Application and progress of CRISPR Cas9 gene editing in B-cell lymphoma

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Abstract

In latest years, with the gradual perception of CRISPR Cas9 technology, coupled with the many advantages of the CRISPR Cas9 system, ease of development, simplicity of use, and low material prices, its mediated gene modifying has been higher used in diagnosing and treating genetic ailments and enhancing patients' prognosis. Moreover, CRISPR Cas9 gene screening library is another hot issue which can be used to discover pathogenic genes, pick drug intervention sites, and analyze drug resistance mechanisms. Rapidly emerging gene editing technology has a huge market and is expected to cure B-cell lymphoma. This article reviews the application and progress of CRISPR Cas9 gene editing in B-cell lymphoma for further study.

Introduction

Lymphomas are malignant hematologic tumors associated with malignancy of certain immune cells produced by proliferation and differentiation of lymphocytes, and are classified into two categories according to histopathologic changes: non-Hodgkin's lymphoma (NHL) and Hodgkin's lymphoma (HL). They can be divided into B-cell, T-cell and NK-cell lymphomas, in accordance to the origin of the lymphocytes. 2016 World Health Organization (WHO) reclassify of hematopoietic and lymphoid tissue tumors [1], lymphomas are mostly B-cell in origin. Malignant lymphoma is becoming more frequent in China, which is comparable to leukemia, and present remedy alternatives have no longer improved outcomes.

There is presently no therapy for B cell lymphoma; it can only be slowed or controlled. The stunning effects of gene editing in duchenne muscular dystrophy (DMD)[2] and sickle cellphone anemia (SCD)[3] have researchers interested. Jeffrey C Miller et al [4] developed zinc finger nucleases (ZFNs) to target genes, and with the rapid development of science and technology, transcription activator-like effector nucleases (TALENs)[5,6] and clustered regularly interspaced short palindromic repeats (CRISPR) Cas9 [7,8] have emerged one after another, with CRISPR/Cas9 being the most thoroughly studied and most commonly used.

1 Concept and mechanism of CRISPR Cas9 gene editing

1.1 Discovery of CRISPR Cas9 gene editing

A mechanism to withstand exogenous viral and plasmid DNA invasion, named CRISPPR Cas9, has emerged from bacteria and archaea below the long-term evolution of nature [9,10]. It consists of type 1 (containing I, III and IV) and type two (containing II, V and VI), with multisubunit complexes and individual proteins as effectors, respectively [11,12]. The type II CRISPR system is the most familiar, whilst crRNA-tracRNA (trans-activated crRNA) is its special structure that can chimerize into single-guided RNA (sgRNA)[13-15].

DNA endonuclease Cas9 in the type II CRISPR machine acknowledges bound dsDNA substrate and then cleaves the corresponding DNA strand via HNH or RuvC nucleases [16].

1.2 CRISPR Cas9 screening

Currently, three different categories of functional gene screening libraries are offered: cDNA libraries, RNA interference libraries, and CRISPR Cas9 gene editing libraries. The latter is further classified into CRISPR-Cas9 knockout libraries, CRISPR-dCas9 activation libraries, and CRISPR-dCas9 interference libraries [17], with the gene-scale CRISPR-Cas9 knockout (GeCKO) [18] being the most prevalent CRISPR library screen. As shown in Figure 1, GeCKO accomplishes this through a series of processes to achieve the screening of genes [19] and drug sites of action[18]and the identification of the mechanism of action of various impact factors[20].

2 Advances in CRISPR Cas9 Gene Editing in B-Cell Lymphoma

CRISPR Cas9 technology has been used to identify some genes, signaling pathways and cytokines that affect the development and prognosis of B-cell lymphoma (as shown in Table 1), which may become potential targets for future treatment of B-cell lymphoma.

2.1 CART therapy and CRISPR Cas9

One of the most successful innovations in the area of hematology is the CRISPR/Cas9-edited chimeric antigen receptor (CAR) T cell (CAR-T cell), which is additionally a primary leap forward in the area of immunotherapy and was approved by the FDA for the treatment of leukemia and lymphoma [21,22].

