Small Molecule Stabilization of the CARD11 G-quadruplex Represses Transcription: Developing a Therapeutic Target for Diffuse Large B Cell Lymphoma

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Abstract

Diffuse large B cell lymphoma (DLBCL) involves abnormal B cell growth in the lymphatic system. Abnormalities such as recurrent genetic mutations cause critical components of the B-Cell Receptor signaling pathway to be overactive. This constitutes an oncogenic defect that drives uncontrolled B cell growth. Caspase recruitment domain-containing protein 11 (CARD11) is a scaffold protein critical to BCR pathway, and its recurrent gain-of-function mutations are frequently found in DLBCL. This study aims to investigate the potential of *CARD11* gene silencing as a therapeutic for DLBCL treatment by targeting the DNA secondary structure-G-quadruplexes (G4s) formed within the gene, as G4s usually act as physical barriers to gene expression. Using circular dichroism (CD), stable G4's were identified within the highly guanine-rich promoter region. Small molecules were screened using a fluorescence-resonance energy transfer (FRET) assay to identify compounds that stabilize G4 structures. To quantify the effects of G4 stabilization on gene expression, qPCR was then used to determine that stabilization of G4s led to repression of transcription and subsequent reduction in mRNA levels of the oncogene *CARD11* and a few others,, which are crucial for DLBCL progression. This silencing extended to co-regulated oncogenes (*BCL2, MYC*), disrupting survival signals in the NFκB pathway. The small molecule R575 demonstrated dose-dependent efficacy across DLBCL cell lines of varying biopsy origins, suggesting applicability to relapsed/refractory cases. These findings indicate that stabilizing the G-quadruplex structures formed in the *CARD11* promoter region could inhibit DLBCL growth by silencing *CARD11* gene expression and downstream oncogenic signals in the BCR pathway.

I. Introduction

Diffuse large B cell lymphoma (DLBCL) is a highly aggressive and heterogeneous subtype of non-Hodgkin lymphoma, characterized by the uncontrolled growth of abnormal B cells in the lymphatic system. DLBCL represents the most common form of non-Hodgkin lymphoma, accounting for approximately 30-40% of cases [1,5]. Despite significant advancements in DLBCL treatment, 60% of DLBCL patients still remain refractory to the standard combinatory treatment R-CHOP (*rituximab, cyclophosphamide, doxorubicin, vincristine*, and *prednisone*), or experience relapse after achieving initial remission [1,4,7]. The B cell receptor (BCR) signaling pathway plays a crucial role in DLBCL development and progression [6]. Upon encountering specific antigens, B cells activate their BCRs, leading to intracellular signaling cascades that promote cell survival and proliferation [1,2,3]. In Activated B cell (ABC) DLBCL, this pathway is constitutively active due to genetic mutations in other key component proteins of the BCR signaling pathway, such as *BCL2* and *MYC*, resulting in enhanced BCR signaling independent of antigen binding [3,6]. Upon activation, BCR sends signals downstream through a multi-protein complex that in turns activates other signaling pathways like the NFκB pathway, which is critical for cell survival and proliferation. The aberrant propagation of survival signals from uncontrolled BCR signaling is a key factor promoting the initiation and progression of DLBCL [1].

The present research focuses on the CARD11 protein, an important component of the multi-protein signaling complex. Since *CARD11* sits at a critical signaling node and its mutations play a vital role in driving lymphoma growth, it represents a promising therapeutic target [1,2,4]. Specifically, silencing *CARD11* could overcome a central DLBCL growth mechanism by cutting off key survival signals at their origin--the mutated CARD11 protein itself. S tabilized G-quadruplex structures (G4s), which

are guanine-rich DNA or RNA sequences folded into stacked tetrads, within the *CARD11* gene promote likely downregulate *CARD11* and other oncogenes by physically blocking transcriptional machinery and interfering with regulatory elements, leading to reduced gene expression (Figure 1). These double stranded DNA secondary structures are extremely stable and can inhibit gene expression when localized to promoter regions, acting as roadblocks to RNA polymerases and other proteins attempting to bind. By screening a library of small molecules for the ability to selectively bind to and stabilize G4 structures, this innovative approach aims to silence the expression of genes involved in the development of DLBCL, offering a promising avenue for developing novel therapeutic strategies for aggressive DLBCL.

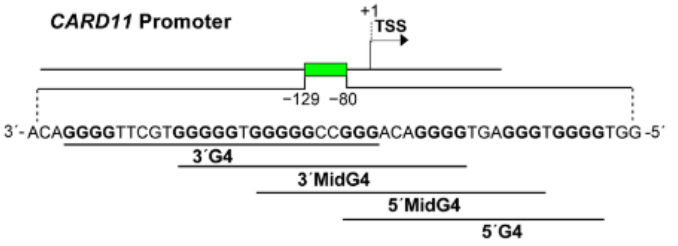


Figure 1. The *CARD11* promoter has potential G4-forming motifs near the transcription start site.

