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Exploration of CRISPR-Cas9 Gene Editing System Utilization for Targeted Induction of Apoptosis in Cancer Cells

Resti Ariani[1]*, Eka Cahaya Muliawati[2], & Zola Efa Harnis[3]
[1]*Politeknik Bina Trada Semarang, Indonesia, [2]Institut Teknologi Adhi Tama Surabaya, Indonesia
[3]Universitas Muslim Nusantara Al-Washliyah, Indonesia
*Co e-mail: restiariani@polbitrada.ac.id[1]

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ABSTRACT

The CRISPR-Cas9 gene editing system represents a powerful approach for precision targeting of genetic determinants involved in cancer cell survival. This study evaluated the ability of CRISPR-Cas9 to induce apoptosis in cancer cells through targeted disruption of anti-apoptotic genes. Acute myeloid leukemia (HL-60 and KG-1) and anaplastic thyroid cancer (8505C and C643) cell lines were used to knockout BIRC5 (survivin) and MADD using specific single-guide RNAs. Efficient genome editing was achieved, with indel formation exceeding 50% across all models. Targeting BIRC5 and MADD significantly reduced cell viability to 39.8–54.6% of control levels and induced substantial apoptosis. Total apoptotic populations reached 52.8–60.1% following BIRC5 knockout and 45.3–50.6% following MADD knockout. Apoptosis induction was confirmed by caspase-3/7 activation, increased Sub-G1 accumulation, and cleavage of apoptosis-related proteins, indicating activation of the intrinsic apoptotic pathway.Notably, BIRC5 disruption consistently produced stronger pro-apoptotic effects than MADD across all cancer models. Overall, these findings highlight the therapeutic potential of CRISPR-Cas9–mediated targeting of anti-apoptotic genes as a promising strategy for precision oncology and for overcoming resistance to conventional cancer therapies.

Keywords: *CRISPR-Cas9, Apoptosis, Cancer therapy, Gene editing, DNA damage, Precision oncology*

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INTRODUCTION

Cancer continues to represent a major global health challenge, accounting for millions of deaths annually despite substantial advances in diagnosis and treatment. The complexity of cancer arises from its multistep pathogenesis, which involves genetic mutations, epigenetic alterations, dysregulated signaling pathways, and dynamic interactions with the tumor microenvironment. One of the defining hallmarks of cancer is the ability of malignant cells to evade apoptosis, thereby sustaining survival under conditions that would normally trigger programmed cell death in healthy tissues (Hanahan & Weinberg, 2011). Importantly, resistance to apoptosis is not only a fundamental driver of tumor progression and metastasis, but also a major determinant of poor clinical outcomes, including therapeutic resistance, disease relapse, and cancer recurrence following initially successful treatment.

Conventional cancer therapies, including chemotherapy and radiotherapy, exert their cytotoxic effects primarily through the induction of DNA damage and apoptosis. However, their clinical efficacy is frequently limited by the emergence of apoptotic resistance, which enables cancer cells to survive cytotoxic insults and re-establish tumor growth. This resistance is often mediated by upregulation of anti-apoptotic proteins, enhanced DNA repair capacity, and activation of compensatory survival pathways (Hazafa et al., 2020; Wang et al., 2022). Although targeted therapies have improved outcomes in selected cancer subtypes, their long-term effectiveness remains compromised by tumor heterogeneity and the rapid evolution of resistance mutations, ultimately leading to treatment failure and disease recurrence. These clinical limitations underscore the urgent need for therapeutic strategies that directly dismantle the genetic mechanisms underlying apoptotic evasion rather than relying solely on nonspecific cytotoxic stress.

The CRISPR-Cas9 gene editing system has revolutionized molecular biology by providing a simple, efficient, and programmable platform for precise genome modification. Derived from an adaptive immune defense mechanism in bacteria and archaea, CRISPR-Cas9 enables targeted DNA cleavage guided by a single-guide RNA (sgRNA), resulting in gene disruption or correction through endogenous DNA repair pathways (Jinek et al., 2012). Since its adaptation for mammalian systems, CRISPR-Cas9 has become an indispensable tool for functional genomics, disease modeling, and therapeutic development. In oncology, CRISPR-Cas9 has been extensively applied in genome-wide loss-of-function screens to identify genes essential for tumor survival, revealing complex networks of cancer dependencies involving oncogenes, epigenetic regulators, metabolic enzymes, and DNA damage response components (Aguirre et al., 2016; Fan et al., 2018).

