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Exploring the Therapeutic Potential of Ribozymes in Cancer Treatment: A Comprehensive Review

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Abstract: ***Ribozymes, RNA molecules with catalytic properties, offer promising therapeutic potential in cancer treatment. This review examines the role of ribozymes in combating Kaposis sarcoma associated herpesvirus KSHV and glioblastoma, highlighting their unique stability and resilience. We discuss the combination of ribozyme therapy with traditional chemotherapy, as evidenced in clinical trials targeting the flt2 receptor. This paper underscores the significance of ribozymes in cancer therapy and the importance of public health policies for equitable technology distribution. This article aims to explore the therapeutic potential of ribozymes in cancer treatment, focusing on their application against Kaposis sarcoma associated herpesvirus KSHV and glioblastoma, while also addressing the implications for public health policy. Ribozymes, RNA-protein complexes where the RNA exhibits catalytic activity, have existed for eons and have remained remarkably consistent throughout evolution. These molecules challenge the traditional view that enzymes are solely composed of proteins, suggesting they have numerous unexplored roles. Our review delves into the nature of ribozymes, exploring the various types that have persisted over time. Notably, ribozymes have been linked to cancer, which offer promising therapeutic potential. We discuss how ribozymes combat Kaposi's sarcoma-associated herpesvirus (KSHC) infection and their therapeutic effects on glioblastomas. Cancer, one of the most debilitating diseases affecting humanity, presents treatment challenges due to therapies' loss of effectiveness over time. However, the inherent stability and unique properties of ribozymes make them resilient to alteration. This study is significant as it highlights the novel role of ribozymes in cancer therapy, offering new avenues for treatment, particularly in overcoming the limitations of traditional therapies. Ribozymes have been predicted to cure many infectious diseases including cancer. A combination of chemotherapy and ribozyme against the flt-2 receptor has been found to cure cancer in phase II clinical trials. Multiple studies mentioned above clearly highlight the novel role of ribozymes in curing cancer. We believe that using ribozymes can provide a cure for this dreadful problem in all low, middle, and high-income group countries. Ribozymes are available in all organisms be it prokaryotes and eukaryotes. It is their extraction that might become a monetary issue but the implementation of policies in place will help different income groups work together and allocate resources to make this world cancer-free. Public health policies that help in the appropriate distribution of technology and products from one stratum to another will help in detecting early cases and curing them when they are not that widespread. Using ribozymes for early detection will be easy as these are quite specific and are able to detect small changes too. By summarizing data from various research organizations, our review addresses one of the most pressing public health issues, proposing ribozymes as a promising avenue for future therapies.***

Keywords: Ribozymes, Cancer Therapy, RNA Protein Complexes, Glioblastoma, KSHV

1. Introduction

Cancer and Public Health

Cancer has a profound impact on public health in the United States, affecting individuals, families, and society as a whole. The burden of cancer includes years of life lost, economic costs, and the physical and emotional toll on survivors. As the population ages and cancer incidence rates rise, the importance of understanding and addressing cancer on a population-wide scale becomes increasingly critical. To tackle this challenge, researchers conduct extensive public health research focused on various aspects of cancer [1][2]. This includes maintaining large registries of cancer data, investigating behavioral patterns and environmental factors that influence cancer risk, addressing disparities in cancer outcomes among different populations, analyzing cancer care delivery and outcomes, assessing the economics of cancer, and studying ways to improve cancer control programs. The National Cancer Institute (NCI) plays a pivotal role in cancer-related public health research by conducting and funding large-scale studies, assembling extensive data collections, and collaborating with federal agencies and communities to develop culturally appropriate prevention and intervention strategies. Examples of NCI-supported activities in public health research include the Surveillance, Epidemiology, and End Results (SEER) Program, which collects data on cancer incidence and survival, and epidemiologic studies that evaluate various factors influencing cancer risk, such as diet, tobacco use, genetics, and environmental exposures. NCI also invests in research on cancer-care delivery, behavioral research related to cancer prevention and screening, and disparities research to address unequal cancer burden among different populations, including racial and ethnic minorities, rural populations, and cancer survivors. Additionally, NCI supports survivorship research to improve the quality of life and long-term outcomes of cancer survivors, identifying risk factors for second primary cancers and other adverse outcomes. Overall, cancer-related public health research provides vital information that informs policies, programs, and practices aimed at reducing the burden of cancer and improving outcomes for individuals and communities across the United States [3].

Cancer survivorship represents a critical area of focus for the public health community, aligning with its mission to prevent disease, prolong life, and promote health. There are an estimated 14.5 million cancer survivors in the United States who face a multitude of physical, psychosocial, and financial challenges [3][4]. The public health community, led by the Centers for Disease Control and Prevention (CDC), has been actively engaged in addressing the needs of cancer survivors through research, surveillance, and programmatic efforts. Since 2004, the CDC has collaborated with various stakeholders to develop and implement strategies outlined in the National Action Plan for Cancer Survivorship (NAPCS). This plan serves as a roadmap for addressing the needs of cancer survivors and their families. CDC's efforts include research, surveillance, program development, and awareness campaigns aimed at improving the experiences of survivors across the cancer continuum [2][4].

