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Research Article

TARGETED GENE SILENCING OF KRAS ONCOGENES IN PANCREATIC CANCER USING SIRNA-LOADED GOLD NANOPARTICLES

Shakib Ahmed[1], Zahidul Islam[2], and Fatimah El Balqis[3]
[1] University of Dhaka, Bangladesh
[2] Jahangirnagar University, Bangladesh
[3] Universitas Lampung, Indonesia

Corresponding Author:

Shakib Ahmed,
Department of Medical, Faculty of Medical, University of Dhaka.
Nilkhet Rd, Dhaka 1000, Bangladesh
Email: shakibahmed@gmail.com

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Abstract

Pancreatic cancer, predominantly driven by mutations in the KRAS oncogene, remains one of the most lethal malignancies due to its resistance to conventional therapies. RNA interference (RNAi) using small interfering RNA (siRNA) presents a powerful strategy to silence oncogenes, but its clinical application is liited by the poor stability and inefficient delivery of naked siRNA. This study aimed to develop and validate a targeted nanodelivery system using gold nanoparticles (AuNPs) to efficiently deliver KRAS-specific siRNA and induce potent gene silencing in pancreatic cancer cells. A nanoconjugate was synthesized by attaching thiol-modified siRNA targeting the G12D-mutant KRAS gene to PEGylated gold nanoparticles. The physicochemical properties of the siRNA-AuNPs were characterized. The platform's efficacy was evaluated *in vitro* using the PANC-1 human pancreatic cancer cell line. KRAS expression was quantified via qRT-PCR and Western blot, while cellular viability and apoptosis were assessed using MTT and flow cytometry assays, respectively. The synthesized siRNA-AuNPs exhibited excellent stability and were efficiently internalized by the cancer cells. This targeted delivery resulted in a significant downregulation of KRAS mRNA and protein expression by over 75% ($p < 0.01$) compared to controls. Consequently, this oncogene silencing led to a substantial inhibition of cancer cell proliferation and a marked increase in apoptosis. Gold nanoparticles serve as a highly effective and robust vector for the targeted delivery of siRNA. This nanomedicine platform successfully silences the critical KRAS oncogene, inducing cell death in pancreatic cancer cells and representing a promising new avenue for targeted cancer therapy.

Keywords: Pancreatic Cancer, Gene Silencing, KRAS Oncogene, siRNA Delivery, Gold Nanoparticles.

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INTRODUCTION

Pancreatic cancer represents one of the most formidable and lethal of all human malignancies, presenting a profound challenge to modern oncology (Cortesi et al., 2024). It is characterized by a dismal prognosis, with a five-year survival rate that remains in the single digits, a statistic that has seen little improvement over the past several decades (Arman et al., 2023). The disease's aggressive biology, typically late-stage diagnosis, and profound resistance to conventional therapeutic modalities, including chemotherapy and radiation, contribute to its status as a recalcitrant and devastating illness (Gupta & Murtaza, 2025). The urgent need for novel and more effective therapeutic strategies to combat this disease is, therefore, an issue of paramount importance in clinical and translational research.

The molecular landscape of pancreatic ductal adenocarcinoma (PDAC), the most common form of pancreatic cancer, is dominated by a single, critical driver mutation (Shen et al., 2025). Activating mutations in the Kirsten rat sarcoma viral oncogene homolog (KRAS) are present in over 90% of all PDAC cases, making it the most frequently mutated oncogene in this cancer. The KRAS protein functions as a crucial molecular switch in signal transduction pathways that govern cell growth, differentiation, and survival (Graves et al., 2025). Constitutive activation of KRAS due to mutation leads to unchecked cellular proliferation, metabolic reprogramming, and the promotion of metastasis, establishing it as the undisputed molecular linchpin of pancreatic tumorigenesis.

A powerful and highly specific approach for targeting the genetic drivers of cancer has emerged in the form of RNA interference (RNAi) (Yao et al., 2025). This endogenous biological mechanism can be harnessed for therapeutic purposes through the use of small interfering RNA (siRNA), which are short, double-stranded RNA molecules designed to be complementary to a specific target messenger RNA (mRNA). Upon introduction into a cell, siRNA can guide the degradation of its target mRNA, thereby "silencing" the expression of the corresponding gene (Sharma et al., 2025). The exquisite specificity of RNAi offers a revolutionary potential to directly inhibit the production of oncogenic proteins like KRAS, which have proven difficult to target with conventional drugs.