Eshhar [23] et al. first suggested structural and functional similarities between endogenous αβT cell receptors (TCRs) and antibodies. Target cells carrying 2,4,6-trinitrophenyl (TNP) semi-antigenic motifs can be recognized by chimeric TCR chains, which consist of immunoglobulin V region and TCR C region, and do not elicit immune response from the receptor as well as rejection. B cells specifically express CD19, while CART cells modified by the CRISPR Cas9 gene themselves in particular recognize CD19 in addition to their T lymphocyte-borne killing function, thus specifically killing tumor cells with long-lasting effects [24,25].

However, there are a couple of elements that have an effect on the efficacy of CAR-T therapy, together with T cell status, individual specificity, etc.

Patients treated with CAR-T cells may develop serious adverse effects, such as tumor lysis syndrome and cytokine release syndrome (CRS), and although these syndromes are manageable, they may become serious complications if not managed properly.

Delivery of bound CAR and CRISPR RNA through electroporation with lentivirus leads to injury of TCR and β-2-microglobulin (B2M) genes, ensuing in homozygous CAR T cells missing TCR, HLA type I molecules and PD1; as Fas receptor / Fas ligand (FasL) induces T cell apoptosis, CTLA-4, PD- 1, LAG-3 and TIM-3 are T cell suppressor receptors or signaling molecules whose expression motives T cell failure [26-28], therefore, knockdown of these elements through CRISPR Cas9 can decrease T cell apoptosis and amplify CAR T cells and therefore decorate their function.

2.2 Diffuse large B-cell lymphoma (DLBCL) and CRISPR Cas9

Diffuse large B-cell lymphoma is the most common non-Hodgkin's lymphoma and has a high heterogeneity, accounting for approximately 1/3 of all cases [29]. The standard first-line treatment regimen today is rituximab (R) + CHOP (cyclophosphamide + adriamycin + vincristine + prednisone), with 5-year survival rates of 60%-70% for patients taking first-line chemotherapy[30], but more than 30% of patients will still develop relapsed/refractory DLBCL due to resistance to targeted and chemotherapeutic agents, resulting in poor prognosis[29,30].

2.2.1 BCL6 gene

DLBCL originates in the germinal center (GC) stage of B-cell differentiation [31], and BCL6, a core transcription factor of the GC response, has been shown to be an oncogene that can develop into lymphoma. Rebecca Caeser et al[32] used steady Cas9 reverse transcription in primary, human GC B cells and proven that the machine can be used for gRNA targeted targeting. In CRISPR Cas9 screen TP53, GNA13, CDKN2A, ATRX, NFKBIA, ZFP36L1, ZNF281, PTEN, FBXO11, FUBP1, S1PR2 and NFKBIE have been strongly enriched and most elements suppressed lymphoma, whilst tumor cell proliferation used to be enhanced with the aid of TSG inactivation. The histone modifiers CREBBP, EP300 and KMT2D are lacking in the CRISPR screen, and these genes exhibit very frequent inactivating mutations in DLBCL and follicular lymphoma [33,34]. The most striking discovering in this study was once the efficient enrichment of gRNAs targeting GNA13 and its upstream receptors S1PR2 and P2RY8.GNA13 is typically viewed an oncogene and its inactivating mutations are frequent in lymphomas however uncommon in different varieties of malignancies, so its enrichment in the display reinforces the specificity of this system for the pathogenesis of GC lymphomas.

2.2.2 Histone acetyltransferase (HAT)

Genes encoding histone acetyltransferases (HATs) CREB binding protein (CREBBP) and EP300 are recurrently mutated in activated B-cell-like and germinal center (GC) B-cell-like subtypes of diffuse large B-cell lymphoma (DLBCL). Hind Hashwah et al [35] used CRISPR Cas9 to edit CREBBP and EP300 genes in the GC B-cell compartment of mice and determined the tumor suppressive impact of HAT in human diffuse large B-cell lymphoma (DLBCL) cell lines and mice. CREBBP and EP300 are tumor suppressors whose gene products contribute to histone H3 acetylation and promote active transcription in DLBCL cells, and affect many gene expression, most notably the MHCII gene, which controls MHCII expression and promotes tumor immune control. CREBBP deficiency also decreases MHCII expression in the B-cell compartment of the germinal center, impairs immunotherapy of tumors, and leads to B-cell overproliferation and predisposition to the development of MYC-driven lymphomas. Loss of CREBBP leads to MYC-driven lymphoma MHCII defects, ensuing in immune escape. CREBBP used to be proven to be a regulator of the enhancer/super-enhancer network, inhibiting enhancer activity via the BCL6/SMRT/HDAC3 complex and regulating GC and plasma cell development as well as antigenic rapture to result in formed lymphomas [36,37].