II. Materials and Methods

A. Oligonucleotides

Oligos from IDT and Eurofins were used for circular dichroism (CD) and fluorescence-resonance energy transfer (FRET) experiments. Their concentrations were determined by absorbance measurements at 360 nm using a nanodrop spectrophotometer and calculating the molar absorptivity (ε) via the Beer-Lambert law. Both CD and FRET samples were stored at 4°C to account for variation errors.

B. CD Spectroscopy Analysis

CD analysis with varying KCl concentrations was used to test G4 formation. Previous research has revealed that potassium ions stabilize G4 structures, so varying potassium chloride concentrations would allow us to determine the optimal stability conditions of G4 and analyze the G4 folding dependence on potassium levels (Figure 2). Oligos were heated and cooled, and CD spectra data was collected at 263 nm using a Jasco-1100 spectropolarimeter. Data was baseline-corrected, smoothed using the Savitzky-Golay method, and thermal stability was then assessed by increasing the temperature by 1°C/min while recording molar ellipticities, allowing T_m determination within ±1°C using Prism software.

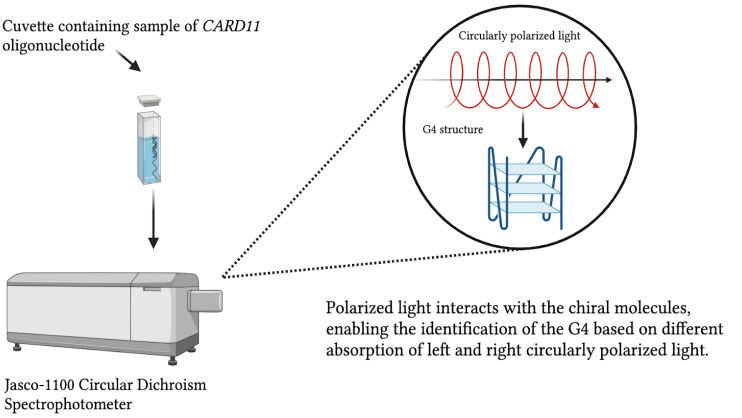


Figure 2. Visual representation of CD methodology to identify G4 structures within the *CARD11* oligonucleotide samples.

C. Small Molecule Screening

Custom Fluorescence Resonance Energy Transfer (FRET) probes were created to target the specific *CARD11* gene sequence, enabling assessment of G4 stability changes induced by small molecules. Probes were prepared with 8 small molecules at 40 µM in Dimethyl sulfoxide (DMSO) at 16% mixed with FAM/TAMRA-labeled *CARD11* probes in 10mM Sodium Cacodylate and 20mM KCl buffers to provide appropriate salt conditions known to facilitate G-quadruplex formation while enabling testing of the small molecule conditions. The probes underwent gentle centrifugation at 2000 rpm to mix the components while avoiding disruption of structure. Transferal to 96-well plates enabled high-throughput and controlled testing of multiple small molecule conditions simultaneously. The FRET assay was performed at 95°C, slightly below melting temperature, to potently evaluate stability modulations detectable by FRET signal changes. Data normalization on a 0-1 scale enabled standardized comparison across probes and conditions for comprehensive analysis. Curve fitting analysis was utilized to determine the logIC50 values for each small molecule, quantifying the molar concentration required to inhibit 50% of G-quadruplex structure stability. Comparison of the logIC50 values provided key insights into whether the small molecules preferentially disrupt or stabilize G4 structures, based on their binding affinity and potency.

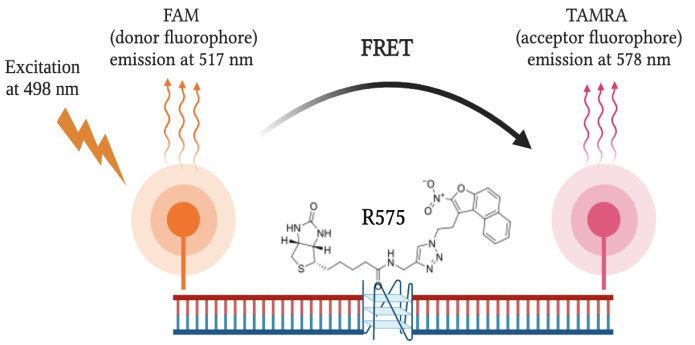


Figure 3. Visual representation of the FRET methodology to test for G4 stabilizing small molecules. R575 used as an example small molecule. In the FRET assay, the G-quadruplex folds upon cooling, bringing the FAM and TAMRA tags on the *CARD11* DNA together, while R575 binding further stabilizes the structure - keeping the tags consistently in close proximity and enabling ongoing energy transfer between them.