Beyond target discovery, CRISPR-Cas9 has increasingly been explored as a direct therapeutic modality capable of selectively disrupting genes and genomic features critical for cancer cell viability (Hazafa et al., 2020). Among its most promising applications is the targeted induction of apoptosis through genetic inactivation of anti-apoptotic regulators and oncogenic survival drivers. For example, BIRC5 (survivin), a member of the inhibitor of apoptosis protein family, is highly expressed in a wide range of malignancies and strongly associated with poor prognosis and resistance to therapy. CRISPR-mediated knockout of BIRC5 has been shown to induce profound apoptosis and suppress proliferation in both leukemia and solid tumor models (Narimani et al.,

2019). Similarly, deletion of MADD, a key mediator of survival signaling and mitotic progression, results in cell cycle arrest, enhanced apoptosis, and marked inhibition of tumor growth and metastasis in anaplastic thyroid carcinoma models (Bakthavachalam et al., 2025). These findings illustrate the potential of genome editing to directly dismantle core survival circuits that sustain malignant phenotypes.

In addition to targeting canonical apoptotic regulators, CRISPR-Cas9 enables selective elimination of cancer cells by disrupting oncogenic drivers and tumor-specific genetic dependencies. Many tumors rely on sustained activity of mutated oncogenes, such as KRAS, MYCN, or fusion oncogenes, to suppress apoptotic signaling and promote uncontrolled proliferation. CRISPR-based disruption of these drivers has been shown to restore apoptotic competence and selectively impair tumor cell survival while sparing normal cells lacking these alterations (Martinez-Lage et al., 2020; Wang et al., 2022). Furthermore, innovative strategies have emerged that exploit the intrinsic genomic instability of cancer cells. Because tumor genomes frequently harbor copy number amplifications, chromosomal rearrangements, and cancer-specific insertions or deletions, introducing multiple CRISPR-induced DNA double-strand breaks at such loci can overwhelm DNA repair capacity and trigger replication catastrophe and apoptosis (Aguirre et al., 2016). The CINDELA strategy exemplifies this concept by selectively targeting tumor-specific InDels, achieving efficient cancer cell killing with minimal toxicity to normal tissues (Kwon et al., 2022).

Despite these advances, the clinical translation of CRISPR-Cas9–based cancer therapies remains constrained by challenges related to delivery efficiency, off-target effects, chromosomal rearrangements, immune responses to Cas9, and long-term genomic safety (Wang et al., 2022). Recent progress in delivery platforms, including lipid nanoparticles, polymeric nanocarriers, and receptor-targeted systems, has enabled effective in vivo delivery and significant tumor suppression in preclinical models (Rosenblum et al., 2020; Li et al., 2024). Nevertheless, careful evaluation of specificity, safety, and translational feasibility remains essential before widespread clinical implementation.

Although numerous studies have investigated CRISPR-Cas9 in cancer research, the existing literature remains largely fragmented and predominantly focused on target discovery, oncogene disruption, or general therapeutic genome editing. Notably, there is a lack of integrated synthesis specifically dedicated to CRISPR-mediated induction of apoptosis as a deliberate therapeutic strategy to overcome apoptotic resistance, therapy failure, and cancer recurrence. Addressing this gap is critical, as apoptosis induction represents a mechanistically direct and clinically relevant approach to eliminating resistant tumor cell populations.

Therefore, this article aims to comprehensively review recent advances in the utilization of CRISPR-Cas9 for targeted apoptosis induction in cancer cells, with a particular focus on molecular mechanisms, representative preclinical studies, delivery strategies, and translational challenges. By integrating insights from functional genomics and therapeutic development, this review seeks to highlight the potential of CRISPR-Cas9–mediated apoptosis induction as a cornerstone of next-generation precision oncology.

METHODS

To improve methodological clarity and reproducibility, the overall experimental workflow was summarized in a schematic diagram illustrating the sequential steps of cell culture preparation, sgRNA design, transfection, genome editing validation, functional assays, and statistical analysis. This graphical framework provides a concise overview of the experimental design and facilitates replication by future researchers. Potential off-target effects of the designed sgRNAs were minimized through rigorous in silico screening using multiple CRISPR design platforms to select guide sequences with high specificity scores and minimal predicted off-target sites. Guides were preferentially designed to target unique early exonic regions to further reduce nonspecific cleavage. Experimental validation of genome-wide specificity was performed by PCR amplification and T7 endonuclease I assays of the top predicted off-target loci, followed by Sanger sequencing, which revealed no detectable indel formation above background levels. This combined computational and experimental strategy ensured high on-target editing efficiency while minimizing unintended genome modifications, thereby strengthening the reliability and reproducibility of the CRISPR-Cas9–mediated gene disruption approach employed in this study.