2.2.3 Locus control region (LCR)

Chi-Shuen Chu et al[38] observed that OCA-B acted particularly on germinal center B cells, promotes GC cell growth, and impacted the BCL6 promoter thru the interaction of OCA-B with MED1. In addition, OCA-B forms a ternary complication with the lymphoid-rich OCT2 and the GC-specific transcription factor MEF2B that occupies and activates the locus control region (LCR) via the octameric form. The LCR is positioned 150 kb upstream of BCL6 on chromosome 3q26 and acts appreciably on adjoining and distal genes consisting of the BCL6 proto-oncogene and its function is GC-specific[39]. CRISPR interference with gRNA targeting OCT2 and OCA-B both resulted in a significant reduction in BCL6 mRNA levels. Knockdown of each gene left the expression level of the other unaffected however MEF2B expression used to be decreased after OCT2 and OCA-B knockdown, and for that reason OCA-B and OCT2 are required for MEF2B-mediated activation of the constitutive enhancer CE1. Furthermore, Bcl6 and its adjacent LCR can function in vivo only if they are located on the same chromosome, reflecting the direct cis-regulatory role of the LCR in the induction of Bcl6 and GC formation. The order of assembly of these key factors on DNA is OCT2-OCA-B-MEF2B. In the early stage of GC formation, these element sequences are activated and inhibit the transcription of BCL6 promoters [40,41]. Thus, a hierarchical model of BCL6 regulation by progressive activation of the LCR can be proposed.

2.2.4 X-chromosome-linked inhibitors of apoptosis (XIAP)

Inhibitors of apoptosis protein cIAP1 and cIAP2 have increased copy number and expression levels in primary diffuse large B-cell lymphoma (DLBCL) tissues, while a second mitochondrial-derived cysteine aspartase (Smac) mimetic activator was designed to antagonize IAP proteins. Anna Dietz et al [42] investigated Smac mimetic BV6 could enhance the proteasome sensitivity of DLBCL cells and analyzed the molecular mechanism. Activation of BAX and BAK co-triggered by BV6 and the inhibitor Carfilzomib (CFZ) promoted cell

death.

BV6/CFZ induces mitochondria-mediated apoptosis mainly by activating BAX and BAK. Pretreatment with the cysteinase inhibitor N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone (zVAD.fmk) rescued BV6/CFZ-induced cell death, confirming the cysteinase dependence. XIAP blocked apoptotic execution by inhibiting cysteinase activity, while cIAP1/2 regulated pro-survival nuclear factor kappa B (NF-ϰB) signaling[43]. Smac mimicry-induced cIAP depletion leads to the accumulation of NF-ϰB-inducible kinase (NIK). Tumor necrosis factor (TNF) α, a well-described NF-ϰB target gene, over binds to cell surface receptors and induces receptor-mediated cell death, further enhancing apoptotic cell death [44].

2.2.5 CC-122

CC-122 is a next-generation brain E3 ligase regulator, and Zhongying Mo et al [45] performed a genome-wide CRISPR / Cas9 screen for CC-122 in the DLBCL cell line SU-DHL-4, enriched for the CUL4/DDB1/RBX1/CRBN E3 ubiquitin ligase complex or regulator, which induced IKZF1 and IKZF3 proteins ubiquitination and degradation, resulting in enhanced antiproliferative effects of CC-122 in DLBCL cells[46]. The sgRNAs targeting the NF-ϰB inhibitory genes CYLD, NFKBIA, TRAF2 and TRAF3 also showed significant enrichment in SU-DHL-4 cells, indicating that inhibition of the NF-ϰB pathway enhanced the anti-tumor activity of CC-122 in DLBCL. The elimination of CRBN showed more resistance to CC-122 than CYLD, NFKBIA, TRAF2 or TRAF3 inactivation, and the knockdown effect of CYLD and NFKB1A was also more pronounced than TRAF2 and TRAF3. Furthermore, knockdown of KCTD5, RFX7 and AMBRA1 in CRISPR screens abrogated the response to CC-122, and accumulation of the Gβγ subunit GNG5 after KCTD5 depletion reduced the growth inhibitory effect of CC-122 in several DLBCL cell lines. RFX7 and AMBRA1 are key factors in CC-122 apoptosis, RFX7 and Ambra1 loss will lead to V-7-AAD cell loss.

