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Etoposide-induced DNA damage in a chromosomal breakpoint of *RUNX1* gene is independent of *RUNX1* expression

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ABSTRACT

In this work, we analyzed the association between *RUNX1* gene expression and the accessibility of BCR3, one of *RUNX1* gene breakpoint regions involved in the chromosomal translocation (8;21), a frequent translocation in treatment-related acute myeloid leukemia patients. To this end, we evaluate DNA damage generation induced by *in vitro* etoposide treatment of KG-1 and Colo320 cells. Our results show that treatment using clinical doses of etoposide for 24 h induces the generation of DNA double strand breaks in the BCR3 of *RUNX1* gene in KG-1 cells, but not in Colo320 cells, even though both cell lines express *RUNX1* gene. These findings suggest that chromatin accessibility and DNA damage generation at the BCR3 due to treatment with etoposide, is independent of *RUNX1* gene expression.

1. Introduction

Etoposide is a drug used as chemotherapeutic treatment for cancer patients. Its main effect is the induction of DNA double strand breaks (DSBs) along the genome, through the inhibition of the enzyme topoisomerase II ligase activity [1]; which results in apoptosis of rapidly dividing cells, such as cancer cells. However, etoposide affects normal cells in the body as well, giving rise to side effects. One of the most severe is the development of treatment-related acute myeloid leukemia (t-AML), which affects about 10% of patients, 2–3 years post-treatment [2]. To date, it is unknown what determines that t-AML is the only type of cancer developed as a side effect of treatment with etoposide, as well as the contribution of etoposide to the onset of t-AML.

t-AML is a type of blood cancer associated with a number of genomic aberrations. One of the most frequent in patients is the chromosomal translocation (8;21), in which the genes *RUNX1* and *ETO* recombine. The chromosomal breakpoints of *RUNX1* gene involved in the t(8;21) were mapped to the intron 5 of the gene, and designated as breakpoint cluster regions (BCRs): BCR1, BCR2 and BCR3 [3]. In myeloid cells, the BCR3 presented DNaseI hypersensitivity [3], a feature that suggests an increased chromatin accessibility of that region in that particular cell type. The expression of *RUNX1* gene in myeloid cells is key for correct hematopoiesis, thus it is possible that chromatin accessibility in *RUNX1* locus may be related to the expression of *RUNX1* mRNA.

Therefore, in this study our aim is to determine if treatment with clinical doses of etoposide for 24 h induces the generation of DSBs in the BCR3 of *RUNX1* gene, in cells that express *RUNX1* gene, using qPCR to assess DSB generation in that specific region.

2. Materials and methods

Cell lines. KG-1 cells were cultured in IMDM medium, supplemented with 10% FBS, 2 mM L-glutamine and 1% penicillin-streptomycin; and Colo320 cells were cultured in RMPI 1640 medium, supplemented with 10% FBS, 2 mM L-glutamine and 1% penicillin-streptomycin. All cells were maintained at 37 °C, with 5% CO_2.

RUNX1 mRNA detection. RUNX1 mRNA was detected by RT-PCR and quantitative real-time PCR (qPCR), using as template cDNA synthesized from total RNA (4 µg) of KG-1 and Colo320 cells. Each cDNA synthesis reaction was diluted 1:25, and 1 µL was used to perform PCR or qPCR, with the following program: [30 s at 95 °C – 30 s at 58 °C – 30 s at 72 °C] for 30 cycles. The reference gene *SNRPD3* was used as an expression control. The primers used were: [*RUNX1* F: GTC GAA GTG GAA GAG GGA AA; R: CCG ATG TCT TCG AGG TTC TC] and [*SNRPD3* F: TCT TCC TGC CAA GAT GTC TA; R: TAA CAT GGG TGC GTT CTT C]. PCR products were resolved using eletrophoresis in 1% agarose gels, dyed with SYBR® Safe DNA Gel Stain (Invitrogen), a flurophore that allows DNA visualization under UV light. Ct data was processed using the ΔΔCt method and statistical significance was assesed with the Mann-Whitney test ($n = 3$ for KG-1 and Colo320 cells).