D. Human Cell Lines

The cell lines RIVA, HBL1, VAL, SUDHL6, and GM16113 from ATCC and DSMZ were stored in RPMI 1640 media with 10% FBS and 1% penicillin-streptomycin. They were grown at 37°C with 5% CO_2. Using these diverse DLBCL cell lines allows the testing of small molecule stabilization of the *CARD11* G4 on malignant cells from varying origins across patients (see Table 1).

Table 1. Cell lines used along their biopsy origins

Cell line	Cell line origin (biopsy status)
RIVA	Peripheral blood
HBL1	Pleural effusion
VAL	Bone marrow
SUDHL6	Peritoneal effusion
GM16113	Peripheral vein
GM22673	Peripheral vein

E. Gene Expression Measured Using qPCR

Lymphoma cell lines were treated with small molecules 9037 or R575 to measure relative gene expression. TaqMan probes for genes including *CARD11* were used in vitro for this qPCR experiment. Master mixes were prepared with added cDNA for the qPCR. The cycle threshold (Ct) values were normalized to housekeeping gene TBP and compared to untreated controls, yielding ΔΔCt values. The relative gene expression changes were assessed based on these values.

F. Statistical Analysis

Data analysis was conducted using GraphPad Prism Software v 9.94. All experiments were performed in triplicate to ensure statistical significance and data reliability. GraphPad Prism facilitated descriptive statistics, t-tests, ANOVA, and non-linear regression analyses. Visualizations, such as bar graphs and scatter plots, aided in interpreting trends and relationships within the data.

III. Results

The guanine-rich promoter oligonucleotide of *CARD11* displayed distinctive G4 CD peaks at ~260 nm (Figure 4A). Additionally, both these peaks and the oligonucleotides' melting temperatures were affected by the presence of a K+ gradient (Figures 4A-B). Consequently, the *CARD11* gene exhibits a Guanine rich sequence (3-4 runs of G consecutively), which is consistent with G4 formation (Figure 1). As the concentration of KCl increases initially, the molar ellipticity decreases, indicating a decrease in the stability of the G4 structures formed by the *CARD11* gene (Figure 4A). The peak at ~ 260 nm with 100mM KCl exhibits the highest molar ellipticity, suggesting that the G4 structure is most stable at this concentration (Figure 4A). As the KCl concentration decreases, the T_m values also decrease, indicating lower stability of the G4 structures, further demonstrating that G4 is most stable at 100mM KCl (Figure 4B).

FRET analysis was performed with the non-interactive small molecule 9037, and we see that 9037 decreased G4 stability in *CARD11, BCL2*, and *MYC* genes--confirming its non-stabilizing nature (Figure 4C). To further demonstrate 9037 as a non-interactive small molecule, qPCR was performed to reveal that 9037 did not significantly lower the fold change values in the key gene's expression while in comparison to the vehicle treatment control (VTC) of DMSO at 16% and no treatment control (NTC) which is distilled water (see Figure 4D). Thus, in subsequent experiments, 9037 was used as a negative control to validate that the screening methodology could differentiate when a small molecule does not stabilize or silence *CARD11* G-quadruplex structures, in contrast to the positive impacts of stabilizing small molecules identified by the screen.

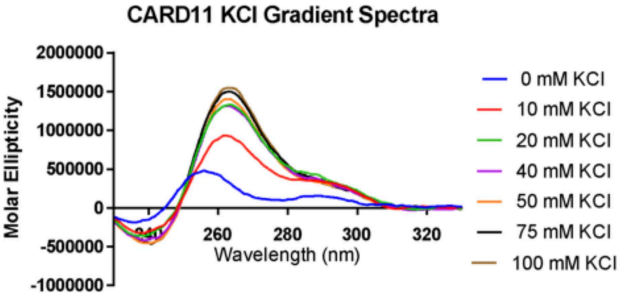


Figure 4A. Upstream G-rich sequence forms a K+-influenced G4 structure, confirmed by circular dichroism peak at ~260 nm.