CDC's surveillance activities, conducted in partnership with state and territorial cancer registries, provide valuable data on cancer survivorship, treatment experiences, and health outcomes. These data inform research, program planning, and policy development to better support survivors and enhance their quality of life. The agency also supports educational initiatives, documentaries, and exhibits to raise awareness about the challenges faced by cancer survivors and to promote healthy behaviors. CDC-led publications highlight key issues such as health status, quality of life, economic burden, and disparities among cancer survivors, shaping research agendas and informing public health practice. Through partnerships with national organizations, state health agencies, and other stakeholders, CDC works to implement comprehensive cancer control plans and provide resources to support survivors. The agency's support of the National Cancer Survivorship Resource Center has led to the development of informational materials addressing survivorship needs and promoting healthy behaviors. The current supplement, "Addressing Cancer Survivorship Through Public Health Research, Surveillance, and Programs," showcases CDC's recent work in cancer survivorship. Articles in the supplement highlight research on health disparities, healthcare utilization, quality of life, behavioral interventions, and innovative uses of cancer registry data. In conclusion, CDC plays a vital role in addressing the needs of cancer survivors through a comprehensive public health approach. By conducting research, collecting surveillance data, and implementing programs, CDC works to improve the lives of cancer survivors and their families, contributing to the overall mission of promoting health and preventing disease [4][5].

The provision of health services within government-sponsored programs like the National Breast and Cervical Cancer Early Detection Program (NBCCEDP) necessitates careful consideration of opportunity costs. As public funds allocated to specific interventions are finite, decision-makers must prioritize strategies that maximize population health while efficiently utilizing resources. Economic evaluation methods play a crucial role in this process by providing insight into the costs and benefits of public health programs, aiding decision-makers in selecting optimal approaches to enhance program effectiveness and efficiency. This paper offers a comprehensive overview of economic evaluation methods and their application to the NBCCEDP, emphasizing the importance of collecting valid, accurate, and reliable economic data to inform program operations and performance. Economic analysis serves as a tool for maximizing value in resource-constrained environments, guiding decision-makers in allocating finite resources among competing health activities. Given the substantial economic burden of cancer and the high value of effective prevention, economic evaluations of cancer prevention programs are essential. Underserved populations often face disparities in accessing preventive services, making economic evaluations of public health programs targeting these groups particularly crucial for demonstrating value to decision-makers and ensuring scalability and replicability. Economic evaluations consider perspectives such as patient, employer, insurer, and society, while categorizing costs into direct (medical and non-medical), indirect (productivity losses), and psychosocial (intangible) costs. Methods for estimating productivity costs, such as the human capital, friction cost, and willingness-to-pay approaches, present challenges when applied to underserved populations due to differences in wages and willingness to pay. Various economic evaluation methods are employed in public health research, including cost-of-illness studies, program cost analysis, cost-effectiveness analysis (CEA), cost-utility analysis (CUA), cost-benefit analysis (CBA), and budget impact analysis (BIA). Each method offers unique advantages and limitations, with the choice depending on the analysis's purpose, audience, and data availability. The NBCCEDP, which provides breast and cervical cancer screening to low-income, uninsured, and under-insured women, serves as a case study for applying economic evaluation methods. Studies have examined program costs, cost-effectiveness, and budget impact, highlighting the program's economic value and its impact on health outcomes. Economic analyses of the NBCCEDP have informed decision-making and resource allocation, contributing to improved efficiency and equity in service delivery. Economic evaluation of public health programs for underserved populations, like the NBCCEDP, plays a vital role in identifying effective and efficient resource allocation strategies. By systematically assessing costs and benefits, economic analyses help demonstrate program value and guide decisions to enhance program effectiveness and equity. Lessons learned from economic evaluations of the NBCCEDP can inform similar programs targeting underserved populations, ultimately improving health outcomes and reducing disparities [5][6][7].

Many studies delve into the intricate relationship between lifestyle factors and cancer risk, highlighting the significant impact of unhealthy diet, tobacco use, and alcohol consumption on the prevalence of cancer in the United States. It underscores that these modifiable behaviors contribute substantially to the occurrence of cancer cases, with estimates suggesting that a reduction in their prevalence could prevent up to 40% of cancer incidences in the country. Central to the discussion is the critical role of public health policies in cancer prevention, with countries worldwide adopting various measures to mitigate the consumption of cancer-causing substances [8]. Examples cited include excise taxes on tobacco and alcohol, implementation of graphical warning labels on cigarette packs, and mass media campaigns aimed at raising awareness about the health risks associated with these behaviors. However, the effectiveness of such policies varies, with outcomes ranging from significant reductions in consumption, as seen in Mexico's sugar-sweetened beverage tax, to mixed results, as observed in studies on alcohol warning labels. Despite variations in effectiveness, public support for health policies generally remains favorable, with a majority of individuals endorsing measures aimed at promoting public health and reducing cancer risk. However, the level of support is not uniform across different demographic groups. Factors such as age, gender, race, education level, political orientation, and beliefs about cancer prevention play crucial roles in shaping individuals' attitudes toward these policies. The text underscores the significance of newly added questions in the Health Information National Trends Survey (HINTS 5 Cycle IV) in providing insights into public support for cancer prevention policies in the United States. By focusing on three key domains—alcohol, tobacco, and diet—the study aims to elucidate the most and least supported policies and identify factors associated with their

