5, pp. 719–729, 2005.

[2] T. Harutyunyan, A. Al-Rikabi, A. Sargsyan, G. Hovhannisyan, R. Aroutiounian, and T. Liehr, “Doxorubicin-induced translocation of mtdna into the nuclear genome of human lymphocytes detected using a molecular-cytogenetic approach,” *Int. J. Mol. Sci.*, vol. 21, no. 20, pp. 1–11, 2020.

[3] A. R. Collins, “The comet assay for DNA damage and repair: Principles, applications, and limitations,” *Appl. Biochem. Biotechnol. - Part B Mol. Biotechnol.*, vol. 26, no. 3, pp. 249–261, 2004.