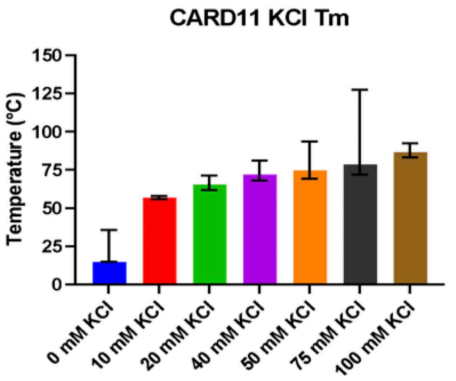
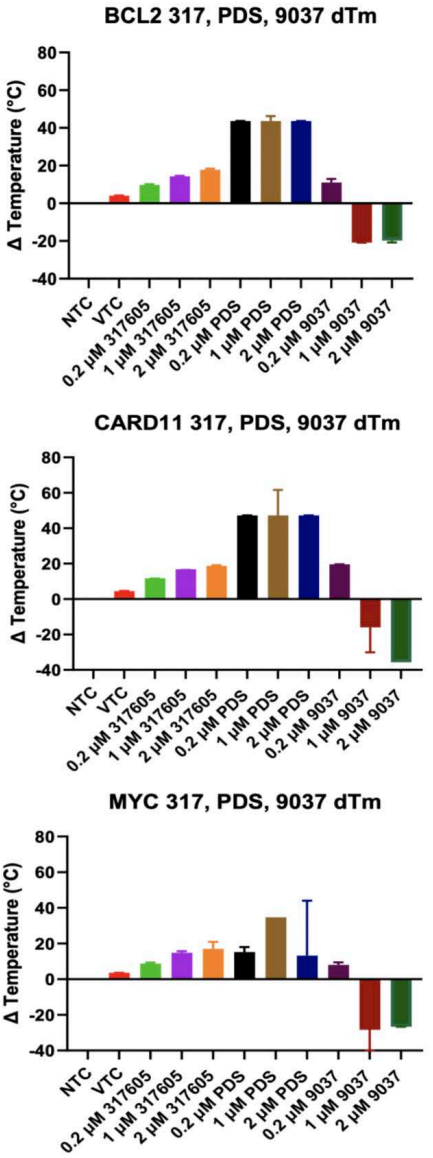


Figure 4B. Corresponding T_m values graphed.



Figures 4C. FRET analysis compared the 9037 impacts (on key oncogenes alongside *CARD11*) to Puromycin Dihydrochloride Salt (PDS, 317605, NTC, and VTC).

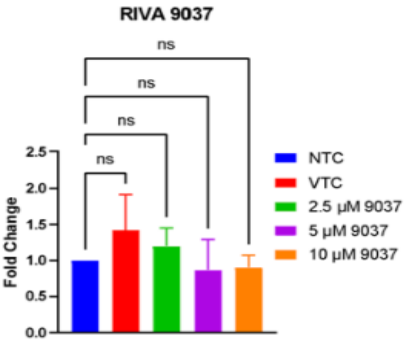


Figure 4D. Fold change *CARD11* mRNA with qPCR data with 9037 tested on RIVA cell line.

FRET assay revealed small molecules R575, 311153, 147481, and 317605 significantly increase G4 Tm, reinforcing structural stability. Small molecules 13248 and 309401 also stabilize G4 but to a lesser extent. Differences arise from diverse chemical structures, binding affinities, and interactions with *CARD11* promoter's G4 motifs. Molecular forces, functional groups, and configurations impact binding.

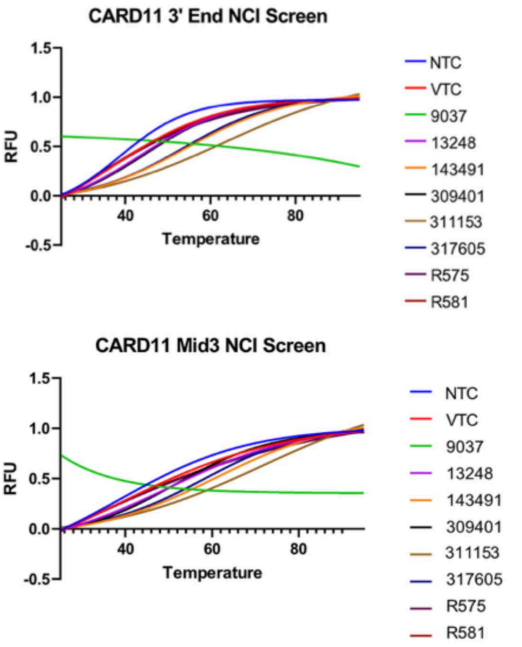


Figure 5. FRET melt curves obtained for small molecules compared to probe + 16% DMSO (vehicle control) at 2.5 μM concentration. Shifts rightwards indicate Tm increase ≥ 4°C, identifying potential candidates. FRET screen used probes in 10 mM NaCac and 20 mM KCl buffer, mimicking the *CARD11* gene's G4-forming conditions.

According to the FRET analysis, small molecule R575 enabled the FAM and TAMRA tagged *CARD11* DNA sequence to retain a consistent 0.7 raw fluorescent unit (RFU) energy transfer plateau from 60°C up to the

highest tested temperature of 100°C, without exhibiting the rapid signal decrease that indicates structure melting seen for other small molecules (Figure 5). We thus conclude that R575 allows for stabilized G4 rigidity and continued folding across an expanded temperature range. This exceptional thermal stability (≥100°C) suggests R575 binds the *CARD11* G4 with high affinity, likely through interactions with the G-tetrad core or loop regions, as observed in other G4-stabilizing ligands [7]. The lack of melting plateau loss contrasts sharply with destabilizing controls (e.g., 9037; Figure 4), reinforcing R575's selectivity.