support. Analysis of HINTS data reveals that while most cancer prevention policies garner considerable support from the U.S. population, there are notable variations in support levels among different policy measures. For instance, policies such as warning labels on cigarette packs and restrictions on tobacco advertising on social media receive higher levels of support compared to measures like banning outdoor alcohol advertising or restricting junk food ads on social media platforms. Further examination uncovers the intricate interplay between demographic characteristics, health behaviors, and beliefs about cancer in influencing individuals' support for these policies. Women, older adults, parents, and those concerned about cancer are more likely to endorse tobacco-related policies. Conversely, conservative political leanings, fatalistic views about cancer prevention, and engagement in risky health behaviors like binge drinking are associated with lower levels of support for alcohol-related policies. The text underscores the need for targeted public awareness campaigns aimed at educating individuals about the link between lifestyle behaviors and cancer risk, thereby potentially increasing support for relevant health policies. It also calls for future research to explore additional factors influencing policy support and devise strategies for implementing effective cancer prevention policies at a broader societal level. In conclusion, the findings underscore the imperative of implementing evidence-based policies to support population-wide cancer prevention efforts, emphasizing the need for tailored interventions to address disparities in policy support among diverse demographic groups and belief systems [8][9].

Public Health Oncology: A Framework for Progress in Low and Middle-Income Countries

The communication addresses the escalating global burden of cancer, emphasizing the need for pragmatic approaches to tackle this pressing public health issue. It proposes the concept of public health oncology or population-affecting cancer medicine as a framework for addressing the challenges posed by rising cancer mortality and incidence rates worldwide. Globally, cancer ranks as the second leading cause of death, with a disproportionate burden borne by low- and middle-income countries (LMCs). The communication highlights the stark reality that a significant majority of new cancer cases and deaths occur in LMCs, and these figures are projected to increase further. Moreover, the high incidence: mortality ratio in LMCs underscores the critical need for improved cancer prevention and management strategies. The discussion focuses on Asian countries due to their substantial population size and increasing cancer burden. It critiques the prevailing individual-centric approach to cancer care, which overlooks the broader ecological context of cancer causation and management. The dominance of private sector, profit-driven models further exacerbates the inequities in access to quality cancer care, particularly in resource-constrained settings. The communication highlights the limitations of current frameworks for addressing global cancer care, which often prioritize technology transfer and infrastructure development without adequately addressing systemic challenges. It argues that weak health systems, governance issues, human rights violations, and incomplete disease knowledge are fundamental barriers to effective cancer control. Specific challenges, such as rural-to-urban migration and cultures of corruption, pose additional hurdles to improving cancer care in LMICs. The discourse underscores the importance of adopting holistic, population-focused approaches that address social determinants of health, governance issues, and human rights considerations. The communication critiques the narrow disease-focused approach in cancer medicine, advocating for broader, more inclusive strategies that address systemic issues. It emphasizes the intersectionality of health with broader societal factors, such as governance, corruption, and human rights, which significantly influence health outcomes. In conclusion, the communication calls for a paradigm shift towards public health oncology and population-affecting cancer medicine to effectively address the global cancer burden. It emphasizes the need for interdisciplinary collaboration, innovative financing mechanisms, and tailored interventions that consider the unique socio-cultural contexts of different communities. Moving forward, the communication advocates for a more nuanced understanding of cancer causation and management, incorporating biological, ecological, and social factors into intervention strategies. It underscores the importance of prioritizing cost-effective interventions, such as early detection programs tailored to local epidemiological data and population needs. Overall, the communication serves as a call to action for practitioners and policymakers to adopt comprehensive, population-focused approaches to cancer control, addressing not only biomedical aspects but also broader societal determinants of health. The communication delves into the emerging model for international development in low and middle-income countries (LMCs), highlighting the paradigm shift towards community empowerment and small-scale enterprise solutions to alleviate poverty. It underscores the increasing role played by non-governmental organizations (NGOs) in spearheading social development initiatives, particularly in regions like South Asia. This shift reflects a broader trend towards local ownership and active citizenship in driving sustainable development processes. The new model places a strong emphasis on harnessing local community assets and fostering self-reliance as fundamental drivers of sustainable development. It advocates for the active involvement of communities in defining and implementing solutions to their own challenges, emphasizing the critical role of intracommunity relationships and citizen participation in shaping developmental outcomes. Within the realm of public health oncology, the community empowerment model underscores the importance of tailoring intervention strategies to the specific ecological and cultural contexts of local communities. It highlights the need for nuanced approaches that take into account anthropological realities, recognizing that effective cancer control efforts must be contextually relevant and culturally sensitive. The communication delineates specific action areas and inaction areas in cancer prevention, early detection, and health systems strengthening. It advocates for intensified efforts in tobacco control, immunization against oncogenic infectious agents, and reducing exposure to carcinogens. However, it also challenges the efficacy of conventional early detection strategies in low-incidence populations and underscores the imperative of improving access to tertiary care for timely diagnosis and treatment. A significant portion of the communication is devoted to advocating for the centralization and coordination of cancer treatment within health systems. It calls for innovative approaches, particularly in leveraging information