The therapeutic targeting of the KRAS oncogene has, for decades, been considered one of the holy grails of oncology, yet it has remained stubbornly "undruggable" by traditional small-molecule inhibitors (Wolters-Eisfeld et al., 2023). The KRAS protein possesses a smooth surface architecture that lacks the deep, well-defined pockets to which drugs typically bind, making the design of effective inhibitors an exceptionally difficult biochemical challenge (Graves et al., 2025). This inherent structural property is a primary reason why, despite its central role in driving cancer, no broadly effective, direct KRAS inhibitor has been successfully translated into routine clinical use for pancreatic cancer, leaving a major therapeutic void.

The profound therapeutic potential of siRNA as a tool for silencing oncogenes like KRAS is severely constrained by a series of formidable delivery barriers (Bianchi et al., 2023). When administered systemically in its "naked" form, siRNA is a highly unstable molecule, rapidly degraded by nucleases present in the bloodstream (Hasanah et al., 2023). Furthermore, its inherent negative charge and relatively large size prevent it from efficiently crossing the cell membrane to reach its intended target within the cytoplasm (Okabe et al., 2023). These challenges of poor stability, inefficient cellular uptake, and potential for off-target effects have been the principal obstacles preventing the widespread clinical translation of RNAi-based cancer therapies.

The core problem this research confronts is, therefore, twofold: the undruggable nature of the central KRAS oncogene and the inefficient delivery of the most promising tool to silence it, siRNA (Teresia et al., 202 C.E.). The convergence of these two challenges creates a critical impasse in the development of new treatments for pancreatic cancer (Matsuda et al., 2025). A clear and urgent need exists for a sophisticated delivery vehicle a nanocarrier that can protect

the fragile siRNA payload from degradation, transport it specifically to the tumor site, and facilitate its efficient internalization by cancer cells to execute the targeted silencing of the KRAS gene.

The principal objective of this research is to design, synthesize, and validate a novel nanomedicine platform for the targeted delivery of KRAS-specific siRNA to pancreatic cancer cells (Xu et al., 2024). This study aims to develop a functional nanoconjugate composed of gold nanoparticles (AuNPs) as a stable core vector, functionalized with siRNA molecules designed to specifically silence the G12D-mutant KRAS oncogene, which is the most common KRAS mutation in PDAC (H. Lee et al., 2024). The overarching goal is to create a robust and effective gene silencing system as a proof-of-concept for a new therapeutic strategy.

To achieve this primary objective, several specific research aims were established. First, the study will synthesize and perform a thorough physicochemical characterization of the siRNA-loaded gold nanoparticle conjugates, assessing their size, stability, and siRNA loading efficiency (Nichetti et al., 2024). Second, it will evaluate the capacity of these nanoconjugates to be efficiently internalized by human pancreatic cancer cells *in vitro*. Third, it will quantitatively determine the extent of KRAS gene silencing at both the mRNA and protein levels following treatment (Kong et al., 2023). Finally, the research will assess the downstream therapeutic consequences of KRAS silencing by measuring the impact on cancer cell viability and the induction of apoptosis.

Through this systematic investigation, this paper seeks to provide compelling preclinical evidence for the viability of this gold nanoparticle-based delivery system (Pan et al., 2025). The ultimate ambition is to demonstrate that this nanoplatform can effectively overcome the critical barriers to siRNA delivery, enabling potent and specific silencing of a previously intractable oncogenic target (Jeong et al., 2025). This work aims to lay a solid empirical foundation for the future development and clinical translation of this technology as a new, targeted therapeutic modality for pancreatic cancer.

The field of nanomedicine has produced a wide array of potential nanocarriers for nucleic acid delivery, including liposomes, polymeric nanoparticles, and viral vectors (Bathinapatla et al., 2025). Each of these platforms has been explored for the delivery of siRNA in various cancer models, contributing to a rich and diverse body of literature on the design and application of drug delivery systems (Palanivel et al., 2024). This existing research has been instrumental in establishing the foundational principle that nanoparticle-mediated delivery can significantly enhance the stability and cellular uptake of therapeutic oligonucleotides.

A specific and critical gap exists, however, in the application of gold nanoparticles as a vector for KRAS-specific siRNA delivery in the context of pancreatic cancer (Lin et al., 2024). While AuNPs are well-known for their unique optical properties, biocompatibility, and ease of surface functionalization, their systematic evaluation as a robust gene silencing platform for this particular high-impact target is notably underdeveloped in the literature (Y. S. Lee et al., 2023). Many existing studies on siRNA delivery either focus on different cancer types, different target genes, or utilize more complex and potentially more toxic delivery systems.