2.2.6 Hippo-Yes-associated protein (YAP)

Xiangxiang Zhou et al [47] determined that YAP expression was up-regulated in DLBCL, and its excessive expression could appreciably increase the growth, invasion and angiogenesis capacity of DLBCL cells. YAP is overexpressed in a variety of cancers and is associated with disease progression. Knockdown YAP by shRNA or CRISPR/Cas9 induced cell cycle arrest and promoted the expression of downstream YAP target genes CTGF and CYR61. The benzoporphyrin derivative Verteporfin (VP) induced a dose-dependent reduction in the expression levels of YAP and TEAD, while inhibiting the expression of the YAP downstream target genes CTGF and CYR61, thus exerting anti-tumor effects. IGF-1R is a tyrosine kinase, inhibition IGF-1R resulting in reduced expression of YAP and its downstream molecules. Supplementation of IGF-1 can reverse IGF-1R decrease the YAP protein level caused by downregulation. To determine how the loss of IGF-1R affects Hippo-Yap signals in DLBCL, PPP or AG1024, a member of the tyrosine molecular class and a cyclic ligand alkaloid, were added to DLBCL cells, both of which inhibit IGF1R activity. Increased expression of MST1(a key protein in Hippo-Yap signaling) was observed after addition. This confirms that IGF-1R may be an upstream regulator of Hippo-Yap signaling pathway, and its depletion can enhance Hippo-Yap signaling pathway.

2.2.7 Sphingosine-1-phosphate receptor 2 (S1PR2)

In diffuse large B-cell lymphoma (DLBCL), the sphingosine-1-phosphate receptor S1PR2 and its downstream signaling pathway are absent and repressed by the transcription factor FOXP1. Anna Stelling et al [48] found that the TGF-β/TGF-βR2/SMAD1 axis was involved in the transcriptional activation of S1PR2. There was a positive trend between SMAD1 and TGF-βR2 and S1PR2 expression, and TGF-β signaling controlled S1PR2 expression through TGF-βR2 and SMAD1. S1PR2-deficient SU-DHL-6 clones failed to undergo apoptosis upon exposure to TGF-β, suggesting that S1PR2 played a key role in TGF-β-driven apoptosis. In CRISPR-mediated DLBCL cell lines, gene editing of any of S1PR2, SMAD1, and TGFBR2 rendered tumor cells unresponsive to apoptosis by means of TGF-β. S1PR2 is an important transcription factor, and its deletion can cause over-proliferation of the developmental center (GC) B cell chamber and also affect the adhesion process of B cells and T cells accordingly promoting the formation of lymphoma.

2.2.8 Mitochondrial deacetylase sirtuin-3 (SIRT3)

Kavita Bhalla et al [49] found elevated activity of the mitochondrial deacetylase sirtuin-3 (SIRT3) in diffuse B-cell lymphoma (DLBCL) triggered via ATM deficiency, resulting in altered mitochondrial structure, lowered TCA flux, and enrichment of the glutamate receptor and glutamine pathways. ATM kinases are vital regulators of the DNA damage response, and B-cell tumors with ATM-zero phenotype mitochondria are poorly dealt with and do no longer respond to either traditional cures or DNA damaging drugs. In the absence of DDR, ATM prompts in response to mitochondrial oxidative stress [50]. Mitochondrial structures are swollen in ATM mutant DLBCL cells, and SIRT3 stimulation does not reverse the structural changes in mitochondria but can repair cell proliferation of DLBCL with CRISPR knockout ATM.

2.3 Burkitt lymphoma and CRISPR Cas9

Burkitt lymphoma (BCL) is a group of non-Hodgkin's lymphoma that originated from B lymphocytes, which has a certain relationship to EBV virus, but the exact pathogenesis is unknown.

