RNAi gene therapy in Cancer treatment

ABSTRACT
Cancer is caused by failures in the genetic control of cell division processes, which alter the function of proto-oncogenes and tumor suppressor genes. Cancer causes and treatments have been studied for years, but the development of effective therapies has its limitations, and conventional therapies frequently have significant side effects. In the search for efficient therapeutic alternatives against cancer cells, the use of non-coding RNA (ncRNA), particularly RNA interference (RNAi), has shown promise. Therefore, the current study aimed to characterize RNAi and their applications in gene therapy against cancer through a descriptive bibliographical review. RNAi consists of a gene silencing mechanism that generally occurs through two pathways: the production of microRNAs (miRNA) and small interfering RNAs (siRNA). Several satisfactory and promising studies have been developed with the use of RNAi in cancer diagnosis and therapy. In general, the strategies investigated in these studies focus on the silencing of genes related to the inhibition of tumor apoptosis, oncogene silencing, inhibition of tumor angiogenesis factors, and chemoresistance reduction. Although the success of using RNAi in the fight against cancer is dependent on the efficiency with which these molecules are delivered to the target tissue, research in this area has been advancing, bringing the possibility of more efficient therapies with fewer side effects increasingly closer.
Keywords: miRNA, genetic silencing, siRNA, gene therapy.

1 INTRODUCTION

Cancer is classified as a non-transmissible pathology and is recognized as the world's second leading cause of death, posing a barrier to increasing the population's survival, particularly in low- and middle-income countries (PEREIRA et al., 2022). Because this pathology is aggressive, complex, and has multiple mechanisms of development and spread, the search for new treatments has become constant and necessary (PRADO et al., 2020). Carcinogenesis, also known as oncogenesis, is a cumulative process that can take years to form a tumor that can be clinically detected, as this process occurs through gene mutation, which can take time. Several carcinogenic or carcinogens agents, including byproducts of cellular metabolism, exogenous chemical compounds, hormones, radiation, and viral infections, are capable of inducing mutations in DNA (LODISH, 2015; INCA, 2021). In general, such agents repeatedly alter the sequence and function of genes, culminating in cell cycle control failures, which alter the balance between apoptosis and cell proliferation, ultimately leading to tumor development.

RESUMO

O câncer se origina a partir de falhas no controle genético dos processos de divisão celular, alterando o funcionamento de proto-oncogenes e genes supressores tumorais. As causas e tratamentos para o câncer têm sido estudadas há anos, porém o desenvolvimento de terapias eficazes possui suas limitações, e as terapias convencionais geralmente trazem consigo importantes efeitos colaterais. Na busca por alternativas terapêuticas eficientes contra células cancerosas, o uso de RNA não codificantes (ncRNA), especialmente os RNA de interferência (RNAi), tem se mostrado promissor. Por essa razão o presente estudo teve objetivos caracterizar os RNAi e suas aplicações na terapia gênica contra o câncer, por meio de uma revisão bibliográfica descritiva. O RNAi consiste num mecanismo de silenciamento gênico que ocorre, geralmente, por meios de duas vias: a produção de micro RNAs (miRNA) e os pequenos RNAs de interferência (siRNA). Várias pesquisas satisfatórias e promissoras têm sido desenvolvidas com a aplicação de RNAi no diagnóstico e terapia contra o câncer. De maneira geral, as estratégias investigadas nesses estudos têm como foco o silenciamento de genes relacionados à inibição da apoptose tumoral, silenciamento de oncogenes, inibição de fatores relacionados à angiogênese tumoral, e redução da quimioresistência. Ainda que o sucesso na utilização de RNAi no combate ao câncer dependa da eficiência na entrega dessas moléculas ao tecido alvo, as pesquisas realizadas nessa área têm avançado, tonando a possibilidade de terapias mais eficientes e com menos efeitos colaterais, cada vez mais próxima.

Palavras-chave: miRNA, silenciamento gênico, siRNA, terapia gênica.
Proto-oncogenes and tumor suppressor genes are the genes whose mutations affect the cell cycle. Proto-oncogenes normally encode growth factors, which signal cells to begin the process of cell division (BROWN, 2021). However, mutations in these genes can cause them to be constantly active, leading to continuous cell proliferation. At this point, these genes are referred to as oncogenes and neoplastic development begins (BORGES-OSÓRIO; ROBINSON, 2013; BROWN, 2021). Tumor suppressor genes, on the other hand, encode proteins that inhibit cell growth and, under normal conditions, regulate cell proliferation and apoptosis. In general, the function of these genes is to maintain genomic integrity, participate in the DNA repair process, and signal the cell to undergo apoptosis, and changes in their function result in tumor development (SUN; YANG, 2009; MEDRADO, 2015; WANG et al., 2018).