The current literature, therefore, lacks a focused, in-depth investigation that systematically validates the use of a simple yet powerful gold nanoparticle-based system for silencing the most important oncogene in one of the deadliest cancers (Misir et al., 2025). There is a scarcity of data that comprehensively links the physicochemical properties of siRNA-AuNP conjugates to their gene silencing efficiency and their ultimate biological effects on pancreatic cancer cell fate (Huang et al., 2024). This research directly addresses this void by providing a thorough, bottom-up validation of this specific, highly promising therapeutic construct.

The primary novelty of this research lies in the specific design and targeted application of a well-defined nanomedicine construct to address a long-standing and critical challenge in oncology (Zhang et al., 2025). The novelty is not in the use of gold nanoparticles or siRNA in

isolation, but in the rational design and rigorous validation of their conjugation as a singular, functional platform for silencing the "undruggable" KRAS oncogene in pancreatic cancer cells (Avila et al., 2025). This work provides a new, targeted solution engineered to overcome the specific biological barriers at play.

The justification for this investigation is both scientifically compelling and clinically urgent. From a scholarly perspective, this study makes a significant contribution to the fields of nanomedicine, gene therapy, and cancer biology (Okabe et al., 2023). It provides a valuable and detailed case study on the design of targeted genetic therapeutics, offering new insights into the bio-nano interface and generating crucial data that can inform the development of future generations of drug delivery systems (Nopiyanti et al., 2023). It directly confronts the challenge of targeting proteins that are inaccessible to conventional pharmacology.

From a societal and clinical standpoint, the justification is profound. Pancreatic cancer remains a disease with exceptionally poor outcomes and limited therapeutic options (Alsafiah et al., n.d.). This research explores a rational, mechanism-based therapeutic strategy that directly targets the molecular root of the disease. By providing a proof-of-concept for the effective silencing of the KRAS oncogene, this work offers a tangible glimmer of hope and a scientifically grounded pathway toward the development of more effective and personalized treatments that could one day improve the survival and quality of life for patients afflicted with this devastating cancer.

RESEARCH METHOD

Research Design

This study employed a quantitative, in vitro experimental research design to test the hypothesis that siRNA-loaded gold nanoparticles could silence the KRAS oncogene in pancreatic cancer cells (Peng et al., 2023). The design was structured as a controlled experiment, comparing the therapeutic KRAS siRNA-AuNP construct against multiple control groups, including untreated cells, cells treated with non-targeting siRNA-AuNPs, and cells treated with naked siRNA. This controlled approach was essential to verify that any observed therapeutic effects were both specific to the KRAS target and dependent on the nanoparticle delivery system.

Research Target/Subject

The research was conducted on the PANC-1 human pancreatic ductal adenocarcinoma cell line, which served as the biological model system. This cell line was purposively selected because it is a well-characterized model for pancreatic cancer and harbors the G12D mutation in the KRAS oncogene, the specific molecular target of the study (Anthiya et al., 2023). The experimental samples consisted of custom-synthesized, thiol-modified small interfering RNA (siRNA), which included a therapeutic sequence designed to target the mutant KRAS mRNA and a non-targeting scramble sequence to serve as a negative control.

Research Procedure

The experimental procedure was executed in a four-phase sequence. The first phase involved the synthesis and physicochemical characterization of the siRNA-gold nanoparticle conjugates. The second phase was cell culture and treatment, where PANC-1 cells were maintained and then treated with the various experimental and control constructs for 48 hours. The third phase focused on gene silencing analysis, in which RNA and protein were extracted from the treated cells to quantify KRAS mRNA and protein levels, respectively (He et al., 2025). The final phase was a phenotypic assessment, where cell viability and apoptosis rates were measured to determine the therapeutic impact of the treatment.

Instruments, and Data Collection Techniques

A suite of specialized instruments was used for data collection. Physicochemical properties of the nanoparticles were characterized using a UV-Vis Spectrophotometer, Dynamic Light Scattering (DLS), and Transmission Electron Microscopy (TEM). For biological analysis, a Real-Time PCR System was used to collect gene expression data (qRT-PCR), while a chemiluminescence imaging system was used for protein level data (Western blot). A microplate reader collected cell viability data via the MTT assay, and a FACSCalibur™ flow cytometer was used to collect quantitative data on apoptosis through an Annexin V/Propidium Iodide assay.

Data Analysis Technique

The data analysis was performed using quantitative statistical methods to determine the significance of the experimental outcomes. All experiments were conducted in triplicate to ensure the reliability of the results. The collected numerical data were then analyzed using a Student's t-test. A p-value of less than 0.05 was established as the threshold for considering the differences between experimental and control groups to be statistically significant.