technology (IT) to enhance outpatient care delivery. Furthermore, it stresses the importance of evidence-based interventions and cost-effective strategies to optimize resource allocation and improve cancer care outcomes. In terms of population-level strategies, the communication advocates for a "search and research" approach in cancer control, prioritizing localized exploratory projects and partnerships with LMC communities. It underscores the need for sustainable efforts that integrate implementation science into medical practice and academic promotion, fostering a culture of innovation and continuous improvement. In conclusion, the communication underscores the importance of embracing horizontal public health oncology perspectives in addressing the multifaceted challenges of cancer control in LMCs. It calls on practitioners to champion efforts that transcend traditional silos and address broader systemic challenges, while also recognizing the value of bottom-up approaches that empower local communities. Ultimately, it advocates for a holistic approach to cancer control that integrates community empowerment, evidence-based interventions, and sustainable health systems strengthening, thus paving the way for meaningful progress in the fight against cancer in resource-constrained settings [10][11].

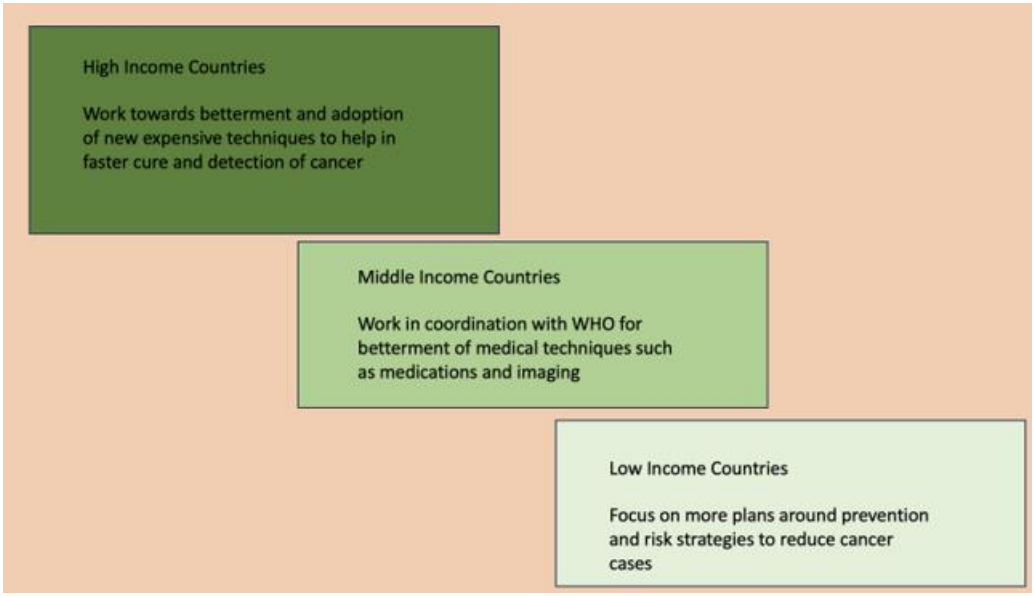

Figure 1: Strategies for different income groups for cancer prevention and cure

Ribozymes

RNA, traditionally considered solely as a messenger for genetic information, has gained prominence in therapeutic applications, ranging from cancer treatment to combating viral infections. Despite having fewer molecular groups compared to proteins, RNA has proven to be an effective tool for therapeutic development. Ribozymes act as a fossil record of ancient molecular evolution on Earth. Its Essential role in macromolecule synthesis across all life forms. It has the potential for new catalyst development, therapeutics, and molecule sensors [11].

Structure of Ribozymes

Studies discuss the discovery and characteristics of five small self-cleaving ribozymes: the hammerhead, hairpin, hepatitis delta virus (HDV), Varkud Satellite (VS), and glmS ribozymes. These ribozymes catalyze sequence-specific intramolecular cleavage of RNA molecules and range in length from 50 to 150 nucleotides. While initially discovered as domains of satellite RNAs, recent studies have shown that they are widespread in genomes across various organisms. Each ribozyme possesses a unique overall architecture and active site organization, which has been elucidated through crystal structures and mechanistic studies. These studies have revealed how RNA active sites can bind preferentially to the transition state of a reaction and how nucleobases within the ribozymes can efficiently perform general acid-base and electrostatic catalysis. Despite their diversity, these ribozymes share common features, such as the ability to fold into complex structures and catalyze reactions without the need for protein cofactors. The hairpin ribozyme, for example, was found to directly participate in catalysis without the need for divalent metal ions, contrary to previous assumptions. Its active site structure resembles that of the protein enzyme RNase A, with conserved nucleotides serving as general acid and base catalysts. Similarly, the hammerhead ribozyme, originally believed to be a metalloenzyme, was later found to be catalytically proficient in the presence of monovalent cations, with conserved nucleotides playing key roles in catalysis. The glmS ribozyme, on the other hand, was discovered as a riboswitch in Gram-positive bacteria, where it regulates gene expression in response to the metabolite glucosamine-6-phosphate (GlcN6P). This ribozyme's active site structure suggests a coenzyme function for GlcN6P, with conserved nucleotides within the ribozyme coordinating its activity. The HDV ribozyme is unique among the five ribozymes in that it employs an active-site divalent cation for catalysis. Its active site structure includes a conserved nucleotide that acts as a general acid/base catalyst and a magnesium ion-activated water molecule that serves as a general base. Overall, these ribozymes highlight the structural and biochemical versatility of RNA and suggest a role for catalytic RNAs early in evolution. They are found in diverse organisms and play essential roles in various biological processes, from viral replication to gene regulation [12][13][14].