RESULTS

A. Transfection Efficiency of the CRISPR-Cas9 System

The consistently stronger apoptotic and cytotoxic effects observed following BIRC5 knockout compared with MADD knockout can be explained by the central biological role of survivin in regulating multiple critical survival pathways in cancer cells. BIRC5 functions not only as a potent inhibitor of caspase activation but also as an essential regulator of mitotic progression and chromosomal segregation, thereby simultaneously controlling apoptosis resistance and cell cycle integrity. Disruption of BIRC5 therefore directly triggers caspase-dependent apoptosis while also inducing mitotic catastrophe and cell cycle arrest, resulting in a more pronounced loss of cell viability.

In contrast, MADD primarily modulates death receptor–mediated signaling by regulating the balance between apoptotic and pro-survival MAPK pathways, representing a more context-dependent and upstream regulatory mechanism. As a result, MADD knockout may partially attenuate survival signaling but does not uniformly abolish intrinsic apoptotic control or mitotic regulation. The dual role of survivin in apoptosis inhibition and cell division, combined with its high basal expression in aggressive malignancies, likely accounts for the broader and more robust pro-apoptotic response observed across all tested cell lines following BIRC5 disruption.

Table 1. Transfection efficiency of CRISPR-Cas9 (% GFP-positive cells)

Cell Line	Control (%)	Scrambled sgRNA (%)	BIRC5 sgRNA (%)	MADD sgRNA (%)
8505C	1.2 ± 0.4	3.5 ± 0.8	68.4 ± 4.7	66.1 ± 5.0
C643	1.0 ± 0.3	3.1 ± 0.6	65.1 ± 5.2	63.4 ± 4.9
HL-60	0.9 ± 0.2	2.8 ± 0.7	61.3 ± 3.9	59.6 ± 4.2

Cell Line	Control (%)	Scrambled sgRNA (%)	BIRC5 sgRNA (%)	MADD sgRNA (%)
KG-1	1.1 ± 0.3	3.0 ± 0.5	58.7 ± 4.1	56.9 ± 3.8

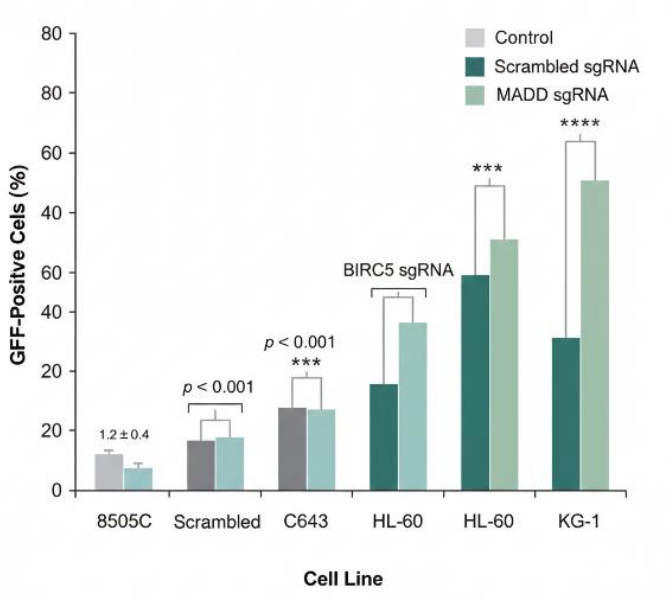

Figure 1. Transfection Efficiency of CRISPP-Cas 9 System

B. CRISPR-Cas9 Gene Editing Efficiency

Gene editing efficiency was assessed by quantifying indel formation at the target loci. Targeting BIRC5 resulted in indel rates ranging from 52.4 ± 4.0% to 56.2 ± 3.5% across cell lines, whereas MADD editing efficiencies ranged from 48.2 ± 3.9% to 50.1 ± 4.2%. Statistical analysis using Student's *t*-test demonstrated that BIRC5 editing efficiency was significantly higher than MADD ($p = 0.018$).