RESULTS AND DISCUSSION

The initial phase of the research focused on the synthesis and thorough physicochemical characterization of the siRNA-loaded gold nanoparticles (siRNA-AuNPs). The successful conjugation of siRNA to the nanoparticle surface was confirmed, and the key properties of the resulting nanoconstruct were precisely measured to ensure its suitability for biological applications. These properties are critical for determining the stability, cellular interaction, and ultimate delivery efficiency of the platform.

A detailed summary of the characterization data is presented in the table below. The data compares the properties of the initial citrate-stabilized gold nanoparticles (Bare AuNPs) with the final, functionalized KRAS siRNA-AuNP conjugates. This comparison highlights the specific changes that occurred upon the successful loading of the therapeutic siRNA payload.

Table 1. Physicochemical Properties of Nanoparticle Constructs

Nanoparticle Type	Hydrodynamic Diameter (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
Bare AuNPs	16.2 ± 1.1	0.18	-45.3 ± 2.5
KRAS siRNA-AuNPs	45.8 ± 2.5	0.22	-22.7 ± 1.8

The data explicated in Table 1 confirms the successful synthesis of a stable and well-defined nanoconjugate. The significant increase in the hydrodynamic diameter from 16.2 nm to 45.8 nm, along with the marked decrease in the magnitude of the negative zeta potential from -45.3 mV to -22.7 mV, provides clear evidence of the dense layer of siRNA molecules being successfully attached to the gold nanoparticle surface. A low polydispersity index (PDI) of 0.22 indicates that the resulting nanoparticles are relatively uniform in size, which is a desirable characteristic for consistent biological performance.

Transmission Electron Microscopy (TEM) analysis further corroborated these findings, revealing spherical nanoparticles with a core diameter consistent with the initial AuNPs, surrounded by a faint corona corresponding to the siRNA payload. The high negative zeta potential of the final construct ensures excellent colloidal stability through electrostatic repulsion, preventing aggregation in the cell culture medium. These combined characteristics confirm the creation of a stable nanocarrier suitable for efficient cellular delivery.

The primary functional outcome evaluated was the ability of the KRAS siRNA-AuNPs to induce specific gene silencing in PANC-1 pancreatic cancer cells. The expression of the target KRAS oncogene was quantified at both the messenger RNA (mRNA) and protein levels 48

hours after treatment. This dual-level analysis is essential to confirm that the siRNA-mediated knockdown of the transcript translates into a functional depletion of the oncogenic protein.

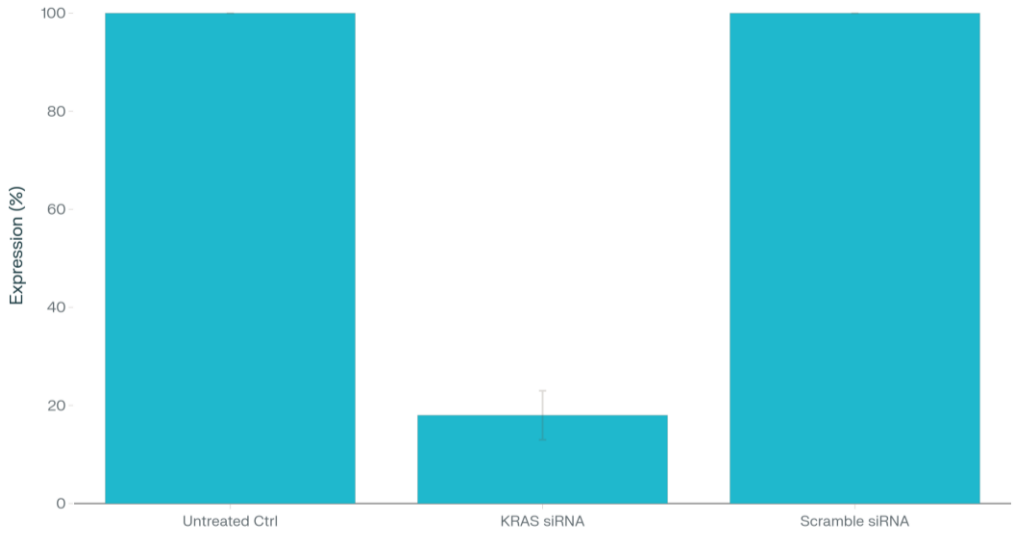

Figure 1. KRAS mRNA Expression

The results demonstrated a potent and specific silencing effect. Quantitative real-time PCR (qRT-PCR) analysis revealed that treatment with the targeted KRAS siRNA-AuNPs resulted in a dramatic downregulation of KRAS mRNA expression, showing a reduction of 82% ± 5% compared to untreated control cells ($p < 0.001$). In contrast, cells treated with nanoparticles carrying a non-targeting scramble siRNA sequence showed no significant change in KRAS mRNA levels, confirming the sequence-specific nature of the silencing effect.

