RNA Therapeutics in Skin Cancer: Emerging Strategies, Challenges, and Future Perspectives

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ABSTRACT

Skin cancer is one of the most frequently diagnosed malignant diseases worldwide and represents a significant public health challenge. A comprehensive understanding of its natural history is essential for the development of effective preventive and therapeutic strategies. The skin, being the largest and most accessible organ of the human body, offers unique advantages for localized and targeted drug delivery, making it an attractive platform for innovative therapeutic interventions. Current therapeutic strategies for skin cancer include surgery, radiotherapy, topical treatments, cryotherapy, chemotherapy, and systemic options such as immunotherapy and targeted therapy for advanced disease. However, these modalities are often limited by tumor recurrence, development of drug resistance, immune-related adverse effects, and challenges associated with effective drug delivery to deeper skin layers. These limitations highlight the urgent need for less invasive, more precise, and durable treatment strategies.

Although ribonucleic acid (RNA)–based therapeutics have been investigated for several decades, recent advances in molecular biology, chemical modification, and delivery technologies have enabled their emergence as clinically viable treatment modalities. RNA therapeutics offer several advantages, including high target specificity, the ability to modulate previously undruggable molecular pathways, reduced systemic toxicity, and the potential for multiplexed targeting within a single therapeutic platform. These features make RNA-based approaches particularly promising for dermatological applications, including skin cancer.

This review systematically examines emerging RNA-based therapeutic approaches for skin cancer, including RNA interference, antisense oligonucleotides, and messenger RNA–based therapies. Additionally, it discusses key biological, technological, and translational challenges and explores strategies to overcome barriers to clinical implementation. Overall, RNA therapeutics represent a promising and transformative platform for advancing skin cancer treatment and improving dermatological care.

KEYWORDS: Skin cancer; RNA therapeutics; RNA interference; Antisense oligonucleotides; Messenger RNA; Dermatological drug delivery; Targeted therapy; Molecular dermatology

Introduction

Skin cancer ranks among the most frequently determined malignant diseases worldwide and represents a significant public health challenge. It is the abnormal and uncontrolled growth of skin cells caused by DNA damage or mutations in the genetic material. Its global incidence and mortality rates have shown a consistent yearly increase, making it a growing public health concern. It is primarily divided into two major classes- keratinocyte carcinomas (non-melanoma) and malignant melanoma skin malignancies. Non- melanoma is further distinguished into squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). BCC is the most common type, which accounts for 80 percent of the total skin malignancy cases. Malignant melanoma is a rare form of skin cancer, making up to about 5 per cent of all cases worldwide (Linares et al., 2015). However, due to its aggressive nature and characteristics, it has a huge mortality rate. According to GLOBOCAN 2022 data, skin cancer represents a significant proportion of the global cancer burden. Melanoma, a malignancy of melanocytes, was reported as the 17th most commonly occurring cancer worldwide, which has over 331,000 new cases and about 59,000 deaths globally. Non-melanoma skin cancers constitute the most common malignant tumours globally, ranking as the 5th most commonly diagnosed cancer, with even more than 1.23 million new cases and

approximately 69,000 deaths, making them significantly more prevalent than melanoma (Wang et al., 2024). Most skin cancers generally stem from the amalgamation of uncontrollable factors, both non-modifiable (e.g., genetic) and modifiable (e.g., environmental) risk elements. The biggest risk component for skin cancer is the exposure to ultraviolet radiation (UV). The survival rate of the patients can significantly increase if the early detection of the disease is achieved and treated efficiently. The most common diagnostic technique is biopsy, whereas optimal modalities to improve the non-invasive diagnosis of skin cancer include multispectral imaging, three-dimensional topography, deep learning algorithms, and techniques. Current therapeutic strategies for skin cancer include surgery, radiotherapy, topical treatments, cryotherapy, chemotherapy, and systemic options such as immunotherapy and targeted therapy for advanced disease. However, these modalities are often limited by tumour recurrence, development of drug resistance, immune-related adverse effects, and challenges associated with effective drug delivery to deeper skin layers. These limitations highlight the urgent need for less invasive, more precise, and durable treatment strategies. Although ribonucleic acid (RNA)–based therapeutics have been investigated for several decades, recent advances in molecular biology, chemical modification, and delivery technologies have enabled their emergence as clinically viable treatment modalities. RNA therapeutics offer several advantages, including high target specificity, the ability to modulate previously undruggable molecular pathways, reduced systemic toxicity, and the potential for multiplexed targeting within a single therapeutic platform. This review systematically examines emerging RNA-based therapeutic approaches for skin cancer, including RNA interference, antisense oligonucleotides, and messenger RNA–based therapies, representing a promising and transformative platform for advancing skin cancer treatment and improving dermatological care (Chai et al., 2024).