Small molecule R575 was then investigated for its impact on genes *KRAS* and *TERT* (which contain G4 structures), and *UBC* and *EEF1A1* (which do not contain a G4 structure). In the RIVA cell line, derived from peripheral blood, 10 μM of R575 led to significant downregulation of *BCL2* and *CARD11* genes, indicating effective silencing (top panel, Figure 6). This suggests R575's potential to modulate *CARD11* gene expression, crucial in DLBCL progression. Similarly, the VAL cell line, derived from bone marrow, showed reduced *BCL2* expression with R575 (middle panel, Figure 6). Cell line GM16113, derived from peripheral vein, also experienced silencing effects on *BCL2, CARD11*, and *MYC* genes with R575 (bottom panel, Figure 6). The consistent silencing of *CARD11* and co-regulated oncogenes (*BCL2, MYC*)—but not non-G4 genes (*UBC, EEF1A1*)—across all cell lines (RIVA, VAL, GM16113) underscores R575's specificity for G4-containing promoters. This aligns with the FRET stability data (Figure 5) and suggests downstream disruption of NFκB signaling, a key survival pathway in DLBCL [1,3].

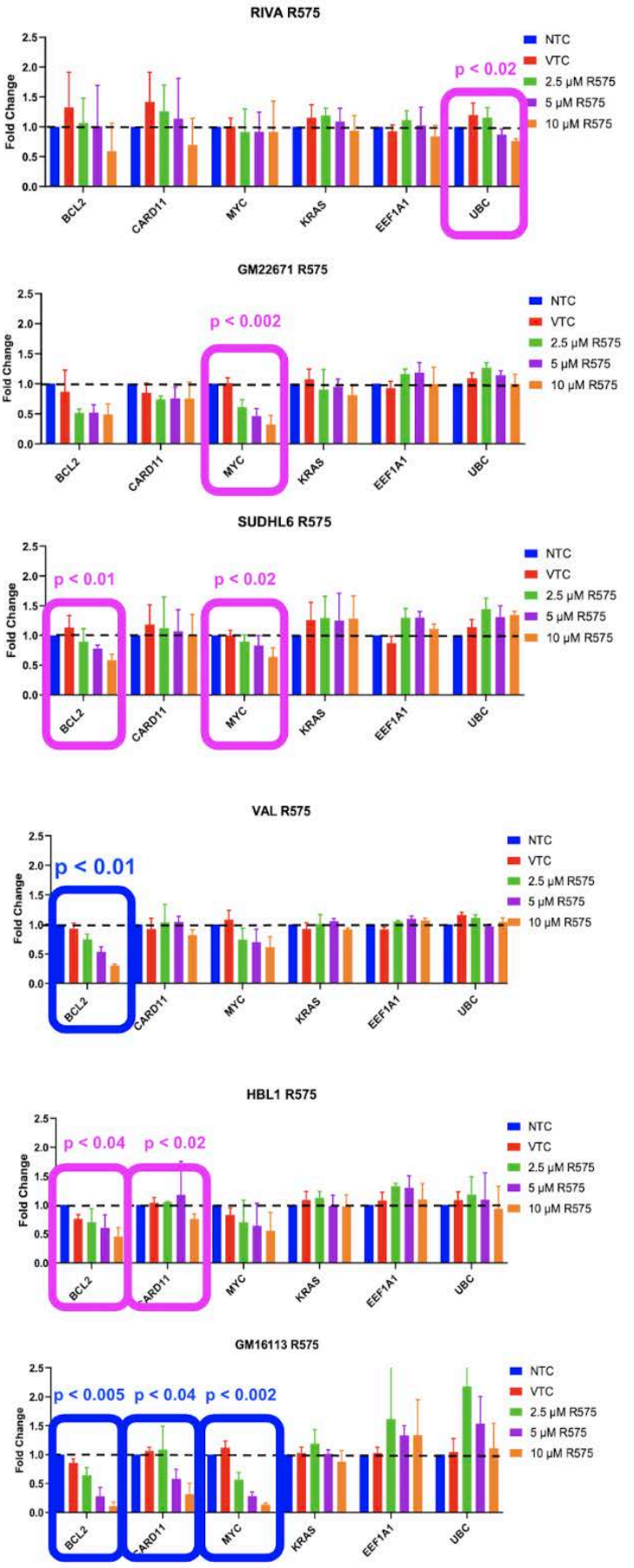


Figure 6. qPCR results for genes *BCL2, CARD11, MYC, MYD88, KRAS, TERT, UBC*, and *EEF1A1* in the RIVA (A), VAL (B), and GM116113 (C) cell lines with varied concentrations of small molecule R575. Boxed experiments show dose-dependent reduced levels of gene expression using ANOVA (purple) or two-sample t-tests (VTS vs. highest dose, pink) statistical analyses. Figures generated by student researcher.