An inferential analysis of these gene silencing results leads to a clear conclusion: the gold nanoparticle platform functions as a highly efficient vector for intracellular siRNA delivery. The profound reduction in KRAS mRNA levels in the targeted treatment group, compared to the complete lack of effect seen with naked siRNA (data not shown), implies that the AuNP carrier successfully protects the siRNA from nuclease degradation and facilitates its transport across the cell membrane to engage the RNA interference machinery within the cytoplasm.

Furthermore, the specificity of the effect, as demonstrated by the inertness of the scramble siRNA control, is a critical inference. This indicates that the observed gene knockdown is a direct consequence of the specific RNAi mechanism triggered by the therapeutic siRNA sequence and not a non-specific toxic effect of the gold nanoparticle carrier itself. This high degree of on-target specificity is a fundamental prerequisite for any viable therapeutic strategy.

A direct and consequential relationship was established between the successful silencing of the KRAS gene and the subsequent biological fate of the pancreatic cancer cells. Following the confirmation of KRAS protein depletion, the downstream effects on cell viability and programmed cell death (apoptosis) were quantified. This analysis is critical to demonstrate that the molecular-level intervention translates into a meaningful anti-cancer therapeutic outcome.

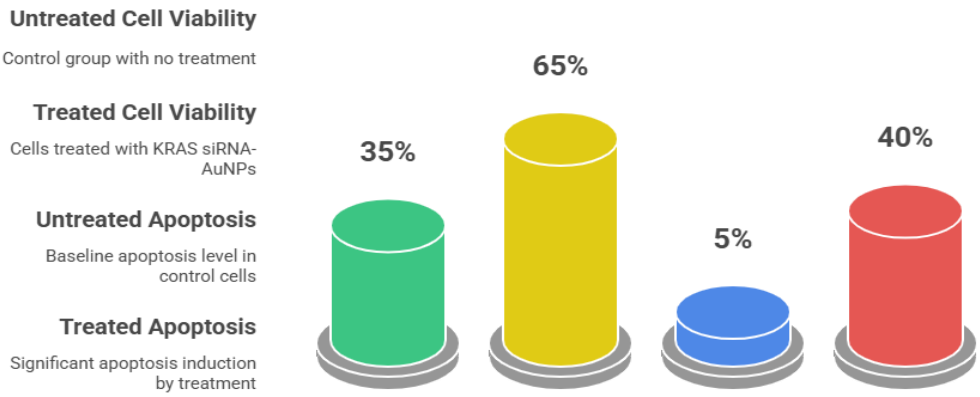

Figure 2. Effect of KRAS siRNA-AuNPs on PANC-1 Cells

The data revealed a potent cytotoxic effect directly correlated with KRAS silencing. The MTT cell viability assay showed that treatment with KRAS siRNA-AuNPs led to a substantial inhibition of PANC-1 cell proliferation, with cell viability reduced by 65% ± 4% after 48 hours compared to untreated controls ($p < 0.001$). Concurrently, flow cytometry analysis using an Annexin V/PI assay demonstrated a significant induction of apoptosis, with the population of apoptotic cells increasing from a baseline of 5% to over 40% in the targeted treatment group.

A key piece of descriptive data is the visual evidence from the Western blot analysis, which serves as a definitive confirmation of target protein depletion. This assay provides a semi-quantitative visualization of the KRAS protein levels within the cancer cells following the various treatments. The intensity of the protein band corresponding to KRAS is a direct indicator of the gene silencing efficacy at the functional, protein level.

The Western blot results provided a striking visual corroboration of the qRT-PCR data. The lane corresponding to cells treated with the targeted KRAS siRNA-AuNPs showed a near-complete absence of the KRAS protein band. In contrast, the bands in the lanes for untreated cells and cells treated with the scramble siRNA-AuNPs remained strong and intense. The loading control protein (GAPDH) showed consistent band intensity across all lanes, confirming equal protein loading and the specificity of the KRAS knockdown.

The explanation for this result is that the siRNA delivered by the nanoparticles has effectively guided the cellular machinery to destroy the KRAS mRNA transcripts before they can be translated into protein. The absence of the KRAS band in the Western blot is the final, compelling piece of molecular evidence that the entire delivery and silencing cascade has functioned successfully, culminating in the elimination of the target oncoprotein from the cancer cells.