Types of skin cancer

Cutaneous melanoma is still known to be one of the most virulent skin cancers worldwide, derived from skin melanocytes, with a progressively increasing incidence every year. Clinicians must maintain a high index of suspicion that pigmented or otherwise strange-appearing lesions may depict melanoma. It is often triggered by intense UV light exposure. It appears as new or changing moles, or spots with irregular borders, varied colours, and increasing size, largely seen in fair-skinned people. Light skin tone has a lesser amount of melanin, a pigment that protects the skin from absorbing UV radiation, therefore giving birth to skin cancer. It is a very invasive type of skin cancer that has the lowest incidence of all skin cancers, poses the highest possibility of disease progression leading to death, and has the highest likelihood of metastasizing to adjacent, distant, and neighbouring cells and tissue. Despite the availability of preventive strategies like sun avoidance and photoprotection, melanoma continues to spread and pose a major and increasing global health challenge, emphasizing the urgent need for efficient preventive strategies, awareness, and advanced therapeutic innovations (*Melanoma: Diagnosis and Treatment*, 2024).

Non-melanoma skin cancer (NMSC), also known as keratinocyte carcinoma, includes cutaneous squamous cell and basal cell carcinoma and is the most frequent human malignancy; rare type includes Merkel cell carcinoma (MCC). BCC typically presents as pale pink, round-shaped blisters with prominent telangiectatic surface vessels. SCC is typically seen as a solid, smooth, or hyperkeratotic bump or plaque, usually with central ulceration. MCC is a highly virulent form of skin cancer that occurs as rapidly multiplying, on the skin usually on the head, neck, eyelids or the body parts that receive heavy sun exposure, as painless red nodules. NMSC is comparatively less lethal since it grows slowly and hardly spreads to other body parts or organs. It is the 5th most frequent occurring cancer all over the world. Although diagnosis, preventive measures, and therapeutics exist, the survival rate is still limited to some months or years, which demands more precise and effective therapeutic inventions (Hyeraci et al., 2023).

Current treatments available

Surgery- Conventional surgical management of NMSC involves lesion with successive assessment of the margins of excision, either by preserved section analysis intraoperatively or after removal and then closure. The understanding of surgical margin analysis between the surgeon and the pathologist should be confirmed. Recurrence of the tumour may be related to the asymmetrical pattern of tumour growth, with the tumour extending

unexpectedly (Lane & Kent, 2005). Mohs micrographic surgery is an outpatient plan of action that provides maximal surgical margin analysis with minimal excision of tissue. MMS has emerged as the preferred treatment for some rare skin cancers and certain variants of melanoma, BCC, and squamous cell carcinoma. It helps in preserving health and provides a very high cure rate. Mohs Micrographic Surgery (MMS) is very successful for certain types of skin cancers but has its drawbacks, such as being a time-consuming procedure, requiring highly specialized personnel, and having limited success for non-contiguous or deeply invasive cancers. The procedure may be prolonged, causing the patient discomfort or pain, potentially halting the surgery (Lang, 2004).

Intralesional chemotherapy- Intralesional chemotherapy for NMSC has been around for more than50 years now. It involves the injection of anti-cancer drugs directly into the tumour to achieve a high local concentration, maximum effect, and low systemic toxicity. It is mainly used as an alternative for nonmelanoma skin cancers (basal cell carcinoma, keratoacanthoma) in patients who are not suitable for surgery or refuse to undergo the surgical procedure. The medications used include methotrexate, 5-fluorouracil (5-FU), and bleomycin, which have shown a high response rate, although it is considered an off-label and less frequent treatment due to the absence of clinical trials, painful injections for the patient, and the possibility of recurrence (Kirby & Miller, 2010).