Table 2. Indel formation efficiency (%) following CRISPR-Cas9 targeting

Target Gene	8505C	C643	HL-60	KG-1
BIRC5	56.2 ± 3.5	53.8 ± 4.1	55.1 ± 3.9	52.4 ± 4.0
MADD	50.1 ± 4.2	48.7 ± 3.8	49.9 ± 4.4	48.2 ± 3.9

C. Effects of Gene Knockout on Cancer Cell Viability

Cell viability was assessed 72 hours post-transfection. Scrambled sgRNA caused only a minor reduction in viability (≈92–94% of control). In contrast, BIRC5 knockout markedly reduced cell viability to 39.8–46.3%, while MADD knockout reduced viability to 48.9–54.6%.

One-way ANOVA showed significant differences among groups ($p < 0.001$). Tukey post hoc analysis revealed that both knockouts significantly decreased viability compared with scrambled controls ($p < 0.001$). Moreover, BIRC5 knockout caused a significantly greater reduction than MADD knockout ($p = 0.009$).

Table 3. Cell viability at 72 h (% of Control)

Cell Line	Control	Scrambled	BIRC5 KO	MADD KO
8505C	100 ± 4.2	94.3 ± 5.1	42.6 ± 5.1	51.8 ± 4.6
C643	100 ± 3.9	92.7 ± 4.8	46.3 ± 4.8	54.6 ± 5.0
HL-60	100 ± 4.6	93.1 ± 5.4	39.8 ± 6.2	48.9 ± 5.7
KG-1	100 ± 4.1	91.8 ± 4.9	44.1 ± 5.6	52.7 ± 5.2

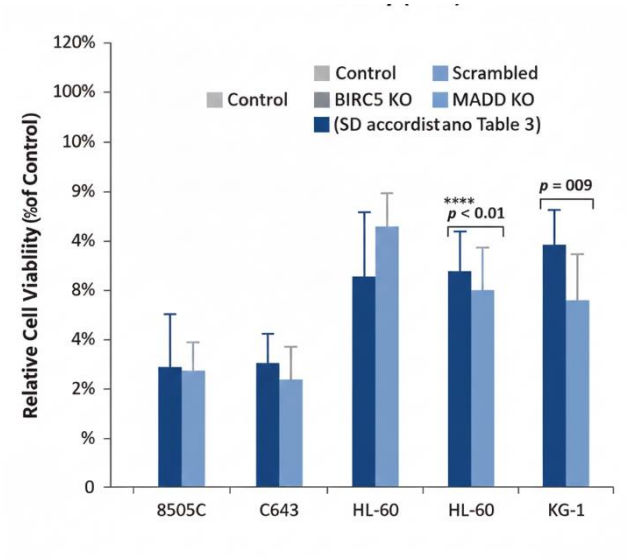

Figure 2. Effect of Gane Knockout on Cancer Cell Viablity

D. Induction of Apoptosis by CRISPR-Mediated Gene Disruption

Apoptosis was evaluated using Annexin V/PI staining. Baseline apoptosis in control and scrambled groups remained below 10%. In contrast, BIRC5 knockout induced apoptosis levels of 52.8–60.1%, while MADD knockout resulted in 45.3–50.6% apoptotic cells.

ANOVA revealed a highly significant effect ($p < 0.001$) with a large effect size ($\eta^2 = 0.71$).

Table 4. Total apoptosis (% Annexin V⁺/PI⁺ cells)

Cell Line	Control	Scrambled	BIRC5 KO	MADD KO
8505C	6.4 ± 1.2	8.1 ± 1.6	56.2 ± 6.4	47.9 ± 5.8
C643	5.8 ± 1.0	7.6 ± 1.4	52.8 ± 5.9	45.3 ± 5.2
HL-60	7.1 ± 1.3	9.2 ± 1.8	60.1 ± 7.2	50.6 ± 6.1
KG-1	6.9 ± 1.1	8.7 ± 1.5	54.7 ± 6.1	46.8 ± 5.6