Etoposide treatment. KG-1 (1 × 10^6 per treatment) and Colo320

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(confluency >90% per treatment) cells were treated with 18 mg/L and 36 mg/L of etoposide (Sigma-Aldrich), its vehicle (0.1% DMSO), or non-treated for 0 and 24 h at 37 °C. After the indicated times, genomic DNA was extracted using the Genomic DNA from Blood kit (Macherey–Nagel).

DNA damage detection. qPCR was performed to detect damage in the region BCR3 of *RUNX1* intron 5, using primers to amplify a fragment of that region. Amplification of a fragment of *RUNX1* intron 4 was used as control. 1 ng of genomic DNA from KG-1 and Colo320 cells was used to perform each independent qPCR, with the following program: [30 s at 95 °C – 30 s at 64 °C – 30 s at 72 °C] for 30 cycles. The primers used were: [BCR3 F: CTT CCT GGT GAC GGT TGT TT; R: GTC CCA TTT CCA GGT ACA CG] and [Intron 4 F: GGC AAC AGA AGA ATC ACA CG; R: CCA TTG TCC CAG TCC TGA GT]. Obtained Ct data was processed using the ΔΔCt method, comparing the amplification of the BCR3 fragment, with the amplification of the control fragment from intron 4. Statistical significance was assesed with the Mann-Whitney test ($n = 4$ for KG-1 and Colo320 cells).

3. Results and discussion

In this study, we adressed the posibility that the expression of *RUNX1* gene could be associated with a higher probability of DSB generation at the BCR3. To test that hypothesis, we treated KG-1 (myeloid) and Colo320 (colon) cells with clinical concentrations of etoposide (18 and 36 mg/L) for 0 and 24 h, and DNA DSB generation at the BCR3 was assessed performing qPCR. Our results show that, although both cell types expressed *RUNX1* gene (Fig. 1A, top panel, Fig. 1B), after 24 h of etoposide treatment only KG-1 cells presented a statistically significant decrease of the BCR3 region relative amplification with 36 mg/L etoposide (Fig. 2A, right panel). These results indicate that DNA DSBs ocurred only in myeloid KG-1 cells, which suggests that *RUNX1* gene expression is not correlated to DNA DSB generation in the BCR3 of *RUNX1* due to treatment with etoposide. A possible explanation is that chromatin in the BCR3 in myeloid cells could be more accessible than in other cell types, not necessarily as results of *RUNX1* expression. For instance, DNase-seq data of hematopoietic cell lines available in the ENCODE Project server reveals colocalization of DNaseI hypersensitivity hotspots with the BCR3 in myeloid cells (Supplementary Figure 1). As well, it has been previously described that in HL-60 cells, the region BCR3 presents acetylated histone H3 [4], an epigenetic mark associated with a more relaxed chromatin state [5]. Performing a complete characterization of *RUNX1* intron 5 could provide answers about other genomic features within intron 5 that might determine an increased probability of DSB generation in myeloid cells.

These results also provide new insights on the effects of etoposide treatment in cells, showing how it affects one of the chromosomal breakpoints of *RUNX1* gene. This type of damage could be one of the initial steps towards t(8;21) generation and subsequent leukemic transformation of myeloid cells due to treatment with etoposide. In conclusion, our results show that, although both KG-1 and Colo320 cell lines express *RUNX1* gene at comparable levels, etoposide treatment induced DSB generation only in KG-1 (myeloid) cells, and not in Colo320 (colon) cells, suggesting that the BCR3 of *RUNX1* intron 5 is more prone to DSBs in myeloid cells, independent of *RUNX1* gene expression.