Radiotherapy- Although there are numerous treatment modalities available for NMSC patients, radiotherapy is an effective and flexible tissue-preserving non-surgical (or medical) treatment modality. In patients where excision isn't feasible (due to medical incapability/technically irremediable) or undesirable (e.g., cosmetic results), radiotherapies are a terrific choice. After the surgical procedure, additional radiotherapy can reduce the risk of recurrence and morbidity in patients with unfavourable pathological conditions. Performance status is poor in elderly, frail, and multimorbid patients, short-term hypo fractionated radiotherapy is beneficial when surgery cannot be performed (Garbutcheon-Singh & Veness, 2019). Radiotherapy for skin cancer is considered effective but is limited by acute reactions (erythema, peeling, pain), late effects (skin atrophy, colour changes, telangiectasias), and possible necrosis or secondary malignancies. It involves multiple treatments, possibly daily, and is contraindicated in patients with certain genetic disorders or in very young patients (Veness & Richards, 2003).

Photodynamic therapy-The procedure of photodynamic therapy (PDT) uses a photosensitizing agent, light, along with oxygen for the destruction of selective cells. PDT is a process that involves the activation by light of wavelengths of the photosensitizing drug that is being inserted or administered, corresponding to the absorption spectrum of the photosensitizer. Due to the accessibility of the skin to phototherapies, PDT using systemic and especially topical photosensitizing compounds has gained prominence in the treatment of dermal disorders. Topical PDT is used for the treatment of superficial or thin NMSCs, actinic keratosis, including some cutaneous lymphomas, and some cases of nodular basal cell carcinoma. It is primarily limited by shallow light penetration, causing depth restriction, making it unsuitable for deep-seated, thick, nodular tumours (Fien & Oseroff, 2007).

Topical treatments- The decision to undergo surgery may be influenced by several factors, such as the co-morbid conditions of the patient, the location of the excision, and the possible incapacity of the patient to undergo frequent excisions; therefore, topical therapy of skin cancer may be indicated in some cases. It may potentially achieve higher concentrations of the drug at the location of the tumour, and may even be less toxic systemically than systemic therapies. Topical therapy of skin cancer as shown in Fig.1, mainly used for superficial BCC and actinic keratosis, is in the form of creams and gels that kill cancer cells or stimulate the immune system. The main agents used are imiquimod and 5-FU, which are directly applied to the skin, providing high concentrations of the drug at the tumoral site with fewer systemic body reactions or side effects while the limitations remain, such as superficial lesions, being incapable to treat deeper or metastatic cancers as well as require high patient compliance over many weeks (Cullen et al., 2019).

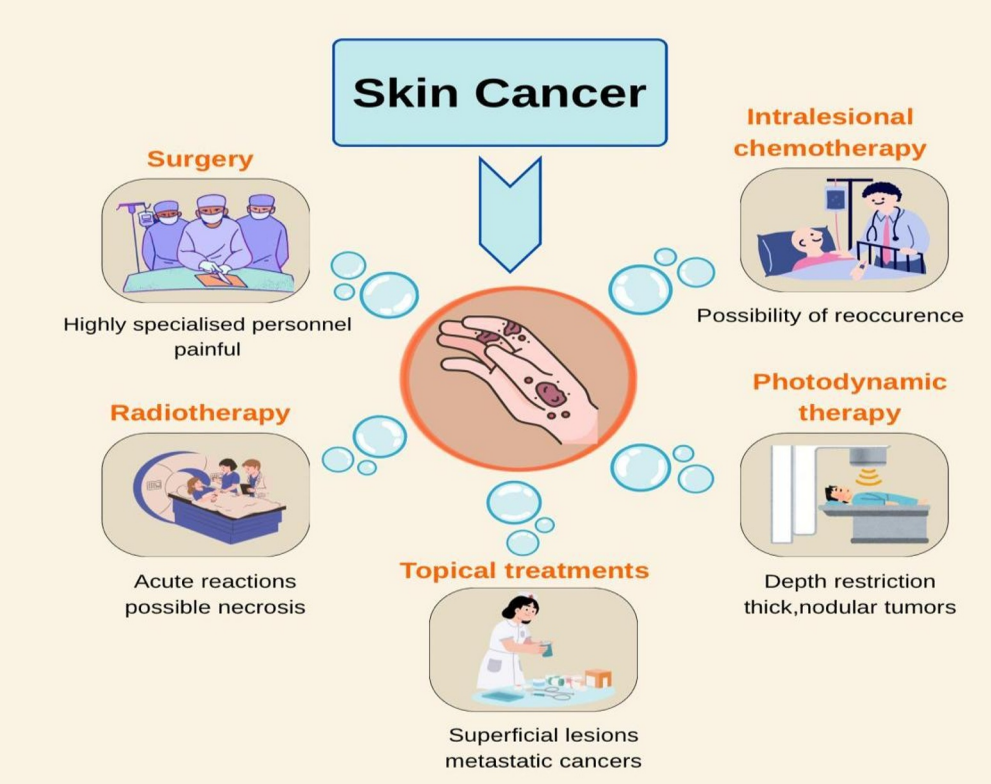

Figure 1. Schematic representation of current skin cancer treatment modalities and their key limitations.

