CRISPR/Cas9 in Chronic Lymphocytic Leukemia

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Contributor: María Hernández-Sánchez

Genome-editing systems such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 technology have uncovered new opportunities to model diseases such as chronic lymphocytic leukemia. CRISPR/Cas9 is an important means of advancing functional studies of Chronic Lymphocytic Leukemia (CLL) through the incorporation, elimination and modification of somatic mutations in CLL models.

Keywords: leukemia ; CRISPR/Cas9 ; editing

B-cell lymphoproliferative disorders are a clonal expansion of the various stages of B lymphocytes in bone marrow, blood, or other tissues. The World Health Organization (WHO) has described more than 30 different entities in the category of mature B-cell neoplasms [1]. Both lymphomas and lymphoid leukemias are included in this classification in which you could find Burkitt Lymphoma, Diffuse Large B-cell Lymphoma, Follicular Lymphoma, and Mantle Cell Lymphoma, as well as Chronic Lymphocytic Leukemia or Hairy Cell Leukemia.

Among the leukemias, **Chronic Lymphocytic Leukemia** (CLL) is the most common adult leukemia in Western countries, with an incidence of 4.2 cases per 100,000 people per year [2]. Its median age at diagnosis ranges from 70 to 72 years, being more frequent in men (2:1). This hematological malignancy is characterized by the presence of mature clonal B lymphocytes that accumulate in the blood, bone marrow, and other lymphoid tissues [1][3][4]. The diagnostics is mainly based on laboratory techniques, namely blood count, morphology, and immunophenotyping [2][3]. Specifically, the diagnosis of CLL is mainly defined by the presence of more than 5×10^9/L B lymphocytes in peripheral blood for at least three months. The clonality of the circulating B-lymphocytes needs to be confirmed by flow cytometry [2][3][4]. The immunophenotype of CLL distinguishes it from other B hematological malignancies by the expression of B cell markers such as CD19, CD23, and weak CD20, along with CD5, a T cell antigen, and low expression levels of surface membrane immunoglobulin [5]. In terms of morphology, the CLL cells found in the blood smear are characteristically small and mature lymphocytes with a narrow border of cytoplasm, and a dense nucleus lacking discernible nucleoli and having partially aggregated chromatin. Large atypical cells, cleaved cells, or prolymphocytic cells, which may be up to 55% of the blood lymphocytes, could also be observed [6]. Gumprecht nuclear shadows, or smudge cells, found as cell debris are other characteristic morphologic features in CLL.

The natural course of CLL is highly heterogeneous, ranging from asymptomatic with no need for therapy to an aggressive disease associated with therapeutic resistance and short overall survival [7]. CLL patients are generally diagnosed with an asymptomatic disease by blood tests performed during a routine physical exam. However, other patients showed symptoms such as fatigue, fever, lymphadenopathy, hepatomegaly, splenomegaly, bone marrow failure, recurrent infections, and/or weight loss [8][9]. In recent decades, the improved understanding of CLL pathogenesis has resulted in the identification of a great number of prognostic markers (clinical systems, serum markers, genetic alterations, et.), significantly improving patient stratification [10]. Prognostication in CLL remains an active research field in order to define not only the prognostic markers able to predict the clinical course at diagnosis but also the predictive markers able to predict the response to treatment in the era of targeted therapies [11].

Numerous studies have demonstrated that the clinical variability is a clear consequence of marked biological diversity [12][13]. During the last decades, Next-Generation Sequencing (NGS) technologies have uncovered a great number of genetic alterations in CLL [14][15], with a long tail of hundreds of genes mutated only in a short fraction of CLL patients [16]. The biological impact of some of CLL genetic abnormalities has been partially understood thanks to CLL models [17][18][19][20]. Since CLL disease is the result of a complex interaction of different lesions, novel models are required to study the biological effects of single and multiple genetic lesions to gain insight into the mechanisms underlying the clonal evolution as well as the treatment response. In this line, models applying genomic engineering will serve as valuable tools to study the effects of CLL drivers on cellular fitness.

The development of genome-editing technologies has broadened new possibilities to model diseases such as CLL. The development of genome editing technologies has opened up the possibility of directly targeting and modifying genomic

sequences in almost all eukaryotic cells. The first approaches used in the field of genome-editing were based on the zinc-finger nucleases (ZNFs) and transcription activator-like effector nuclease (TALENs) [21][22]. The discovery of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems as genome-editing technologies has overcome many of the limitations of the earlier strategies, allowing us to create disease models in a rapid, efficient, and cheap way [23]. Since 2013, when it was first applied in mammalian cells as a tool to edit the genome [24][25], the versatile CRISPR/Cas9 technology has been rapidly expanding its use in modulating gene expression, ranging from genome sequence changes to epigenetic and transcriptional modifications.

CRISPR/Cas9 genome editing technology is based on inducing DNA double-strand-breaks (DSBs) that stimulate the cellular DNA mechanisms: error-prone non-homologous end joining (NHEJ) and homologous recombination (HR). This system—derived from a bacterial adaptative immune system—relies on two key components: the nuclease Cas9 and the single-guide RNA (sgRNA) [24][25]. The sgRNA molecule is complementary to the target region of interest and directs Cas9 to the genomic region of interest, leading to the generation of DSBs [26]. Consequently, on one hand, NHEJ is an error prone repair process that joins broken ends, generally resulting in the introduction of small indels (insertions and deletions), and therefore, the presence of frameshift mutations which generate premature stop codons and mimic loss-of-function (LoF) mutations, which can be useful to generate knock-out models. On the other hand, HR happens in the presence of a donor DNA template, allowing specific DNA edition such as gain-of-function (GoF) mutations, being the ideal strategy for generating knock-in models [27][28].

Besides the application of the generation of isogenic models with LoF or GoF mutations, CRISPR/Cas9 technology allows us to generate chromosomal rearrangements by the introduction of two distant DSBs within the same chromosome to produce chromosomal inversion or deletion, or the induction of two DSBs in different chromosomes leading to a chromosomal translocation [29][30][31][32].

CRISPR-gene editing system also provides a powerful way to switch gene expression on or off at the transcription level [33][34]. In this line, a nuclease-deactivated form of Cas9 termed deadCas9 (dCas9), which is unable to cleave DNA, is necessary. A fused dCas9 with silencer agents or transcriptional activators can bind to the promoter region to efficiently repress or activate gene expression, respectively [35][36].

The possibility to perform multiplex mutagenesis by CRISPR has opened a range of functional genome-screening approaches. These approaches could reveal key genes associated with drug resistance or identify vulnerable target genes for the development of targeted drugs [37][38].

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