2.3.1 MYC

EBV virus will initiate the transcription of MYC after infection, and also activate KC and TLR-4 pathways to maintain its own immune system, so as to escape host immune recognition and clearance, and the depletion of MYC or factors affecting MYC expression causes EBV virus to enter a lysis state. Guo Ran et al[51] performed a genomic screen using CRISPR / Cas9 in Burkitt's lymphoma B cells and identified a MYC-centered gene network in which MYC and endoglin, FACT, STAGA, and mediator collaborate to repress transcription of the BZLF1 promoter. BZLF1 primarily regulates B-cell lysis, and its transcription causes more than 30 early lytic genes, including viral DNA polymerase, the synthesis factor BMRF1, kinases and other factors important for cleaved DNA replication. The expression of Epstein-Barr nuclear antigen (EBNA), latent membrane protein (LMP), and BZLF1 was reduced after the knockdown of ubiquitin-like PHD and ring finger-containing 1 (UHRF1), DNA methyltransferase 1 (DNMT1), and polycomb repressor complex 1 (PRC1) by Guo Rui et al[52]. The incapability of the immune system to recognize BV virus led to a long-term latent infection in the dormitory. Sidorov et al [53] investigated the impact of IgH/c-myc translocation in primary B cells the use of CRISPR/Cas9 knock-in method and confirmed that CD4+ T cells inhibit eBL by using killing pre-eBL cells lacking IgH/c-myc translocation in vitro on the one hand; on the other hand, by means of reducing the EBNA2 expression promoter, they induced EBV transition between latencyIIII and latency I, thereby indirectly stimulating eBL development. EBNA2 has an anti-apoptotic effect, but its loss can lead to decreased viability of the LCL. CD4+ T lymphocytes increased the expression of bcl6 mRNA in the LCL, which is an important marker of eBL.

2.3.2 Kinases MLKL (Mixed lineage kinase domain-like protein), RIPK1 and RIPK3 (receptor-interacting protein kinases 1 and 3)

Annkathrin Koch et al [54] proposed that if cystathionase was blocked with zVAD.fmk (TBZ), the combination therapy of Smac mimicking BV6 and TRAIL induced BL necrotic apoptosis. The higher the expression of MLKL (mixed lineage kinase domain-like protein), the better the therapeutic effect of TBZ on BL cells, confirming that MLKL expression is one of the influencing factors of TBZ treatment on BL cells. Knockdown of MLKL in BL-2, RAMOS, Seraphine, and BL-30 cells showed that cell death was completely inhibited, suggesting that its mediated cell death was heavily dependent on MLKL. Necroptosis is based on the generation of necrosome, the kinase MLKL, RIPK1 and RIPK3 (receptor-interacting protein kinases 1 and 3) constitute the necrosome [55], the place phosphorylation of MLKL and RIPK1 is a necessary link in apoptosis. MLKL is a direct provider of RIPK3 and its phosphorylation allows MLKL to oligomerize and translocate at the plasma membrane, thereby binding it to the membrane and inflicting its dying [56]. In BL cell lines, expression of TRAIL-R1/2 [57], a Smac mimic that binds to TRAIL (TNF-related apoptosis-inducing ligand), prompts cysteinase-non-dependent necrotic cell dying in an MLKL-dependent manner after cysteinase inhibition.

2.3.3 Chromatin assembly factor 1 (CAF1)

Yuchen Zhang et al [58], via a genome-wide human CRISPR screen, observed that an essential element in

preserving Burkitt latency is the chromatin assembly factor CAF1. Its depletion leads to the conversion of Burkitt cells from latency to lysis, ensuing in the expression of early Burkitt cell BZLF1, BMRF1, and the secretion of viral particle. Meanwhile it reduced histone 3.1 and 3.3 as well as inhibited tri-methylation of lysine 4 on histone H3 and histone H3 lys-27-specific trimethylation (H3K9me3 and H3K27me3) occupy several regulatory elements of the viral genomic cleavage cycle. The CAF1 complicated is composed of three subunits, CHAF1A, CHAF1B, and RBBP4, and their depletion similarly inhibits the expression of EBV lytic genes. EBV The predominantly expressed protein elements are EBNA2 and EBNA-LP, and inactivation of EBNA2 reduces the expression of CAF1 subunit mRNA [59]. In addition, EBNA-LP, EBNA3A, EBNA3C and LMP1-activated NF-ϰB subunits co-occupy the CHAF1A, CHAF1B and RBBP4 promoters, suggesting that they may support CAF1 expression. The loss of histone chaperone is similar to that of CAF1, which can up-regulate BZLF1 and BMRF1, but weaker than CAF1.