The future of RNA-based therapeutics

RNA therapeutics hold great potential for the development of advanced dermatological treatments due to the ability to treat previously undruggable targets, high specificity with fewer side effects, and the ability to target multiple RNA molecules simultaneously in a single formulation. Although there have been studies and research relating to RNA therapeutics for several decades, there have not been many products developed for actual clinical use until recently. This could be due to the following challenges to the application of RNA therapeutics, including the lack of effective methods of delivery to the target site, as well as the short lifespan and rapid degradation of RNA molecules in the human body and environment. This review proposes to offer some insight into, firstly, the vast possibilities of RNA therapeutics in the dermatological field, and secondly, how the aforementioned challenges can be addressed, so as to encourage the development and usage of novel dermatological therapies (Johari, 2024).

Therapeutic technologies based on nucleic acids, like anti-sense oligonucleotides, small interfering RNA (siRNA), and therapeutic mRNA (mRNA vaccines), hold tremendous potential as anticancer agents. Unlike any other therapeutic modalities, the druggable universe is not constrained by these approaches because therapeutics can be practically formulated using the hybridization rules of the Watson-Crick principles or mRNA inhibitors. Nucleic acid-based therapeutic proteins can be formed using the protein code. Moreover, these modalities provide the specificity of a selective gene which is not be attained by other different approaches, especially when the aim is to target protein classes which might have multiple and highly homologous family members. Thus, with RNA-based therapies, “undruggable” targets can be specifically targeted. This review aims to discuss the progress made in RNA-based therapies for skin cancer treatment and highlight the need for efficient research to establish these novel modalities as a promising cancer drug.

1. Anti-sense Oligonucleotides (ASO)

ASO typically range from 15-20 base pairs in size and are mono-stranded deoxynucleotide analogues. They are short mono-stranded polymers that are derived from RNA or DNA chemistry, and in a sequence-specific manner, they bind to the RNA target, which inhibits gene expression. Antisense sequence is (3’ to 5’), which is

complementary to the target mRNA sense sequence(5'to3'). Oligonucleotide (OGN)-based therapeutics are a new generation of sanative, including anti-sense oligonucleotides as one of its subtypes, which are a promising new approach for gene-specific modulation. Antisense works on an approach of reduction in expression by inhibiting the process of translation from mRNA to proteins. A prime illustration of this is how normal cells can become a malignant tumor when they don't undergo apoptosis anymore. Apoptosis is employed in normal cells to regulate cell damage. When normal cells lose this vital role, they transform into malignant. Apoptosis is usually triggered by chemotherapeutic agents; when melanoma cells become resistant to cellular suicide from this lethal attack, the therapy will fail. Research has shown that genetic, biochemical and functional transformations of melanoma cells indicate that they are no longer able to undergo apoptosis as a normal cellular means to perform any action. Additionally, melanoma cells have developed a method of utilizing the pathways responsible for their survival, as part of some sort of "program," as well as through their ability to prevent being destroyed by chemotherapy through decreased apoptosis (Chi et al., 2017).