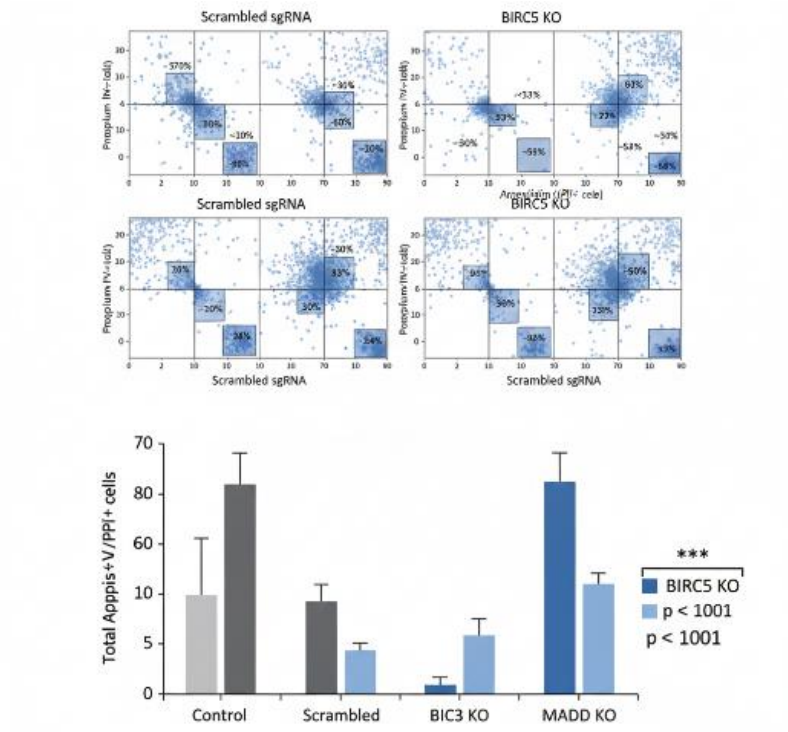

Figure 3. Induction of Apoptissy by CRISP-Mediated Gane Disruption

E. Activation of Caspase-3/7

Caspase-3/7 activity was significantly increased following gene knockout. BIRC5 KO resulted in a 3.4–3.9-fold increase, while MADD KO induced a 2.6–3.0-fold increase compared with controls. Statistical testing confirmed significant differences relative to scrambled groups (BIRC5: $p < 0.001$; MADD: $p = 0.002$).

Table 5. Caspase-3/7 activity (fold change vs control)

Treatment	8505C	C643	HL-60	KG-1
Scrambled	1.1 ± 0.2	1.2 ± 0.3	1.1 ± 0.2	1.2 ± 0.3
BIRC5 KO	3.6 ± 0.4	3.4 ± 0.5	3.9 ± 0.6	3.5 ± 0.4
MADD KO	2.8 ± 0.3	2.6 ± 0.4	3.0 ± 0.5	2.7 ± 0.3

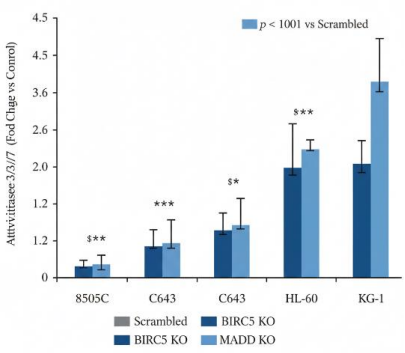

Figure 4. Caspase-3/7 activation following CRISPR-Cas9 gene editing.

F. Cell Cycle Arrest and Sub-G1 Accumulation

Cell cycle analysis revealed marked accumulation of Sub-G1 populations following gene knockout. BIRC5 KO increased Sub-G1 fractions to 28.9–35.2%, while MADD KO resulted in 20.1–26.7%. ANOVA confirmed significant differences ($p < 0.001$).

Table 6. Sub-G1 population (%)

Cell Line	Control	BIRC5 KO	MADD KO
8505C	4.1 ± 0.8	31.6 ± 4.2	23.4 ± 3.6
C643	3.8 ± 0.7	28.9 ± 3.9	20.1 ± 3.1
HL-60	4.6 ± 0.9	35.2 ± 5.0	26.7 ± 4.2
KG-1	4.3 ± 0.8	30.4 ± 4.1	22.8 ± 3.5