The nucleolytic ribozymes constitute a diverse group of

catalytic RNA molecules that facilitate site-specific cleavage reactions through nucleophilic attack of a 2′-hydroxyl group on adjacent phosphodiester linkages, with some also capable of catalyzing reverse ligation reactions. Identified through bioinformatic analyses, this group currently comprises nine distinct species, with four discovered in the last five years. Their increasing diversity offers an opportunity to discern common catalytic strategies and extract general principles of RNA catalysis. While these ribozymes employ general acid-base catalysis as a common strategy, utilizing nucleobases for proton transfer, the specific catalytic functional groups vary among them. The majority utilize guanine N1 as the general base, with adenine and guanine nucleobases serving as general acid and base, respectively, in some ribozymes. However, variations exist; for instance, the HDV ribozyme employs metal-ion-bound water as the general base and a cytosine nucleobase as the general acid. The GlmS ribozyme is an exception, using the amine group of bound glucosamine 6-phosphate as the general acid. The hammerhead ribozyme, a well-studied member, utilizes a guanine nucleobase as the general base, but its mechanism of general acid catalysis has been subject to debate, with proposals suggesting involvement of G8 O2′ or a hydrated divalent cation. The pistol ribozyme, a recent discovery, exhibits structural similarities with the hammerhead ribozyme but differs mechanistically. While both utilize a guanine nucleobase as the general base, the pistol ribozyme likely employs a divalent cation as the general acid. This difference is supported by experimental data showing distinct pH dependencies upon 2′-amino substitution. In this paper, we present structural and mechanistic data on the pistol ribozyme, highlighting its differences from the hammerhead ribozyme. Additionally, we detail the chemical synthesis of RNA oligonucleotides, providing insights into the experimental techniques employed in our study. Oligonucleotides were synthesized using t-BDMS phosphoramidite chemistry, with specific phosphoramidites synthesized as needed. Deprotection methods varied depending on the modifications present, with oligonucleotides eventually redissolved and purified for further analysis. The crystallographic analysis of the pistol ribozyme reveals a structure consistent with previously determined models, with minor differences near the cleavage site. The ribozyme consists of four double-stranded segments, forming a pseudoknot and a three-way junction. The core of the ribozyme, containing highly conserved nucleotides, serves as the active site [15][6][17]. The structure was solved at 3.1 Å resolution using single-wavelength anomalous dispersion (SAD) data, with phases obtained by locating bromine atoms. Comparison with the hammerhead ribozyme shows similar helical architectures but distinct curvature and nucleotide arrangements. In the pistol ribozyme, a variable-length section connects P1 and P2, forming interactions in the minor groove of P1, crucial for ribozyme activity. A conserved guanine nucleobase (G40) in the central core plays a catalytic role, positioned for general base activity. The central core of the ribozyme displays an approximately in-line geometry necessary for the cleavage reaction, with nucleobases flanking the scissile phosphate creating the required arrangement. G40, analogous to G12 in the hammerhead ribozyme, is well-positioned for general base catalysis. The presence of a deoxyribose at position -1 prevents cleavage, but G40's role is evident. Overall, the crystallographic analysis provides structural insights into the pistol ribozyme's catalytic mechanism, highlighting similarities and differences compared to the hammerhead ribozyme. The central core of the pistol ribozyme consists of three strands: (a) linking PS and P3 helices with G40, C41, and G42; (b) linking P3 and P2 helices with G-1 and U+1 flanking the scissile phosphate; and (c) the 180° turn connecting P2 and PS. A similar structure is observed in the hammerhead ribozyme, with a longer sequence in the turn. For catalytic cleavage, an approximately in-line geometry between the O2′ nucleophile, scissile phosphate, and O5′ leaving group is crucial. Comparison with the hammerhead ribozyme reveals a similar arrangement, with nucleobases flanking the scissile phosphate creating the necessary in-line conformation. In the pistol ribozyme, G-1 is stacked between P3 and G40, held in place by hydrogen bonding with G40 and G42. U+1 contributes to the first base pair of P2, resulting in an angle close to the optimal 180°. G40, positioned adjacent to the O2′ nucleophile, is suggested to act as a general base for deprotonating the nucleophile during cleavage. This role mirrors that of G12 in the hammerhead ribozyme. Experimental investigation of the pistol ribozyme's cleavage rate as a function of pH, in the presence of Mg2+ and Na+ ions, reveals a narrow bell-shaped profile with apparent pKa values of 7.8 and 9.7. Substitution of G40 with inosine (G40I) lowers the higher apparent pKa to 8.5, indicating G40's involvement in proton transfer during cleavage. The perturbation of activity in the G40I variant suggests G40's critical role as a general base. This raises the question of what serves as a general acid, which warrants further discussion. The structural comparison between the central cores of the pistol and hammerhead ribozymes reveals key differences in their nucleotide arrangements and interactions. In the pistol ribozyme, the strand connecting PS and P3 consists of three nucleotides, with G40 forming a triple base interaction with the G33-C41 base pair. Above this plane, G42 is stacked on the G33-C41 base pair, with G-1 forming hydrogen bonds with G40 and G42. In contrast, the equivalent strand in the hammerhead ribozyme forms a helical structure, with G12 positioned by a base pair with A9. The junction sequence between P2 and PS in the pistol ribozyme forms a loop interacting with P3 on the minor groove side. A31 at the turn between P2 and PS is stacked on G32, and hydrogen bonds stabilize the narrow minor groove at the turn. C30, likely protonated at pH 6, forms a hydrogen bond with G33. The loop in the hammerhead ribozyme shares similar features but is more elaborate, with additional interactions between the loop and adjacent helix. In the hammerhead ribozyme, G8 O2′ is positioned close to the O5′ leaving group, potentially acting as the general acid in the cleavage reaction or assisting in the positioning of a catalytic Mg2+ ion. However, in the pistol ribozyme, the equivalent nucleotide, A or G32, is unlikely to participate in catalysis, as the ribozyme is equally active with either nucleotide and mutations of A32 have little effect on activity. Instead, the O2′ of A32 is crucial for activity, indicating its importance in the reaction. However, G32 O2′ is too far from O5′ for direct involvement as the general acid, suggesting a need for structural alteration for its participation in catalysis. The comparison of the central cores of the pistol and hammerhead ribozymes highlights structural differences in their nucleotide arrangements and interactions. While the pistol ribozyme features a triple base interaction involving G40 and G42, the hammerhead ribozyme forms a helical structure with G12 positioned by a base pair with A9. Additionally, the loop structures in both ribozymes stabilize