A common feature of melanomas is that they frequently exhibit drug resistance, which is related to the overexpression of an anti-apoptotic protein (BCL-2) that is very important for regulating the overall process of apoptosis. The BCL-2 protein is a key part of the membrane that surrounds the mitochondria, and serves to inhibit the release of cytochrome C, which consequently prevents the activation of the apoptotic cascade that would normally occur when cytochrome C is released, leading to the activation of caspase 9 in response to cytotoxic chemotherapeutic agents. There are two subclasses of inhibitors of apoptosis (i.e., anti-apoptotic members of the BCL-2 family). The subclasses of BCL-XL, BCL-W, and BCL-2 are grouped together as one subclass; the subclasses of BCL-2A1 and MCL-1 are grouped together as a second subclass. To promote apoptosis, it is necessary to inhibit all of the inhibitors from both subclasses. The effectiveness of BCL-2 Antisense Therapy has been assessed in clinical trials with melanoma sufferers. The results indicated that regulating BCL-2 and BCL-XL can be an important target for developing antisense therapies for melanoma, and that concurrently inhibiting BCL-2 and BCL-XL provides clinical benefits that are additive to each other. As an example of this, oblimersen sodium is an 18-base phosphorothioate antisense oligonucleotide that targets the first six codons of the open reading frame of BCL-2 mRNA and causes RNA cleavage via RNase H activity. Oblimersen lowers BCL-2, an apoptotic protein expression and in human cancer xenografts, it increases the chemotherapy-induced apoptosis. Oblimersen quickly undergoes metabolic breakdown into both forms of its respective metabolites and multiple forms of the parent compound. The process of exonucleolysis utilizes exonucleases to systematically degrade parent oligonucleotides by removing one nucleotide at a time. The result of this process includes both a mononucleotide metabolite and a truncated form of the parent oligonucleotide (i.e., the N-1 oligonucleotide is one base shorter than the parent oligonucleotide). In pharmacokinetic studies, N-1 and N-2 are the major metabolites of oblimersen and both have shown activity in getting therapeutically useful results. In some of the earlier studies performed, only the total amount of oblimersen and its metabolites was used to describe oblimersen pharmacokinetic parameters because there were no means of analysing the parent drug and its shortened version separately. There was a finding in one study that demonstrated that combining oblimersen with dacarbazine to treat patients with advanced melanoma improved clinical measurement endpoints and extended overall patient survival in a cohort of patients who had elevated serum LDH at study initiation. The initial clinical trials of continuously delivering 14-day subcutaneous dosages of oblimersen showed that some side effects were fever, thrombocytopenia, and exhaustion.

Survivin, an anti-apoptotic protein, may be useful as a therapeutic target through antisense oligonucleotide therapy in melanoma. Survivin is an inhibitor of apoptosis, is an IAP family member, and is predominantly expressed in fetal and embryonic tissues and in a variety of human cancer types, such as melanoma, at elevated levels. In adults, survivin is minimally present or absent in most normal adult tissues; furthermore, it has not been detected in terminally differentiated human tissues. Survivin interacts with procaspase-9 when a cofactor is present and selectively inhibits the mitochondrial/cytochrome c pathway for apoptosis. During the processes of mitosis, survivin can be overexpressed and will bind to the microtubules of the mitotic spindle, potentially having oncogenic effects based on bypassing the checkpoint G2-M. Eli Lilly and Company developed and manufactured a compound i.e., LY2181308, which uses antisense oligonucleotides to inhibit survivin expression. The purpose of this application of an MOE- modified second-generation ASO to an 18-mer sequence was to inhibit survivin expression within a tumor cell population. The inhibition is achieved through the binding of the ASO at the

translation initiation site of survivin mRNA to block the ability of the mRNA to be translated; therefore, leading to the deterioration of the RNA transcript. A study to evaluate the effects on survivin protein expression and apoptosis was performed in patients who received LY218308 intravenously before and after breast tumor biopsy, using an escalating dose of the drug. The objective of this study was to demonstrate a decrease in survivin protein expression and restoration of apoptosis signalling in vivo. The oligonucleotides have some considerations, which include the instability of these oligonucleotides in vivo and their inefficacy in the targeting and inhibition of survivin mRNA. In addition, in patients who are chronically treated with antisense oligonucleotides, the assessment of kidney function is important. People who take angiotensin converting enzyme inhibitors or non-steroidal anti-inflammatory agents along with ASOs for survival typically have a reduced incidence of apoptosis. A common characteristic of MCC is the lack of apoptosis, regardless of whether Merkel cell polyomavirus (MCV) is present, as has been demonstrated in several studies. One possible reason that MCC is able to continue to grow could be due to the overexpression of the BCL-2 protein, which inhibits the apoptosis of transformed MCC cells by preventing them from undergoing apoptosis.

