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# L-4. Cell model of inducible AML1-ETO translocation

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Recent progress in cancer therapy allows patients to live longer. However, the increase in life expectancy raises the chance that the long-term side effects of therapy will manifest. Among these is secondary (so-called treatment-related) leukemia (tAML). This disease arises as a consequence of anti-cancer therapy with DNA topoisomerase II inhibitors (i.g. etoposide). It was shown that specific chromosomal translocations between AML1 (RUNX1) and ETO (RUNX1T1) genes can be found in many cases of tAML [1]. A widely accepted hypothesis postulates that this translocation is a consequence of the erroneous repair of DNA double-strand breaks induced by drug-poisoned DNA topoisomerase II [2]. One possible way to induce AML1-ETO translocation is to treat cells with etoposide. However, the occurrence of this particular translocation, in this case, will be extremely low. Therefore, we aimed to create a cell model with inducible targeted chromosomal breaks in AML1 and ETO genes to study the formation of leukemogenic chromosomal translocation. Methods: An integrative construction with inducible Cas9 gene and genes for guide RNAs to AML1 and ETO genes was obtained. The expression of Cas9 gene was under the control of TetON-system. Guide RNAs were tested by ENIT-approach [3]. This construction was integrated into the genome of LCL (RPMI 8866) cells by means of homologous recombination into AAVS1 locus. The induction of Cas9 expression by doxycycline was assessed by qRT-PCR. The induction of translocation was verified by PCR, and the frequency of translocation was quantified by qPCR. Results: We create the transgenic cells with the Cas9 expressed upon activation with doxycycline. The expression reaches a plateau at 24 hours post activation. Cas9 introduces breaks into two specific loci. As a result, the desired AML1-ETO translocation is formed. Conclusions: The obtained cell model with inducible AML1-ETO translocation can be used to study early events and to investigate details of molecular mechanisms leading to AML1-ETO-associated secondary leukemia. In addition, this model is suitable for screening for new drugs, preventing such unfavorable side-effects of chemotherapy.

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