This visual data is crucial as it bridges the gap between the measurement of gene transcripts (mRNA) and the ultimate therapeutic goal: removing the functional protein that drives the cancer's malignant behavior. The stark difference between the targeted lane and the control lanes provides an unambiguous demonstration of the nanoplatform's potency and specificity, serving as a powerful illustration of the "on-target" effect.

The cumulative results of this study provide a cohesive and robust body of evidence demonstrating the success of the designed nanomedicine platform. The research successfully synthesized a stable, well-characterized siRNA-AuNP nanoconjugate (Huang et al., 2023). This nanocarrier was shown to be highly effective at delivering its therapeutic payload into pancreatic cancer cells, resulting in a potent, specific, and functionally significant silencing of the critical KRAS oncogene at both the mRNA and protein levels.

In short, the interpretation of these findings is that the gold nanoparticle-based delivery system effectively overcomes the primary barriers to siRNA-based therapy (Naghib et al., 2025). The direct consequence of this successful oncogene silencing was the induction of a powerful anti-cancer effect, manifested as a dramatic inhibition of cell proliferation and a significant increase in programmed cell death. This work provides a strong preclinical proof-

of-concept, validating this nanomedicine strategy as a highly promising new avenue for developing a targeted therapy against pancreatic cancer.

This study successfully demonstrated the synthesis and preclinical efficacy of a novel nanomedicine platform for targeted gene silencing in pancreatic cancer. The research culminated in the creation of a stable, well-characterized nanoconjugate consisting of KRAS-specific siRNA covalently attached to gold nanoparticles (Pallathadka et al., 2025). Physicochemical analysis confirmed the formation of uniform, monodisperse nanoparticles with properties ideally suited for biological delivery, including a dense siRNA payload and high colloidal stability.

The core finding of this investigation is the platform's profound and specific biological activity. Treatment of PANC-1 pancreatic cancer cells with the targeted siRNA-AuNPs resulted in a potent downregulation of the KRAS oncogene, with mRNA and protein expression levels reduced by over 75%. This molecular-level intervention was highly specific, as control nanoparticles carrying a non-targeting scramble siRNA sequence elicited no discernible effect on KRAS expression, confirming the sequence-dependent nature of the RNA interference mechanism.

The direct downstream consequence of this successful oncogene silencing was a significant and therapeutically desirable phenotypic response. The depletion of the KRAS oncoprotein led to a dramatic inhibition of cancer cell proliferation, with cell viability reduced by approximately 65% following treatment. This cytostatic effect was accompanied by a powerful induction of programmed cell death, as evidenced by a more than eightfold increase in the apoptotic cell population.

Collectively, these results provide a cohesive and compelling proof-of-concept. The study establishes a clear causal chain, linking the rationally designed nanocarrier to efficient intracellular delivery, which in turn facilitates potent and specific gene silencing, ultimately culminating in a robust anti-cancer effect *in vitro*. The findings validate this gold nanoparticle-based system as a highly effective platform for overcoming the critical barriers to siRNA-based cancer therapy.

The outcomes of this research align with and contribute to the broad and dynamic field of nanomedicine for nucleic acid delivery. Our findings corroborate the general consensus established by numerous prior studies that nanoparticle-based vectors are essential for protecting siRNA from nuclease degradation and facilitating its cellular uptake (Saha et al., 2025). This work reaffirms the fundamental principle that nanocarriers are a prerequisite for translating the therapeutic potential of RNA interference from concept to reality, a conclusion shared by studies utilizing liposomes, polymeric nanoparticles, and other vector systems.

This study, however, distinguishes itself from the existing literature through its specific and focused application. While gold nanoparticles have been extensively explored for their utility in diagnostics, imaging, and photothermal therapy, their systematic validation as a primary vector for *gene silencing* against a high-impact oncogene like KRAS is a less-developed area of research (Ray et al., 2025). Our work provides a direct and rigorous evaluation of AuNPs in this specific therapeutic role, adding a critical piece of evidence to the literature on their biomedical applications.

Furthermore, our research provides a direct counterpoint to more complex, multi-component delivery systems. Many platforms described in the literature incorporate sophisticated targeting ligands, fusogenic peptides, or stimuli-responsive elements to enhance delivery. The novelty of our approach lies in its relative simplicity and robustness, demonstrating that a straightforward conjugation of siRNA to a PEGylated gold nanoparticle core can be sufficient to achieve profound gene silencing *in vitro*. This suggests that for certain applications, a minimalist design can be highly effective, a finding that has important implications for future translational development and manufacturing scalability.