Therefore, modulating BCL-2 expression may provide a useful therapeutic approach to treat MCC; for example, two independent studies demonstrated that 75% of MCC tumors tested were positive for antiapoptotic BCL-2 expression. In one of the aforementioned studies, human MCC was grafted onto SCID (severe combined immunodeficiency) mice (T cells and B cells are lacking in this type of mouse) & BCL-2 antisense phosphorothioate oligos were employed. Results evaluated using Western blotting of tissue samples obtained from tumors due to treatment with oligodeoxynucleotides showed that there was a decrease in BCL-2 protein levels (30%) in antisense treated group, in contrast to the untreated control samples, despite no differences in all of the other treatment groups. Although this was a relatively small decrease, an obvious reduction in the rate of tumor growth in the antisense oligonucleotide-treated animals occurred at two weeks into treatment. However, these same antisense oligonucleotides were shown to have limited to no effect in humans with Merkel Cell Carcinoma during a phase II clinical trial. The results of the study on MCC suggest that the antisense oligonucleotide had an antisense mechanism; however, there could also have been non-antisense mechanisms involved. This supports the notion that using an oligonucleotide as an antisense agent would be justified to treat skin cancer (Laikova et al., 2019).

2. mRNA vaccines

Many cancers are caused by mutations that occur in specific cells throughout our lives, called Somatic Mutations. These could occur due to environmental exposures (i.e., carcinogens), inherited risk factors (i.e., genetic predisposition), and environmental influences during development. These mutations cause proteins made from these genes to function abnormally. Many of the mutated proteins only exist in cancer cells and are not found in healthy cells. Because of this abnormal (mutated) protein, the immune system will view that cell as foreign and therefore target it for macrophages and T-cells to kill the affected cancer cell.

Several neoantigens will exist in many cancers. The development of targeted neoantigen therapies has been an exciting new direction for the treatment of cancers at a lower-cost and more universal level than current treatments. The introduction of immunotherapy as a cancer treatment method has completely changed the approach to treating cancer, as it allows physicians to utilize the power of immunology against cancer cells. The global response to the COVID-19 pandemic has rapidly advanced the field of mRNA technology, which has led to increased advancement in the expansion of cancer vaccines. An mRNA cancer vaccine is composed of a single strand of RNA (the mRNA) that codes for a specific mutated protein from a particular cancer. The mRNA vaccines work by delivering a copy of the genetic instructions of the mutated cancerous protein to antigen-presenting cells (APCs) that reside within the cytoplasm of cells, including dendritic cells (DCs). This mRNA is read in the cytoplasm of the APCs, where it will produce the mutated protein, the neoantigen, and allow the APCs to present the neoantigen to naïve T-cells. Neoantigens stimulate DCs through Toll-like receptors and trigger an intense immune activation. In addition to stimulating DCs, mRNA vaccines also produce a strong type I interferon response and are therefore significantly better able to induce T cell responses against cancer cells. DCs are recognized as the most important type of antigen-presenting cell within the immune system. They can also uptake antigens by endocytosis, then break them down and present them via Major Histocompatibility Complexes (MHC), such as MHC Category I and MHC Category II Molecules; the antigen will be presented to both CD8+

T Cells and CD4+ T Cells. Based on their unique properties as well as their important role in initiating the adaptive immune system's response, the major biological functions of DCs and their relationship to both are very much at odds with any potential for using DCs as targets for future cancer prevention strategies involving vaccination. Furthermore, Dendritic cells are able to produce numerous cytokines and chemokines, which play a critical part in T cell proliferation, activation, and recruitment. Trimix adjuvant consists of three 'naked' mRNA strands that code for three different proteins. These are: (I) the CD40 ligand, or CD40L, which activates CD4+ T lymphocytes, or T cells; (II) a second mRNA codes for CD70, which activates CD8+ T cells; and (III) an mRNA codes for a constitutively active form of TLR4, or TLR4c, that is used to activate DCs and provide them with enhanced ability to present antigen to T cells. The combination of the Trimix adjuvant and mRNA encoding aircraft has been tested in numerous clinical trials of DC-based mRNA vaccination and has been demonstrated to have good safety and immunogenicity profiles. Multiple cancer vaccination approaches are under investigation, including DC-based vaccines, recombinant viral vaccines, DNA vaccines, and mRNA vaccines, although mRNA-based vaccines are notable for their adaptability and expeditious development. mRNA vaccines also have a considerably more favourable safety profile than traditional vaccine strategies such as viral vectors, because they contain only the components necessary for antigen production. The creation of mRNA vaccines can provide numerous advantages, including the ability to produce single-use antibodies, induce apoptosis within cancer cells, induce changes to the microenvironment of the tumor, and generate T cells with specificity to the cancer. One major benefit of mRNA vaccines is that they can create antigens unique to the tumors, activating the immune system against the cancerous cells. mRNA vaccines have been shown to have significant potential advantages compared to traditional methods of vaccination, namely the preferential stimulation of CD8+ T cells, which are essential for the destruction of tumors, in their generation of an effective immune response. The route of administration of the mRNA vaccine plays an important role in determining both the quantity and quality of the immune response. Delivery of the cancer mRNA vaccine can be accomplished via the use of intradermal, subcutaneous, or intramuscular routes of administration. Additionally, mRNA vaccine administration does not carry any of the significant side effects associated with DNA vaccine administration, such as the potential for integration of the DNA into the genomic DNA of the patient. Integration of genomic DNA can result in adverse events that include disruption of a gene, insertional mutagenesis, death of the cell, and even the development of tumors. Furthermore, mRNA vaccines operate in the cytoplasm of the cell and therefore can be delivered to the target cells with ease because they are not able to penetrate the nucleus of a cell. Another key advantage of mRNA vaccines is the ease and cost-effectiveness of manufacturing them, resulting in high production rates of the final product when they are cultivated in vitro environment (Bidram et al., 2021).