2.4 Mantle Cell Lymphoma (MCL)

Most patients with MCL respond well to treatment and have an excellent prognosis. However, as with most other B-cell lymphomas, MCL patients are highly heterogeneous and have a different proportion of different genes and mutation types in the tumor. Up to 84% of the cyclin D1 (CDC10) gene rearrangement in tumors of MCL patients occurs in one or more B-cell lymphomas.

2.4.1 5-lipoxygenase (5-LOX)

Human B lymphocytes express 5-lipoxygenase (5-LOX) and 5-LOX activating protein (FLAP), which convert arachidonic acid to leukotrienes. 5-LOX gene expression was found to be up-regulated in B-lymphocytic leukemia (B CLL) and mantle cell lymphoma (MCL) [60], and levels of 5-LOX were associated with disease progression and recurrence in patients with B CLL. Chuanyou Xia et al [61] discovered that loss of ALOX5 gene caused a big decrease in CRISPR / Cas9-mediated migration of JeKo-1 cells, and that 5 - LOX and FLAP inhibitors reduced the adhesion of JeKo-1 cells to stromal cells [62]. These results suggest that inhibition of 5-LOX may be a new therapy for MCL and certain other B-cell lymphomas.

2.4.2 Reactive oxygen species (ROS)

Sudjit Luanpitpong et al [63] first confirmed a subpopulation of CSC (cancer stem cells) that was regulated by using ROS (reactive oxygen species) in MCL cell lines and patient-derived primary cells and inversely correlated with the sensitivity of bortezomib (BTZ). ROS are necessary signaling molecules in the tumor microenvironment. O2- has a vast inhibitory impact on CSC-like cells, while H2O2 has an extensive enriching and apoptosis-inhibiting impact on CSC-like cells. Mcl-1 and Zeb-1 (also regarded as TCF8) are effective targets of O2- and H2O2. Using CRISPR/Cas9 gene editing technology, the expression of Mcl-1 was inhibited, and the expression of Zeb-1 was decreased, while the expression of TCF8 was increased. It was found that intracellular ROS levels increased significantly after Mcl-1 gene silencing, and Mcl-1 is a critical target gene of O2 and has a regulatory role in CSC-like cells, suggesting that ROS has an impact in BTZ-induced apoptosis and CSC-like cells can resist BTZ-induced apoptosis by apoptosis-regulating protein Mcl-1. What's more, mitochondrial membrane potential is also an important factor in the protective impact of the mitochondrial antioxidant system on BTZ-induced apoptosis.

2.4.3 CG-806

The dual BTK/SYK inhibitor, CG-806 (luxeptinib), downregulates the expression of the anti-apoptotic proteins Mcl-1 and Bcl-xl, inhibits the Akt/mTOR signaling pathway, and mitochondrial inner membrane depolarization, accompanied by metabolic reorganization of glycolysis, thereby inducing apoptosis in MCL cells[64]. the BCR signaling pathway can drive NFϰB, induce Bcl-2 protein expression, and Bcl-2 is a key factor in BCR cross-linking, and BCR cross-linking, which activates the Akt/mTOR pathway, can also promote the interaction between SRC family kinases (e.g. LYN) and CD79A/B and activate splenic tyrosine kinase (SYK). Elana Thieme et al [64] showed that the dual BTK/SYK inhibitor CG-806 interferes with the BCR signaling pathway in the above manner. The loss of BAX, an activator of apoptosis, makes CG-806 anti-apoptotic. They also found that Bcl-2 and MOMP bind to CG-806, suggesting that Bcl-2 and MOMP

play an important role in the induction of apoptosis by CG-806. Inactivation of type I interferons and cell cycle control pathways, as well as Wnt/β-linked protein and mTOR signaling pathways, promote CG-806 resistance. Downregulation of NFϰB pathway was associated with increased BCL2/BAX expression, NFKBIA is a negative regulator of NFϰB signaling, and CG-806 became less effective in treating MCL cells after knockdown of NFϰB and BAX. The above confirms that the NFϰB pathway and the Bcl-2 family network play a very important role in BTK / SYK dual inhibition.