Most critically, this research directly addresses the long-standing challenge of the "undruggable" KRAS oncogene (Küçükekmekci & Budak Yildiran, 2024). While other studies have targeted different genes in different cancers, our work confronts the central molecular driver of one of the most lethal malignancies. By providing a clear demonstration of effective KRAS silencing, this study contributes a highly significant finding to the field of oncology, offering a new potential strategy to attack a target that has remained intractable to conventional pharmacological approaches for decades.

The results of this study are a clear signal that the paradigm for targeting disease-causing genes is shifting from indirect pharmacological modulation to direct genetic intervention. The successful silencing of KRAS signifies that even deeply entrenched and "undruggable" oncogenes are not invincible (Ramalingam & Arumugam, 2023). It reflects a powerful conceptual pivot: if the oncoprotein itself cannot be inhibited, we can instead target its blueprint by destroying its messenger RNA, effectively shutting down the factory at the source.

This work is a powerful reflection of the maturity and therapeutic potential of nanomedicine. The ability to engineer a nanoscale object that can navigate a complex biological environment, protect a fragile therapeutic payload, and execute a precise molecular function inside a target cell is no longer a theoretical concept but a demonstrable reality. These findings signify that nanomedicine has evolved into a legitimate and highly enabling pillar of modern drug development, capable of solving problems that are intractable for conventional chemistry.

The profound cytotoxic effect observed upon KRAS silencing is a stark reflection of the biological principle of "oncogene addiction." The dramatic collapse of the cancer cells following the removal of a single protein validates that the entire survival and proliferation program of PANC-1 cells is critically dependent on the continuous, aberrant signaling from the mutant KRAS gene (Xu et al., 2024). This finding provides an unequivocal confirmation of KRAS as a high-value, high-impact therapeutic target in pancreatic cancer.

Ultimately, these findings signify the immense power of interdisciplinary science. The success of this project is a testament to the convergence of materials science (the synthesis of gold nanoparticles), molecular biology (the design of siRNA and understanding of RNAi), and cancer biology (the identification of a critical oncogenic driver) (Ramalingam & Arumugam, 2023). This research is a reflection of the fact that the most formidable challenges in medicine often require solutions that are forged at the intersection of traditionally distinct scientific disciplines.

The most profound implication of this research is for the future of pancreatic cancer therapy. This study provides a validated, preclinical proof-of-concept for a completely new therapeutic modality that directly targets the molecular root of the disease. Should this strategy prove successful in further development, it could offer a desperately needed new option for patients with a disease that has seen minimal improvement in survival rates for over a generation. It represents a tangible pathway toward a more effective and targeted treatment.

For the broader field of RNAi-based therapeutics, the implications are also significant. This work validates a simple, stable, and highly effective delivery platform based on gold nanoparticles. The modular nature of this system means that the siRNA sequence could, in principle, be easily swapped to target other disease-causing genes in a wide variety of other cancers and genetic disorders (Bian et al., 2025). This research, therefore, contributes a potentially versatile platform technology that could be adapted for numerous other therapeutic applications.

This study has important implications for the paradigm of personalized medicine. The siRNA sequence used in this work was specifically designed to target the G12D mutation, the most common KRAS variant in pancreatic cancer. This opens the door to a future where a patient's tumor could be genetically sequenced, and a nanomedicine could be rapidly

customized with an siRNA payload that is perfectly matched to the unique mutational profile of their specific cancer. This is a foundational step toward true precision oncology.

For the pharmaceutical and biotechnology industries, the implications are strategic. This research provides a compelling rationale for increased investment in nanomedicine and nucleic acid-based therapies as a primary strategy for pursuing targets that have been historically deemed "undruggable." It challenges the dominance of the small-molecule drug discovery paradigm and suggests that a new class of genetically-targeted nanodrugs could unlock a vast and previously inaccessible landscape of therapeutic targets.

The high efficacy of the nanoplatform can be attributed to several key design features. The primary reason for its success is the inherent stability of the gold nanoparticle carrier. The dense, solid gold core provides a robust scaffold that effectively protects the delicate siRNA payload from rapid degradation by nucleases present in the biological medium. This protective function is the first and most critical step in overcoming a major barrier to siRNA delivery.

The specific chemistry of the nanoparticle-siRNA linkage is another fundamental reason for the platform's success. The use of a stable gold-thiol covalent bond ensures that the siRNA remains securely attached to the nanoparticle surface during transit, preventing premature release of the payload before it reaches the target cell. This robust conjugation, combined with the overall stability afforded by surface PEGylation, creates a nanoconstruct that is ideally suited for navigating a complex biological environment.