Cancer vaccines offer a hopeful new immunotherapy strategy for producing specific, durable immune responses to tumor-associated antigens (TAAs). Cancer vaccines are an artificial method for activating an immune response against TAAs.

The goal of a cancer vaccine is to stimulate the body's immune response against TAAs. This response can be cellular or humoral, and both types are effective at suppressing the growth of cancerous tumors and eliminating them. Two categories of TAAs are simply unmodified proteins that are produced at an increased or abnormal rate in tumorigenic cells; they may be found on many different types of cells, but will not be visible on noncancerous (normal) cells. Tumoral-related substances consist of an antigen related to differentiation and protein made only by an individual, a type of universal tumoral antigen, and an antigen produced by oncoviruses. Clinical trials testing different types of vaccines for treating patients have demonstrated limited efficacy. In some studies, vaccines target multiple types of antigens that can also be found in unaffected cells, thereby creating the possibility of an autoimmune toxicity due to the administration of a vaccine against this antigen. Tumor-specific antigens are unique to cancer cells and are absent in healthy tissues. They are produced during the process of genetic mutations occurring within a tumor and are therefore referred to as neoantigens because they are newly formed from DNA that was not present before. These novel antigens possess a significantly higher binding ability with MHC molecules and a greater immunogenic response compared to normal antigens. Tumor cells express neoantigens specifically, which in turn generate an anti-tumor immune response through T cells with minimal "off-target" activity. As a result, neoantigens have become the major target of modern vaccine development. Currently, two personalised mRNA-based cancer vaccines have been developed (mRNA 4157 from Moderna encodes for up to

34 neoantigens; and BNT122 from BioNTech encodes for up to 20 neoantigens) (Vishweshwaraiah & Dokholyan, 2022).

3.Rna interference

Although RNA interference (RNAi) is relatively new, having only been discovered in the past 12 years, recent studies have shown that it is an effective method for silencing genes and has been further developed rapidly over the last few years due to its extreme importance in genetics, molecular biology, and physiology. RNAi uses a naturally occurring mechanism that involves small interfering RNA (siRNA) duplexes to selectively silence homologous genes at the post-transcriptional level via the binding of complementary mRNA and the subsequent degradation of that mRNA. RNAi has been used as a new and powerful way to silence genes for basic research into cancer therapies, as well as having promising results in the treatment of cancer patients.

RNAi refers to a method by which genes are silenced after they have already been transcribed into mRNA and consists of two types of small RNA molecules, such as short hairpin RNA (shRNA), microRNA (miRNA), and siRNA. RNA molecules of <30 nucleotides are incorporated into the RISC (RNA-induced silencing complex), where they are separated into single strands. One strand is used to provide direction to the RISC to find an area of complementary or near-complementary base pairing to the targeted mRNA. After the RISC has located the target, there are now two possibilities for inhibiting the gene expression: the RISC can either degrade the mRNA or block the translation of the mRNA to protein. The primary component of RISC is the Argonaute (AGO) protein. In humans, there are 8 members of the AGO family. Four are members of the AGO subgroup, and four are members of the P-element induced wimpy testis (PIWI) subgroup. Only one member of this family, AGO2, can mediate the degradation of the target RNA. Thus, AGO2 is the only member of the AGO protein family that fulfils the role of RISC executor for siRNA-mediated silencing (Deng et al., 2014).