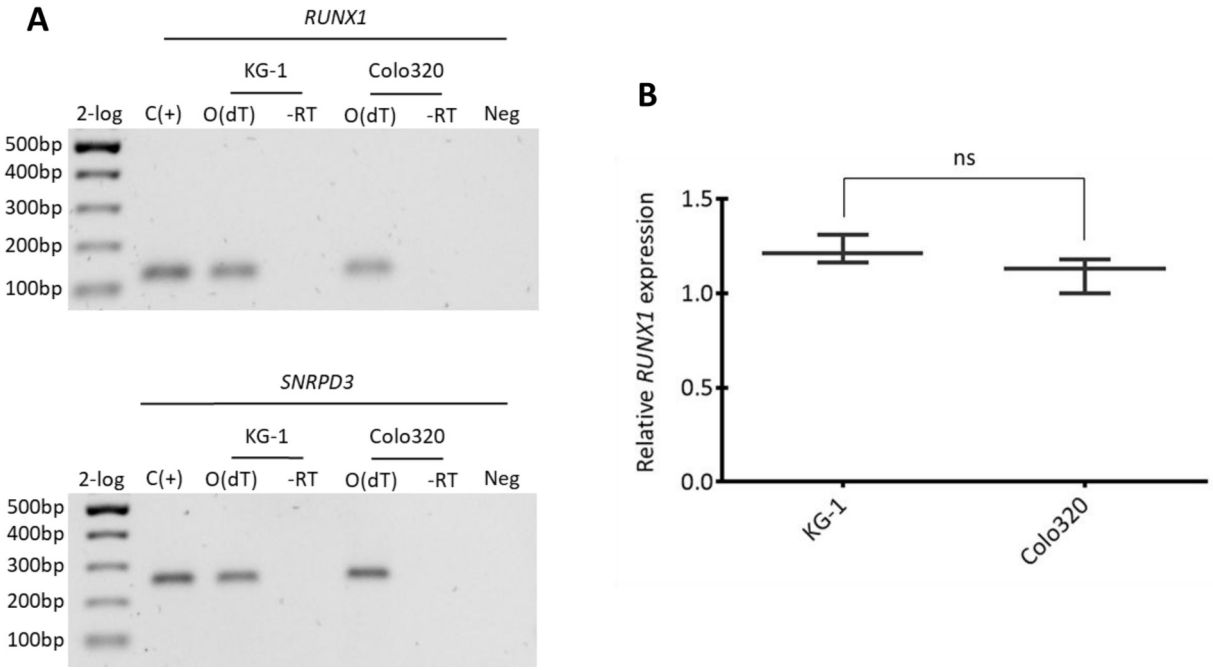

Fig. 1. *RUNX1* gene is expressed by both KG-1 and Colo320 cells. (A) Top: detection of *RUNX1* mRNA by RT-PCR in KG-1 and Colo320 cells. Bottom: detection of the reference gene *SNRPD3* mRNA by RT-PCR in KG-1 and Colo320 cells. [2-log: DNA ladder; C(+): positive control; O(dT): cDNA synthesized using oligo(dT) as primer; −RT: minus RT control; Neg: water as template]. (B) Expression of *RUNX1* mRNA, relative to *SNRPD3* mRNA expression, in KG-1 and Colo320 cells by qPCR. The symbol ns indicates $p > 0.05$, according to the Mann–Whitney test. (KG-1 and Colo320: $n = 3$).