the junction regions, with A31 in the pistol ribozyme and U4-G5-A6 in the hammerhead ribozyme playing key roles. In the hammerhead ribozyme, G8 O2′ is positioned near the O5′ leaving group, potentially serving as the general acid, whereas in the pistol ribozyme, the equivalent nucleotide, A or G32, is unlikely to participate directly in catalysis. These structural insights provide valuable information about the mechanisms of catalysis in these two ribozymes. The investigation of the mechanistic role of G8 O2′ in the hammerhead ribozyme involved atomic mutation coupled with pH titration of cleavage rates. Substitution of O2′ with an amino group resulted in a plateau in cleavage rates between pH 6 and 8, supporting the direct participation of G8 O2′ in general acid–base catalysis. However, in the pistol ribozyme, substitution of A32 O2′ with an amino group did not significantly alter the pH dependence of cleavage rates, indicating a mechanistic difference between the two ribozymes. Further examination suggested a potential role of divalent metal ions in the pistol ribozyme cleavage reaction, with a log–linear dependence of cleavage rate on the pKa of hydrated metal ions, indicating their involvement in proton transfer. The binding site of a divalent metal ion close to the active center of the pistol ribozyme was identified, suggesting its role in facilitating proton transfer through inner sphere water molecules. The investigation into the active site of the pistol ribozyme revealed a possible presence of a hydrated Mg2+ ion, supported by unassigned electron density consistent with a hydrated Mg2+ ion and strong anomalous scatter from Mn2+ ions at the same position. This suggests a potential role of metal ions in catalysis. Atomic mutation of functional groups in the cation binding pocket and core of the ribozyme resulted in significant changes in cleavage rates. Notably, substitution of the proR non bridging oxygen of the scissile phosphate with a phosphorothioate group led to a 45,000-fold decrease in activity, with Mn2+ ions providing partial rescue. This suggests a possible outer sphere interaction between a bound Mg2+ ion and the proR oxygen. Similarly, modification of A32 2′OH and G42 with 2-aminopurine substitutions also reduced cleavage rates, indicating their involvement in catalysis. Modeling suggests that a pentahydrated Mg2+ ion bound to G33 N7 could interact with the proR oxygen of the scissile phosphate and A32 2′OH, positioning an inner sphere water molecule as a general acid. However, certain modifications did not align with the available structures, suggesting potential reorganization of the active site during catalysis. Overall, these findings provide insights into the complex mechanisms underlying ribozyme catalysis and highlight the dynamic nature of the active site during the transition state [17][18] [19][20].