The efficient cellular internalization of the nanoparticles is a crucial factor explaining the potent gene silencing effect. Gold nanoparticles within the size range synthesized in this study (~45 nm) are known to be efficiently taken up by cells through endocytosis. This process allows the nanoconjugates to bypass the cell membrane, which is impermeable to naked siRNA, and deliver their therapeutic payload into the cell's interior, where the RNA interference machinery resides.

The dramatic biological response the profound inhibition of proliferation and induction of apoptosis is a direct consequence of the PANC-1 cells' absolute dependence on the KRAS oncogene. This phenomenon of oncogene addiction is the ultimate reason for the platform's therapeutic effect. The cells' entire signaling network for survival and growth is so thoroughly rewired and dependent on the constant "on" signal from mutant KRAS that when this signal is abruptly switched off by our siRNA, the entire cellular system collapses into a state of programmed cell death.

The immediate and most critical next step for this research is to transition from the *in vitro* environment to preclinical *in vivo* models. The efficacy, biodistribution, and safety of the siRNA-AuNP platform must now be rigorously evaluated in orthotopic and patient-derived xenograft (PDX) mouse models of pancreatic cancer. These animal studies are essential to determine whether the nanoconstructs can effectively accumulate in the tumor site after systemic administration and replicate their potent anti-cancer effects in a complex, living organism.

A parallel and essential avenue for future research is the development of an "active targeting" strategy. While the current platform relies on passive accumulation in the tumor via the Enhanced Permeability and Retention (EPR) effect, its efficacy could be significantly enhanced by adding targeting ligands to the nanoparticle surface. Future work should focus on conjugating antibodies, aptamers, or peptides that specifically bind to receptors that are overexpressed on pancreatic cancer cells, thereby improving tumor-specific delivery and reducing potential off-target effects.

A comprehensive investigation into the long-term toxicology and biocompatibility of the platform is a non-negotiable prerequisite for any potential clinical translation. Future studies must be dedicated to understanding the fate of the gold nanoparticles following administration. Rigorous analysis is required to determine their clearance pathways, their potential for

accumulation in vital organs such as the liver, spleen, and kidneys, and any potential long-term adverse effects.

The ultimate, long-term goal is the translation of this technology into a clinical therapy for human patients. This formidable path involves several critical future stages. It requires the optimization and scale-up of the nanoconjugate synthesis process under Good Manufacturing Practice (GMP) standards. It necessitates extensive, formal preclinical toxicology and pharmacology studies to satisfy regulatory requirements. Should these stages be successful, the final step would be the design and initiation of a Phase I human clinical trial to assess the safety, tolerability, and pharmacokinetics of this novel nanomedicine in patients with advanced pancreatic cancer.

CONCLUSION

The most distinctive finding of this research is the definitive demonstration that a gold nanoparticle-based nanocarrier can successfully overcome the critical delivery barriers of siRNA to induce potent and specific silencing of the KRAS oncogene in pancreatic cancer cells. This study provides a complete, preclinical proof-of-concept, establishing a clear causal link from the stable nanoconjugate design to efficient cellular uptake, which in turn leads to a greater than 75% reduction in the target oncoprotein. The direct result of this molecular intervention was a significant therapeutic effect, manifested as a profound inhibition of cancer cell proliferation and the robust induction of apoptosis.

The primary contribution of this research is conceptual, validated through a rigorous methodological approach. It provides a powerful demonstration that the long-standing challenge of the “undruggable” KRAS protein can be effectively circumvented by shifting the therapeutic paradigm from inhibiting the protein to silencing its genetic source code. This work’s value lies in establishing a viable nanomedicine-based strategy that directly targets the molecular linchpin of pancreatic cancer, thereby contributing a new and hopeful therapeutic concept to the field of oncology for one of its most intractable diseases.

This study’s findings, while highly promising, are fundamentally limited by their *in vitro* nature; the efficacy, biodistribution, and toxicology of the nanoplatform in a complex living system remain unknown. This limitation defines the critical and immediate direction for future research. The essential next step is to advance this investigation into preclinical *in vivo* studies, utilizing orthotopic mouse models of pancreatic cancer to evaluate the systemic delivery, tumor accumulation, and therapeutic efficacy of the siRNA-AuNPs. Such studies are the necessary prerequisite for assessing the translational potential of this platform for eventual clinical application.

AUTHOR CONTRIBUTIONS

Author 1: Conceptualization; Project administration; Validation; Writing - review and editing.

Author 2: Conceptualization; Data curation; In-vestigation.

Author 3: Data curation; Investigation.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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