IV. Conclusions

This study demonstrates the potential of targeting G-quadruplex structures within the *CARD11* promoter as a therapeutic strategy for DLBCL. We investigated G-quadruplexes forming in the *CARD11* gene using circular dichroism, identifying stable structures that can act as gene expression barriers. Through screening, the study found a small molecule R575 that substantially stabilizes G4. As stabilized G4 leads to gene silencing, there is no surprise that gene expression analyses revealed that R575 consistently downregulates *CARD11* across DLBCL cell lines, indicating potential for modulating oncogenic BCR signaling. We thus propose G4 in the *CARD11* promoter as a prospective therapeutic target for silencing *CARD11* and disrupting downstream signaling in DLBCL. Small molecule stabilization of these structures shows promises for precise, personalized treatment strategies. Further translational research on G4-targeted therapeutics *in vivo* is warranted to validate and optimize findings toward improved patient outcomes. This innovative approach signifies a potential paradigm shift towards direct DNA/RNA targeting to limit translation of key oncoproteins driving aggressive DLBCL growth.

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References

[1] Swafford K., Acharya, B., Xu, Y.Z., Raney, T., McCrury, M., Saha, D., Frett, B., & Kendrick, S. Targeting a Novel G-Quadruplex in the CARD11 Oncogene Promoter with Naptho(2,1-b)furan-1-ethanol,2-nitro- Requires the Nitro Group.Genes,13(7),1144 https://doi.org/10.3390/genes13071144 (2022).

[2] Candelaria, M., & Dueñas-Gonzalez, A. Rituximab in combination with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in diffuse large B-cell lymphoma. Therapeutic advances in hematology, 12, 2040620721989579 (2021). https://doi.org/10.1177/2040620721989579

[3] Kendrick, S., Muranyi, A., Gokhale, V., Hurley, L. H., & Rimsza, L. M. Simultaneous Drug Targeting of the Promoter MYC G-Quadruplex and BCL2 i-Motif in Diffuse Large B-Cell Lymphoma Delays Tumor Growth. Journal of medicinal chemistry, 60(15), 6587–6597. https://doi.org/10.1021/acs.jmedchem.7b00298 (2017).

[4] Davis, R. E. et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature 463, 88-92, doi:10.1038/nature08638 (2010).

[5] Testoni, M., Zucca, E., Young, K. H. & Bertoni, F. Genetic lesions in diffuse large B-cell lymphomas. Ann Oncol 26, 1069-1080, doi:10.1093/annonc/mdv019 (2015).

[6] Davis, R. E. et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. Nature 463, 88-92, doi:10.1038/nature08638 (2010).

[7] De Cian, A., Guittat, L., Kaiser, M., Saccà, B., Amrane, S., Bourdoncle, A., Alberti, P., Teulade-Fichou, M. P., Lacroix, L., & Mergny, J. L. Fluorescence-based melting assays for studying quadruplex ligands. Methods (San Diego, Calif.), 42(2), 183–195. https://doi.org/10.1016/j.ymeth.2006.10.004 (2007)

CRISPR-Cas9: A Testament to the Value of Basic Research

Ray Wang

Abstract

The CRISPR-Cas9 system is a programmable method of editing DNA sequences and its discovery has transformed the fields of medicine, agriculture, and biotechnology, to name a few. While it has revolutionized modern gene editing approaches, its discovery was not the result of targeted studies. Rather, it was based on years of basic scientific research and the motivation and curiosity of passionate investigators to understand basic biological phenomena. By highlighting key studies, this review traces the historical and experimental trajectory from prior gene-editing techniques, such as restriction enzymes and zinc finger nucleases (ZFNs), to the discovery of the CRISPR-Cas9 system. The story of CRISPR-Cas9, from being investigated as a form of bacterial adaptive immunity to the dawning realization of its massive potential, symbolizes the value and necessity of continued investment in basic scientific research .

Introduction

Since his inauguration on January 20th, President Trump and his administration have enacted policies that significantly reduced federal support for science, signing executive orders that froze billions of dollars in research funding (Glenza, 2025; Nature, 2025). These actions have called into question the national commitment to the advancement of scientific knowledge, once deemed an "essential key to our security as a nation" (Tollefson et al., 2025). In a time when the importance of science has somehow become debatable, basic research, or "a systematic study directed toward greater knowledge of phenomena without specific applications in mind" is under even greater threat, as parties interested in shaving off government inefficiencies may view basic research studies as aimless ventures that waste taxpayer dollars (NIAID, 2023). However, as evidenced by our understanding of the revolutionary gene-editing CRISPR-Cas9 system, which can be programmed with RNA to make precise, sequence-specific cuts in DNA, this view of basic research could not be further from the truth. The discovery of CRISPR-Cas9 has opened worlds of new possibilities in fields such as medicine, agriculture, and bioenergy, and it was discovered through basic research.