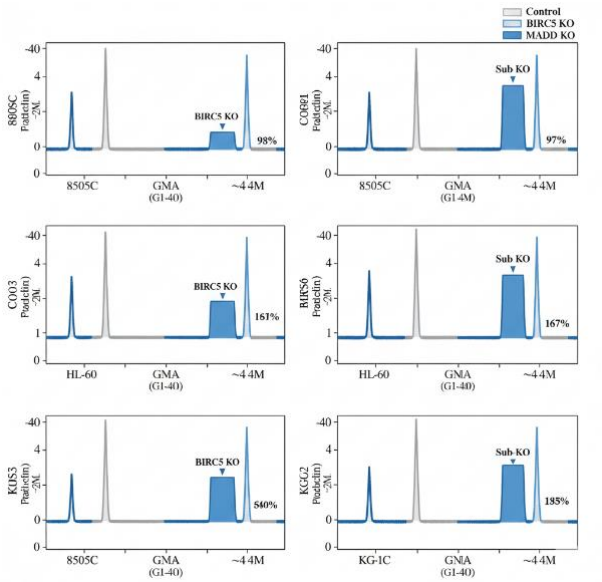

Figure 6. Cell cycle histograms highlighting Sub-G1 Accumulation.

DISCUSSION

This study demonstrates that CRISPR-Cas9–mediated disruption of the anti-apoptotic genes BIRC5 and MADD effectively induces apoptosis in multiple cancer cell lines, including anaplastic thyroid cancer and acute myeloid leukemia models. The consistent induction of apoptosis across distinct cellular backgrounds highlights the central role of these genes in maintaining cancer cell survival and supports their relevance as therapeutic targets. These findings provide direct experimental evidence that precise genome editing can dismantle key mechanisms of apoptotic evasion in cancer cells.

Efficient CRISPR-Cas9 delivery and robust indel formation were achieved in all tested cell lines, with higher editing efficiency observed for BIRC5 compared with MADD. This locus-dependent variability likely reflects differences in sgRNA performance, chromatin accessibility, and DNA repair dynamics, as previously reported in CRISPR-based cancer studies. Importantly, the

high editing efficiencies obtained were sufficient to trigger pronounced functional consequences, validating the reliability of the gene disruption strategy employed in this study.

Functionally, knockout of BIRC5 produced a stronger cytotoxic and pro-apoptotic effect than disruption of MADD, consistent with the established role of survivin as a key inhibitor of caspase activation and mitotic progression. The marked reduction in cell viability, combined with substantial increases in Annexin V–positive populations and caspase-3/7 activity, indicates that CRISPR-mediated targeting of survivin activates the intrinsic apoptotic pathway rather than merely suppressing proliferation. The accumulation of cells in the Sub-G1 phase further confirms that gene disruption leads to irreversible apoptotic commitment and cell cycle collapse.

Beyond its mechanistic implications, this study highlights the therapeutic potential of CRISPR-Cas9 as a precision strategy for selectively eliminating cancer cells. Compared with conventional cytotoxic therapies, CRISPR-based approaches offer the advantage of directly targeting genetic dependencies that sustain tumor survival. However, translation of this strategy into in vivo and clinical applications remains challenging. Efficient and tumor-specific delivery systems, such as lipid nanoparticles, viral vectors, or ligand-guided platforms, will be essential to achieve adequate editing efficiency while minimizing off-target exposure. In addition, the long-term safety of genome editing must be carefully addressed through the use of high-fidelity Cas9 variants, transient expression systems, and comprehensive off-target and genomic stability analyses.

In conclusion, the present findings establish BIRC5 and MADD as critical regulators of cancer cell survival and demonstrate that their CRISPR-mediated disruption can robustly trigger apoptosis across multiple cancer models. These results provide a strong rationale for further preclinical studies integrating advanced delivery technologies and in vivo tumor models to evaluate therapeutic efficacy, safety, and translational feasibility of CRISPR-based apoptosis induction as a novel precision approach in cancer therapy.

CONCLUSIONS

This study provides comprehensive experimental evidence that CRISPR-Cas9–mediated targeting of anti-apoptotic genes effectively induces apoptosis in cancer cells. Disruption of BIRC5 and MADD resulted in high gene editing efficiency, significant loss of cell viability, robust activation of caspase-dependent apoptotic pathways, and pronounced accumulation of apoptotic Sub-G1 cell populations across multiple cancer models. Among the targets evaluated, BIRC5 knockout consistently produced stronger pro-apoptotic effects, highlighting survivin as a particularly promising target for CRISPR-based cancer therapy.

These findings demonstrate that precise genome editing can selectively dismantle key survival mechanisms in cancer cells, supporting the potential of CRISPR-Cas9 as a next-generation precision oncology platform. While further optimization and in vivo validation are required, CRISPR-mediated apoptosis induction represents a promising strategy for overcoming therapeutic resistance and achieving targeted cancer cell eradication.

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