2.5 Other B-cell lymphomas with CRISPR Cas9

Primary mediastinal B lymphoma (PMBL) is a tumor characterized by genetic variants with a high degree of heterogeneity, the most common of which is the XPO1 gene located in the chromosome 12p25.2 region. XPO1 point mutations are less frequent but are positively expressed in most PMBL, and amplification of this gene is associated with poor prognosis in patients with PMBL. Genomic abnormalities are also frequently present in cHL, giving both a similar clinical presentation. The XPO1 gene encodes export protein 1 (XPO1), which transports cargo proteins to the nucleus by binding to cargo binding sites in the hydrophobic groove of the cargo protein nuclear export region; it also prevents RNA degradation by RNA polymerase II (Pol II) by reducing cargo protein nuclear export and increasing the amount of cargo protein in the nucleus. High heritability of E571K mutations in PMBL and cHL, but not in other types of B lymphocytes. Hadjer Miloudi et al[65] studied XPO1 E571K mutation in PMBL and cHL by editing E571K gene using CRISPR-Cas9. XPO1 gene is expressed in each solid and hematological tumors, whilst XPO1E571K gene is spectrum-specific and is only observed in B cells[66]. Every mRNA and protein contains XPO1 mutations, which primarily change the subcellular localization of XPO1 and impact protein transport to the cytoplasm, lysosomes, and mitochondria. On the cytoplasmic surface of the nuclear envelope, XPO1 co-localizes with nucleoprotein 1, also known as importin 1, KPNB1, or IPO1. Mutant XPO1 can attach to IPO1, which alters the dynamics of associated cargo's nuclear export and import.**2.6 Other B-cell lymphoma impact factors and CRISPR Cas9**

2.6.1 JAK-STAT signaling pathway

Verena Barbarino et al [67] found that the combination of Bruton tyrosine kinase inhibitor and ibrutinib significantly reduced the antiboy-dependent cytophagocytotic effect of JAK2 on macrophages mediated, while ADCP was enhanced with JAK inhibitor or knockout of JAK2 with CRIPR Cas9. It is suggested that JAK-STAT signaling pathway can be used as a new direction in the treatment of B cell lymphoma. Furthermore, Janus kinase 2 (JAK2) was significantly absent only under ibrutinib treatment, with no significant difference in ADCP augmentation using second-generation BTKis. ADCP is a complement cascade mediated mainly by macrophages, mediated by complement receptor (CAR) and complement activator (CIC). Together with complement activation, ADCP contributes to pathogen eradication. Furthermore, JAK2 and JAK3 kinases can strengthen ADCP caused by ibrutinib.

2.6.2 CD40

The proliferation and differentiation of B cells depend on the nuclear protein CD40, which is mediated by CD40L during development and binds to the surface receptors on B cells. In tumor tissues of patients with autoimmune disorders, upregulation of CD40/CD40L results in aberrant lymphangiogenesis and loss of lymphocyte function. In addition, autoimmune disorders might arise as a consequence of reduced CD40 activation. Uncertainty exists regarding the precise mechanisms by which CD40 influences the expression of other cell surface molecules. As multimeric CD40 ligands stimulate Daudi B cells and Chang Jiang et al[68] discovered that cells with upregulated Fas expressed cd40l substantially more than other CD40 ligands, elevated Fas protein membrane (PM) content could be employed as a marker to identify this cell. The nuclear factor-B (NF-B), mitogen-activated kinase (MAPK) and phosphatidylinositol 3 kinase (PI3K) pathways that control Daudi B-cell differentiation and proliferation are affected by the accumulation of TNFR-related proteins after CD40 activation, which causes an increase of Fas in B cells. A non-classical CD40 transcription factor called FBXO11 is crucial for B-cell development. Reduced FBXO11 inhibits CD40 expression, mainly decreasing CD40L induced Fas expression without impairing TNF-α induced Fas expression. After knockout

ELF1, CD40 induced Fas generation decreases, inhibiting the classical and non-classical MAPK pathway of CD40; ICAM-1 expression is suppressed, which is the target gene of CD40. High expression of m6A in cancer cells, m6A encoding protein WTAP and CD40 bind, can regulate the expression level of m6A, affect cell proliferation. Bispecific serine/threonine phosphatase DUSP10 In the CD40 negative regulator screen, DUSP10 is the primary B-cell negative feedback regulator of the CD40 / MAPK pathway.