Therapeutic uses of Ribozymes

RNA-based therapeutic approaches have gained prominence in recent years, offering versatile tools for various medical applications, from cancer treatment to combating viral infections. Despite having fewer molecular groups compared to proteins, RNA has proven to be highly effective for therapeutic purposes. The discovery of ribozymes and riboswitches has revolutionized our understanding of RNA's functional capabilities, highlighting its crucial role in biological processes such as metabolic processing and gene regulation. Through techniques like SELEX (systematic evolution of ligands by exponential enrichment), researchers have harnessed the potential of RNA to develop a wide range of applications, including cellular detection and gene expression control. This review aims to summarize the latest advancements in utilizing ribozymes and riboswitches for therapeutic interventions, some of which have progressed to clinical trials. Ribozymes, classified based on their size, encompass a variety of enzymatic reactions. Large ribozymes (>300 nt) include group I and group II introns, as well as protein-assisted RNase P. On the other hand, small nucleolytic ribozymes like the hammerhead, hairpin, hepatitis delta virus (HDV), Varkud satellite (VS), and glmS ribozymes are well-characterized catalysts. Among these, the hammerhead ribozyme stands out as the smallest and most extensively studied. Its compact size and catalytic efficiency make it particularly appealing for therapeutic applications. Riboswitches, predominantly found in the untranslated regions of bacterial mRNA, play a crucial role in regulating gene expression. Comprising an aptamer and an expression platform, riboswitches undergo conformational changes upon ligand binding, thereby modulating gene expression. Metabolite-binding riboswitches present a novel approach to combatting multiple drug resistance (MDR) by serving as potential targets for therapeutic intervention. Ribozymes and riboswitches offer promising avenues for therapeutic intervention in various human diseases. Ribozymes, in particular, show potential as an alternative to RNA interference (RNAi) technology, with no observed off-target effects thus far. Specific requirements, such as the need for complementary nucleotides for ribozyme hybridization and cleavage specificity, underscore the importance of careful design and optimization in therapeutic applications. Overall, the continued exploration of ribozymes and riboswitches holds great promise for advancing RNA-based therapeutic strategies in the future [18][21].

Ribozymes, RNA molecules capable of catalyzing biochemical reactions, have garnered significant attention due to their potential applications in gene regulation and therapy. Initially identified in the 1980s, natural ribozymes like the hammerhead, hairpin, and group I and II intron ribozymes catalyze specific reactions such as self-cleavage or self-splicing. The hammerhead ribozyme, for instance, is known for its ability to cleave phosphodiester bonds at specific sites, making it a valuable tool for genetic therapy through RNA-mediated inhibition of gene expression. Trans-acting ribozymes, such as the hammerhead ribozyme, can down-regulate gene expression by cleaving target RNA molecules in trans. They consist of a catalytic core flanked by arms that bind to the target RNA via complementary base pairing. This allows for sequence-specific cleavage and has been extensively explored for applications in inhibiting viral gene expression, particularly in the context of HIV infection. Moreover, trans-cleaving ribozymes have shown promise in targeting dominant mutations in genetic disorders like Marfan syndrome. By designing ribozymes specific to mutant alleles, it's possible to preferentially down-regulate the production of faulty proteins, potentially restoring normal cellular function. Another approach involves utilizing group I intron ribozymes for RNA repair through trans-splicing. These ribozymes can replace faulty RNA sequences with wild-type counterparts, offering a potential therapeutic strategy for genetic diseases like myotonic dystrophy. By targeting mutant RNA molecules and editing them in situ, RNA repair holds promise for maintaining natural gene regulation while correcting genetic

defects. Despite the progress made, challenges remain in optimizing ribozyme specificity and delivery systems for efficient targeting and expression. However, with ongoing advancements in understanding ribozyme structure-function relationships and the development of delivery technologies, ribozymes continue to be promising tools for genetic therapy and gene expression studies. In conclusion, ribozymes represent a versatile class of molecules with significant potential for applications in gene regulation and therapy. Continued research into their design, specificity, and delivery mechanisms will further enhance their utility in treating a wide range of inherited diseases and understanding gene expression processes [22].

Ribozymes, catalytic RNAs, are pivotal players in various biological processes, including RNA replication, splicing, translation, and degradation. Despite their significance, deciphering the evolutionary paths and functional versatility of ribozymes remains a complex endeavor. Recent methodological advancements, such as in vitro evolution techniques, high-throughput sequencing, and structural elucidation methods, have provided deeper insights into the intricate relationship between ribozyme genotype and catalytic phenotype. This comprehensive review delves into the multifaceted landscape of ribozyme evolution and function, exploring the rugged terrain of ribozyme fitness landscapes, the potential for functional plasticity through subtle genomic alterations, and the implications for both natural evolution and biotechnological applications. Ribozymes, RNA molecules endowed with catalytic prowess, constitute a fascinating realm within molecular biology. Their involvement in diverse cellular processes underscores their fundamental importance. However, unraveling the evolutionary trajectories and functional repertoire of ribozymes presents a formidable challenge. This review sets out to elucidate recent breakthroughs in experimental methodologies aimed at unraveling the intricate interplay between ribozyme sequence, structure, and function, with profound implications for our understanding of RNA biology and biotechnology. The emergence of ribozymes as catalytic entities has revolutionized our understanding of RNA's capabilities beyond its traditional role as a passive carrier of genetic information. This section provides an overview of the diverse classes of natural ribozymes, spanning self-splicing introns, ribonuclease P, and small self-cleaving ribozymes. Despite their sparse distribution in nature, ribozymes exhibit catalytic efficiencies on par with protein enzymes, prompting inquiries into the origins of their catalytic prowess. Central to understanding ribozyme evolution is the concept of fitness landscapes, which delineate the relationship between genotype and phenotypic fitness [22][23][24]. However, mapping ribozyme fitness landscapes poses considerable experimental challenges due to the vast sequence space and complex biochemical interactions involved. Recent advancements in high-throughput sequencing methodologies have provided invaluable insights into the rugged terrain of ribozyme fitness landscapes and the nuanced interplay between sequence and function. One intriguing aspect of ribozyme evolution is the existence of neutral pathways that facilitate the acquisition of new catalytic functions. By dissecting these pathways, researchers have uncovered potential evolutionary trajectories connecting ribozymes with disparate catalytic activities. Case studies, such as the evolution of the glmS ribozyme, shed light on the mechanisms underlying the rapid acquisition of novel biochemical functions through minimal genetic changes. Ribozymes exhibit remarkable versatility, catalyzing a broad spectrum of chemical reactions using a limited repertoire of functional groups. This section explores examples of promiscuous ribozymes capable of performing multiple catalytic functions using a shared structural scaffold. Understanding the molecular basis of ribozyme promiscuity holds profound implications for elucidating their evolutionary trajectories and harnessing their biotechnological potential. The glmS ribozyme stands out as a unique example of a ribozyme reliant on an exogenous small molecule coenzyme for catalysis. Structural and biochemical analyses of the glmS ribozyme offer insights into its evolutionary origins and the intricate interplay between RNA structure and function. Case studies elucidating the dual catalytic mechanisms of the glmS ribozyme provide a glimpse into the evolutionary trajectories that shape ribozyme function. Ribozymes have emerged as powerful tools in bioengineering, with applications spanning gene expression control, RNA therapeutics, and synthetic biology. This section explores recent advancements in ribozyme engineering and synthetic biology, showcasing the potential of in vitro evolution methods to design novel ribozymes with tailored catalytic activities. Case studies elucidating the engineering of allosteric ribozymes and aptazymes underscore the versatility of ribozymes in biotechnological applications. In conclusion, ribozymes represent a captivating frontier in molecular biology, with far-reaching implications for both natural evolution and biotechnological innovation. Recent methodological advancements have provided unprecedented insights into ribozyme evolution and function, shedding light on the intricate interplay between sequence, structure, and catalytic activity. Continued research in this field promises to unveil new facets of ribozyme biology and unlock their full potential in medicine, biotechnology, and synthetic biology [25][26].