Prior Approaches to Gene Editing

A gene-editing tool that could be programmed to modify specific sequences of DNA was sought after by biologists for many decades. At the heart of potential gene-editing technologies were nucleases, enzymes able to cleave DNA or RNA, which are found in bacterial restriction enzymes. A vast range of restriction enzymes exist in nature, each of which recognizes and cleaves its own unique DNA sequence, but they cannot be modified to recognize different sequences. Some researchers attempted to create a programmable nuclease by linking zinc finger proteins, a class of DNA-binding proteins that recognize specific nucleotides, to the Fok I endonuclease, creating a zinc finger-nuclease (ZFN) hybrid (Kim et al., 1996; Urnov et al., 2005). The Kim group confirmed with SDS/PAGE that cleavage at the desired sequence was achieved with their "hybrid restriction enzymes" (Kim et al.). However, in one of their sample lanes demonstrating the success of the CP-QDR ZFN, there were two additional products present (at ~9.2 kb & ~15 kb) that were not addressed or acknowledged in the article. This calls into question the specificity and fidelity of the CP-QDR ZFN. The Urnov group advanced the applications of ZFNs by demonstrating that these enzymes could be used to create double-stranded breaks in human lymphoblast cells. These types of breaks induce homologous recombination (HR), a natural DNA repair process that uses a matching DNA template to fix broken strands, to correct mutations in the gene responsible for the SCID genetic disorder (IL2Rγ). Successful HR introduced a restriction enzyme cut site into the genomic DNA, which after being digested, presented itself as a distinct band on PAGE: using this method, they found that HR occurred at a frequency of 18% (Urnov et al.). Using RT-PCR, a method used to detect RNA expression levels, they confirmed that the production of γC mRNA was restored in cells where HR was able to successfully restore both copies of the IL2Rγ gene, demonstrating the therapeutic potential of ZFNs (Urnov et al.). However, a few inherent problems with

ZFNs remained. First, to target a novel sequence of DNA, a new ZFN must be made from scratch by linking a set of zinc finger proteins to Fok I, which is a laborious and time consuming process. Next, the specificity and efficiency of ZFN cleavage is not robust, as demonstrated by the additional CP-QDR products and the low HR frequency.

Discovery of CRISPR Arrays

Unlike ZFNs, which require labor-intensive protein engineering for each new target, the CRISPR-Cas9 system can be easily reprogrammed by simply altering the guiding RNA sequence, allowing for faster and more versatile gene editing. Clustered, regularly interspaced, short palindromic repeats (CRISPR) are short DNA repeat sequences separated by spacer DNA sequences; together, these stretches of CRISPR and spacer sequences are known as CRISPR arrays. To elucidate the function of these motifs, Francisco Mojica, a microbiologist at the University of Alicante in Spain, used BLAST, a bioinformatics technique, to identify any homology that these spacer sequences shared with known genes and found significant similarities between 47 spacers and bacteriophage genomes (Mojica et al., 2005, Table 2). This finding, along with previous studies showing that strains of phage-resistant bacteria containing a spacer similar to a viral gene were resistant to said virus, led Mojica to be the first to suggest a relationship between the CRISPR array and bacterial adaptive immunity. Mojica's findings and hypotheses were read by food science biologists working at Danisco, one of the world's largest producers of food ingredients and pharmaceutical excipients, who observed that some of the bacteria that they were using as dairy cultures were randomly developing resistance to phage attacks. Could it be possible that, in accordance with Mojica's hypothesis, the CRISPR array was somehow involved in conferring resistance to these mutant strains? After sequencing the CRISPR loci of the phage-resistant strains, the Danisco researchers found that the resistant strains contained spacers homologous to genes found within the bacteriophages responsible for the phage attacks on their dairy cultures (Barrangou et al., 2007). To determine whether this exciting association between spacer and phage resistance was due to a causal relationship, they altered the CRISPR locus both by adding homologous spacers to a non-resistant strain, which granted resistance, and deleting the spacers from a resistant strain, which restored susceptibility to phage attack. This established that the spacers were responsible for phage resistance. While their primary focus was on the spacer DNA sequence, they also generated another mutant in which they disrupted the cas5 gene, which encodes an enzyme containing an HNH-type nuclease motif. This strain lost its resistance, despite containing the spacers responsible for phage resistance. They didn't know it at the time, but the Danisco researchers had identified the nuclease responsible for CRISPR activity.