2.6.3 Phosphatidylinositol-3-phosphate 5-kinase (PIKfyve)

Sophia Gayle et al [69] concluded that apimodal was an appropriate and selective antitumor agent in opposition to B-NHL. The PIKfyve lipokinase active site is an apilimod-specific binding site, and their binding exerts antibody-dependent cell-mediated cytotoxic (ADCC) effects, thus allowing the use of PIKfyve inhibitors (such as rituximab and cetuximab) to selectively kill b-cell lymphoma (bcL-NHL) without damaging normal cells. PIKFYVE knockdown assays confirmed that PIKFYve inhibition resulted in reduced apilimod activity. In addition, using a genome-wide CRISPR screen, they discovered that lysosomal dysfunction significantly contributed to the cytotoxicity of apilimod. Although PIKfyve activity is lost, endosomal and lysosomal membrane trafficking is disrupted. Genome-wide loss genetic screening of SU-DHL-10 B-NHL cell lines using GeCKO library to identify several genes such as TFEB (Major transcription regulator of lysosomal organisms) and endosomal/lysosomal genes CLCN7, OSTM1 and SNX10; After knockout OSTM1 and CLCN7, it was found that apilimod resistance increased, confirming that these genes affect apilimod sensitivity. CLCN7/OSTM1 and TFEB are downstream factors of the B-cell receptor (BCR) and key regulators of B-cell development that can serve as potential therapeutic targets. Knockdown of CLCN7 and OSTM1 using CRISPR in B-NHL cells resulted in complete apilimod resistance, while knockdown of TFEB resulted in only partial resistance, confirming that CLCN7 / OSTM1 is a key factor in apilimod resistance in B-NHL, while TFEB is a regulator of apilimod resistance mechanisms. Altogether, these findings introduce a novel therapeutic strategy to kill B-NHL tumor cells by disrupting lysosomal homeostasis through apilimod-mediated PIKfyve inhibition.

4 Summary and outlook

CRISPR/Cas9 technology, as a novel technology, has the potential to permanently destroy tumor genes, which is not only simpler and faster in design and synthesis, but also creates new avenues for the study of tumor pathogenesis, screening of drug-acting gene targets and precision medicine, and has become a hot spot for many researches in recent years.

At present, B cell lymphoma is mostly treated by chemotherapy. However, patients are susceptible to drug resistance, so it is important to develop a new treatment for the disease. Based on this, the researchers developed a new treatment, targeting gene therapy, which gives hope to B-cell lymphoma patients and is expected to solve the treatment challenges of the disease. But more research is needed to learn more about CRISPR gene editing and its role in disease and to combine it with other treatments to cure B-cell lymphoma. While some problems remain unresolved, I believe that with continuous improvement, this technology can be applied to areas such as medicine in the future.

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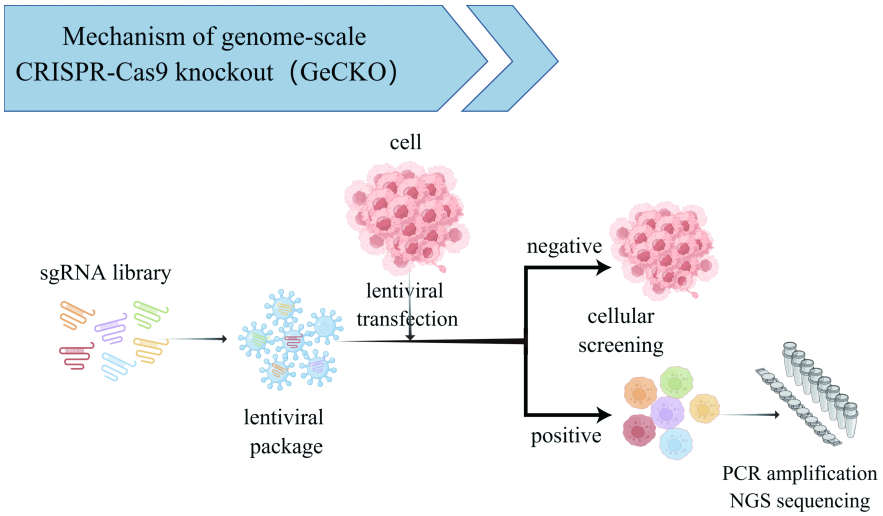
Figure 1. Mechanism of genome-scale CRISPR-Cas9 knockout(GeCKO)

The main steps are construction and design of gRNA libraries, lentivirus transfection, cell screening (positive/negative screening), and gene analysis.



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`Table 1.docx` available at https://authorea.com/users/603764/articles/633957-application-and-progress-of-crispr-cas9-gene-editing-in-b-cell-lymphoma