The RNAi pathway is a complex mechanism by which eukaryotes regulate gene expression at the post-transcriptional level through the use of siRNA as well as other types of double-stranded RNA (dsRNA) molecules that degrade target mRNA via homology-based processes. siRNAs are approximately 21-23 nucleotides in length, have a very specific and unique structural configuration, including 2-3 nucleotide 3' overhangs as well as 5' phosphate and 3' hydroxyl functional groups, to ensure that only the intended gene(s) are silenced. In addition, each siRNA contains both a sense (passenger) strand and an antisense (guide) strand.

RNAi in mammalian cells dsRNA can come from either within the cell (endogenous) or from outside the cell (exogenous). When a long dsRNA is present in a cell, it undergoes processing by Dicer, which is an RNase III-type endonuclease, resulting in the production of siRNAs. Once siRNA has been produced, it binds to a multifunctional protein called Argonaute-2 (Ago-2) and becomes part of an RISC. Within these RISCs, the dsRNA is separated into single strands; Ago-2 then cleaves the so-called 'passenger' strand. By creating intermolecular base pairing with one or more complementary bases on a single-stranded guide RNA molecule, the RISC is activated to be specific to its target. Upon activation, endonucleolytic cleavage occurs between positions 10 and 11 relative to the 5′ end of the antisense siRNA strand, after which (Ago-2) seeks out and cleaves the complementary mRNA. By breaking down additional mRNA targets, this activated complex can also prolong gene silencing. In rapidly dividing cells, this process can continue for a few days, while it can continue for several weeks in non-dividing cells. To put this into practice, siRNA can be produced in a lab and delivered straight to target cells, avoiding the Dicer mechanics. It is also possible to discuss other forms of siRNA, such as Piwi-interacting RNA, shRNA, and miRNA. But the most widely used type of RNA interference in treatment is siRNA (Gulino, 2012).

Additionally, it is assumed that RNA interference is gene-specific, targeting only the gene that has a complementary sequence. In actuality, though, RNA interference can occasionally result in nonspecific off-target effects. Delivering these big molecules to the target cells is the main obstacle in the development of RNAi-based treatments. SiRNAs' size and negative charge make it difficult for them to flow through cell membranes. The main cellular uptake mechanism for non-viral siRNA delivery is endocytosis (Zhong et al., 2016).

Limitations

Although RNA-based therapeutics have great potential, there are still many hurdles to overcome. The main problem with these therapeutics is the delivery issue, since RNA molecules are large, negatively charged, and very prone to degradation by ubiquitous RNases. Although RNAi is viewed as a potent gene-silencing tool, siRNA has difficulty penetrating target tissues and cells because of poor penetration properties in solid tumours (Zhong et al., 2016). There are a number of reasons why the potential of OGNs as therapeutic agents has not been fully explored. Certain issues will remain with mRNA vaccines, like how easily they are digested by nucleases and their difficulty reaching target tissues following systemic administration, as well as potentially life-threatening side effects such as glomerulonephritis, vasculitis, and thrombocytopenia.

While mRNA vaccines have the potential to significantly impact cancer prevention and treatment, many obstacles remain before they will be implemented widely. Foremost among these is the immunosuppressive nature of the tumor microenvironment and the immune evasion strategies that cancer cells use to avoid detection. Scientists are working very hard to find new ways to increase the ability of mRNA vaccines to stimulate immune responses, including developing strategies for enhancing the stability and distribution of mRNA molecules and optimizing the formulation of vaccines to provide very strong immune stimulation. Overall, developing mRNA as a form of immunotherapy represents an exciting new direction in cancer treatment (Bidram et al., 2021).

Conclusion

Therapeutic technologies using RNA represent an expanding, rapid class of therapeutics with the potential to fundamentally change how we can manipulate cellular biology in ways we could never have done before.

RNA therapeutics also carry deep implications for drug development, including inhibiting previously "undruggable" protein targets, changing the expression of genes, and creating proteins based solely on sequence.

This opportunity is not available through other forms of therapeutic modalities, and especially not with conventional forms of treatment. Although challenges still exist to achieving these innovations of therapy, continuous advancement holds a substantial opportunity for revolutionizing how we treat cancer by providing us with a significant new tool in our ongoing mission to restore health through overcoming the devastating impact that cancer has on our society.

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