CRISPR-Cas as a Programmable System

In addition to functioning as a method of adaptive immunity, CRISPR was also demonstrated to interfere with horizontal gene transfer (HGT) between bacteria (Marraffini & Sontheimer, 2008). After observing a CRISPR locus in a clinically isolated strain of Staphylococcus aureus, the researchers were interested in the requirements for HGT interference, specifically the target of CRISPR interference. After introducing silent mutations to the target gene in the conjugative plasmid, which was identified by its homology with a spacer in the isolated strain's CRISPR locus, CRISPR interference activity was lost. To determine whether this was the result of DNA or mRNA interference, a self-splicing intron was introduced into the target gene, modifying the DNA target, but leaving the mRNA product unchanged. This change disrupted CRISPR interference, indicating that the target of CRISPR is DNA. The findings about the DNA target of CRISPR and the importance of the cas5 HNH nuclease-like enzyme for phage resistance came together when Garneau et al. were able to demonstrate that the CRISPR-Cas system was cleaving DNA (2010). After introducing a plasmid containing proto-spacers (pNT1) to S. thermophilus, which contains its own CRISPR locus, they were able to use both gel electrophoresis and Sanger sequencing to confirm that the pNT1 plasmid was being precisely cut three bases upstream of the proto-spacer adjacent motif (a PAM sequence, essential for CRISPR-Cas9 target recognition). When cas5 was disrupted, in accordance with Barrangou's findings, cleavage of pNT1 failed. Garneau's group concluded their paper by suggesting that the CRISPR-Cas system can be used to generate organisms with increased bacteriophage resistance, not knowing that their results would soon help redefine the boundaries of gene editing.

Conclusion

The programmability of the CRISPR-Cas system was discovered when the specificity of the cas5 enzyme (now known as cas9) was determined to be dictated by crRNA, the transcribed products of spacer sequences (Jinek et al., 2012). RNA constructs ('chimeras') were synthesized, linking crRNA to tracrRNA, which activates crRNA, and when they were used to program the Cas9 nuclease, they found that the Cas9 was able to successfully cleave their desired DNA target. The long-awaited, heavily-anticipated, programmable nuclease had finally arrived, and it was the product of decades of basic research. If not for the curious biologists, geneticists, and biochemists tinkering with

conserved bacterial DNA motifs and invisible-to-the-naked-eye nuclease enzymes, the revolutionary CRISPR-Cas9 technology would never have been discovered. Basic research is more than a venture to appease curious scientists; it is practically essential for future innovation and the advancement of humankind.

Works Cited

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., & Horvath, P. (2007). CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes. Science, 315(5819), 1709–1712. https://doi.org/10.1126/science.1138140

Garneau, J. E., Dupuis, M.-È., Villion, M., Romero, D. A., Barrangou, R., Boyaval, P., Fremaux, C., Horvath, P., Magadán, A. H., & Moineau, S. (2010). The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature, 468(7320), 67–71. https://doi.org/10.1038/nature09523

Glenza, J. (2025, February 20). NIH funding cuts are Trump's latest attack on education, professors say. The Guardian. https://www.theguardian.com/us-news/2025/feb/20/trump-nih-cuts

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity. Science, 337(6096), 816–821. https://doi.org/10.1126/science.1225829

Kim, Y. G., Cha, J., & Chandrasegaran, S. (1996). Hybrid restriction enzymes: Zinc finger fusions to Fok I cleavage domain. Proceedings of the National Academy of Sciences, 93(3), 1156–1160. https://doi.org/10.1073/pnas.93.3.1156

Marraffini, L. A., & Sontheimer, E. J. (2008). CRISPR Interference Limits Horizontal Gene Transfer in Staphylococci by Targeting DNA. Science, 322(5909), 1843–1845. https://doi.org/10.1126/science.1165771

Mojica, F. J. M., Díez-Villaseñor, C., García-Martínez, J., & Soria, E. (2005). Intervening Sequences of Regularly Spaced Prokaryotic Repeats Derive from Foreign Genetic Elements. Journal of Molecular Evolution, 60(2), 174–182. https://doi.org/10.1007/s00239-004-0046-3

NIAID. (2023, February 15). What does basic research mean?
Tollefson, J., Kozlov, M., Witze, A., & Garisto, D. (2025, February 20). Trump's siege of science: How the first 30 days unfolded and what's next. Nature. https://www.nature.com/articles/d41586-025-00525-1
Trump 2.0: An assault on science anywhere is an assault on science everywhere. (2025, February 25). Nature. https://www.nature.com/articles/d41586-025-00562-w

Urnov, F. D., Miller, J. C., Lee, Y.-L., Beausejour, C. M., Rock, J. M., Augustus, S., Jamieson, A. C., Porteus, M. H., Gregory, P. D., & Holmes, M. C. (2005). Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature, 435(7042), 646–651. https://doi.org/10.1038/