Ribozymes curing cancer

A recent study investigates the potential of ribozymes derived from ribonuclease P (RNase P) catalytic RNA to combat Kaposi's sarcoma-associated herpesvirus (KSHV) infection, a key player in Kaposi's sarcoma and other AIDS-related diseases. Researchers focused on the KSHV immediate early replication and transcription activator (RTA) mRNA, crucial for viral gene expression and replication. They engineered a functional ribozyme called F-RTA to target the RTA mRNA sequence, demonstrating its effectiveness in cleaving the RNA in vitro. In cells, the expression of F-RTA led to a remarkable 250-fold reduction in KSHV production, and RTA expression was suppressed by 92-94%. In contrast, control ribozymes had little impact on viral production. The study reveals that F-RTA's suppression of RTA expression resulted in decreased KSHV early and late gene expression, ultimately leading to reduced viral growth. The researchers employed ribonuclease P (RNase P) ribozymes, capitalizing on their ability to target and cleave specific RNA sequences. This innovative approach offers a promising avenue for anti-KSHV therapy. The study's findings provide critical insights into potential gene-silencing tools for inhibiting KSHV gene expression and associated infections, offering hope for improved treatments for KSHV-associated diseases [27].

In another scenario of cancer tumors, the study focuses on the potential of hammerhead ribozymes and DNAzymes to target miR-21, a critical player in cancer, especially glioblastoma multiforme (GBM). Glioblastoma is a deadly brain tumor with a grim prognosis, and miR-21 is linked to its development. The researchers designed ribozymes and DNAzymes to combat miR-21 and its precursors effectively, aiming to silence its oncogenic functions. Their experiments demonstrated that these catalytic nucleic acids efficiently reduced miR-21 levels, especially in mature miR-21. In vitro tests revealed that these agents performed better against mature miR-21 than pre-miR-21, suggesting that mature miR-21 is a more suitable therapeutic target. Additionally, cell culture experiments using T98G cells confirmed the potential of ribozymes and DNAzymes to reduce miR-21 levels and upregulate PTEN, a known miR-21 target. Overall, this research highlights the promising role of catalytic nucleic acids in combating miR-21-associated diseases, including GBM [28] [29].

2. Conclusion

Ribozymes have been predicted to cure many infectious diseases including cancer. A combination of chemotherapy and ribozyme against flt-2 receptor has been found to cure cancer in phase II clinical trials. Multiple studies mentioned above clearly highlight a novel role of ribozymes in curing cancer. We believe that using ribozymes can provide a cure for this dreadful problem in all low-, middle- and high-income group countries. Ribozymes are available in all organisms be it prokaryotes and eukaryotes. It is their extraction that might become a monetary issue but the implementation of policies in place will help different income groups work together and provide for resources to make this world cancer free. Public health policies which help in appropriate distribution of technology and products from one strata to another will help in detecting early cases and curing them when they are not that widespread. Using ribozymes early detection will be easy as these are quite specific and are able to detect small changes too.

In conclusion, ribozymes hold significant promise as therapeutic agents in cancer treatment, particularly against KSHV and glioblastoma. Their unique properties make them resilient candidates for therapy, and their potential in early detection and treatment could revolutionize public health strategies worldwide. Future research and public health policies should focus on the equitable distribution and application of ribozymebased technologies to combat cancer effectively.

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