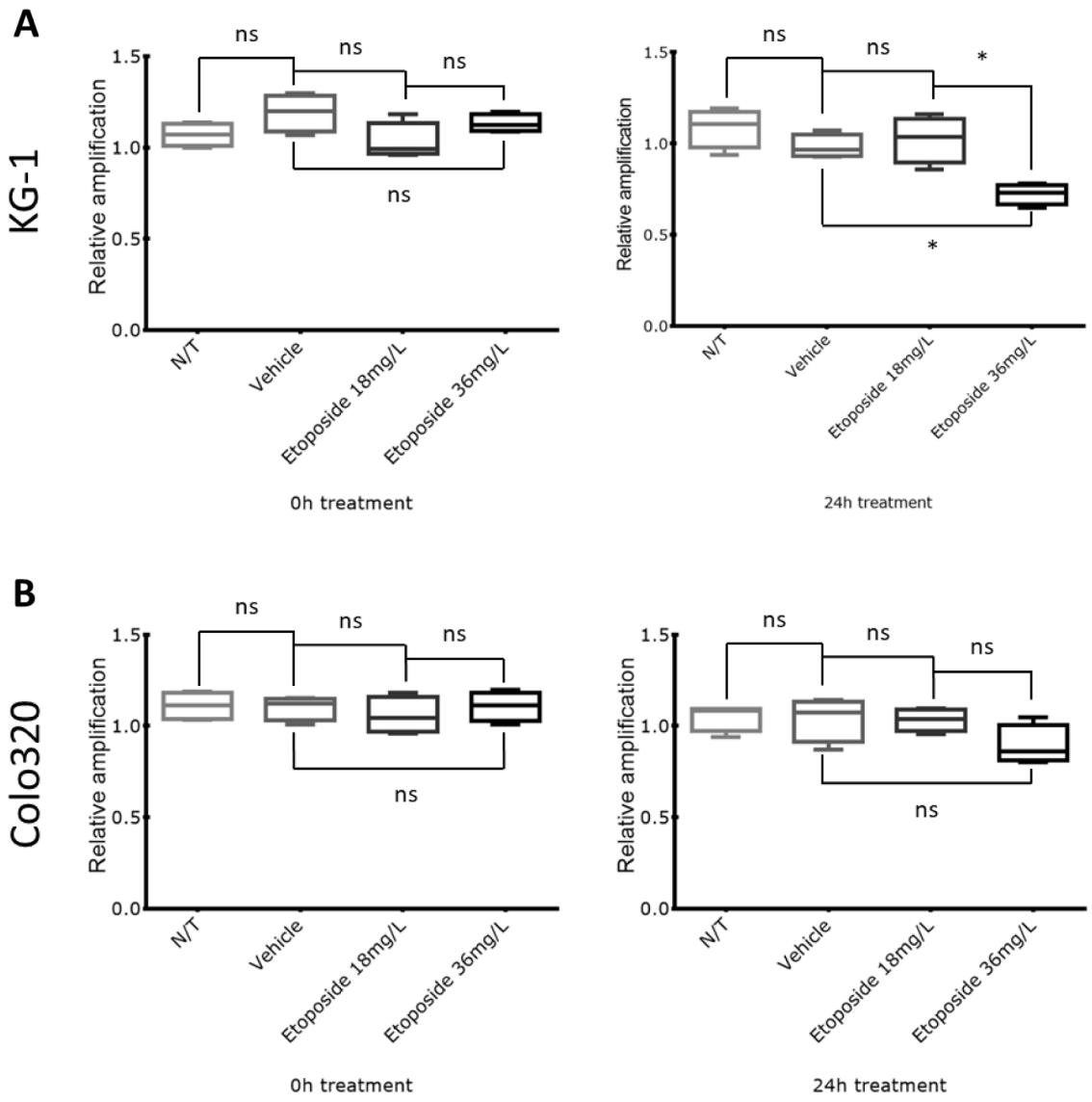

Fig. 2. Treatment with etoposide induces DSBs in the BCR3 in myeloid cells. Relative amplification: amplification by qPCR of a fragment of the region BCR3 of *RUNX1* gene, relative to the amplification of a control region, using as template genomic DNA from KG-1 (A) and Colo320 (B) cells treated with 18 and 36 mg/L etoposide, its vehicle, or non-treated (N/T) for 0 and 24 h (KG-1: $n = 4$; Colo320: $n = 4$). The symbol * indicates $p < 0.05$, and ns indicates $p > 0.05$, according to the Mann–Whitney test.

Declaration of Competing Interest

The authors declare they have no conflict of interest.

Acknowledgments

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lrr.2019.100182.

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