Cancer causes and treatments have been studied for many years, but the development of effective therapies has its limitations, and as a result, the primary methods used to combat this pathology remain surgery, chemotherapy, and radiotherapy (BENJAMIN, 2019; CHEN; KUO, 2017). However, these approaches frequently result in severe side effects that significantly debilitate the patient (PIERCE et al., 2016; TAYLOR et al., 2017; NURGALI et al., 2018). Because cancer is a complex disease mainly due to the tumor microenvironment generated by the imbalance in the control pathways of altered cells, the search for alternatives such as gene therapy becomes critical, as the goal of this therapy is the silencing of an undesirable gene, preventing the synthesis of anomalous proteins, making this therapy a promising possibility (XIN et al., 2017).

One notable option within gene therapy is the use of RNAi (RNA interference), cellular mechanisms that naturally regulate protein synthesis by silencing post-transcriptional genes. The function of these RNAi is so important and versatile that they are currently being studied for use in gene therapy for a variety of diseases, including cancer. Some studies available in the literature have shown promise for disorders caused by a single gene or in the case of protein overexpression, both in vitro and in vivo tests (DYKXHOORN; LIEBERMAN, 2006). Given the foregoing, the goal of this study was to characterize RNAi and its applications in cancer gene therapy.
2 METHODS

This work presents the main aspects of RNAi gene therapy and is thus a qualitative study of narrative review. Vosgerau and Romanowski (2014) point out this type of review as a broad analysis of the literature, providing qualitative information on a given topic. Nonetheless, narrative reviews are of fundamental importance, as they not only contribute to the acquisition of knowledge about a specific theme but also enable the updating of this information and the discussion of related methods and subtopics (ELIAS et al., 2012).

Searches for scientific articles were conducted in the databases "Google Scholar," "LILACS," "PubMed," and "SciELO" using the Boolean operator "AND", resulting in the index term "RNAi AND Cancer AND Gene Therapy." For the development of this work, the most relevant articles published after the year 2013 were chosen.

3 DEVELOPMENT

3.1 RNAi CHARACTERIZATION AND BIOGENESIS

The most studied molecules for therapeutic application in cancer are non-coding RNAs (ncRNAs), recognized for their functions related to gene expression control and genomic integrity maintenance. Among the ncRNA classes, RNA interference (RNAi) has been tested in gene therapies for the treatment of a variety of diseases, including cancer (FRANÇA et al., 2010). RNAi is a gene silencing mechanism that generally occurs through two pathways: the production of microRNAs (miRNA) and small interfering RNAs (siRNA) (MANSOORI; SANDOGHCHIAN; BARADARAN, 2014). miRNA and siRNA are both produced from double-stranded RNA (dsRNA) precursors and have similar biogenesis. Their origins, however, differ because miRNAs are considered endogenous, resulting from products of the organism's genome (miRNA genes) and even from the splicing process (exons and excised introns). On the other hand, siRNAs are generated by double-stranded precursors present in the cell cytoplasm, whose origins can be viruses' genomes, transposons, DNA breaks, or even primary miRNAs (pri-miRNA) (MAIA, 2020).

Essentially, miRNA formation starts with the transcription of a pri-miRNA molecule from a miRNA gene (canonical pathway). However, other primary miRNAs can also be formed from introns and exons cleaved by splicing (non-canonical pathway). While still in the cell nucleus, the pri-miRNAs are cleaved by the microprocessor complex, formed by the ribonuclease enzyme Drosha (RNase III), and the RNA-binding protein DGCR8, also known as Pasha protein in non-human animals, resulting in shorter
precursor miRNAs (pre-miRNA), which are then transported to the cytoplasm by the GTP-binding nuclear protein complex (RanGTP) and the exportin enzyme (XPO5) (KIM; KIM; KIM, 2016; MAIA, 2020).

The pre-miRNA is cleaved in the cytoplasm by the ribonuclease enzyme Dicer, resulting in a mature miRNA with a length of 21 to 28 nucleotides and a double-stranded structure. From here, the Argonaut proteins that make up the RNA-Induced Silencing Complex (RISC) separate the two strands of the miRNA molecule. One strand, complementary to the target mRNA for silencing, remains in the RISC complex (guide strand), while the other is released and degraded. The guide strand in the RISC complex forms the so-called miRISC and induces the complex to bind to the target mRNA. The biogenesis process of siRNA is very similar to that of miRNA, however, the mechanism of action of these molecules differs slightly (JORGE et al., 2021; MAIA, 2020).

Once miRNAs are encoded by a different gene than the genes they will act on, they are called trans regulators. As a result, miRNAs do not have perfect complementarity with the target, and only the seed region of their sequence, which consists of 6 to 7 nucleotides, is complementary to the target mRNA (MAIA, 2020). Because of its lower complementarity, a miRNA can regulate numerous distinct mRNAs (REDDY, 2015). These molecules' mechanism of action is based on the binding of the miRISC complex to the untranslated region of the 3' end (3'UTR) of the target mRNA, which results in gene silencing. miRNAs can cleave the mRNA molecule (slicing), but because of their lower complementarity, they generally silence genes by interrupting the translation process, preventing recognition of the mRNA molecule by translation initiation factors, causing ribosomal subunit disassembly, or degrading newly synthesized peptides (JORGE et al., 2021; MAIA, 2020).

siRNAs, on the other hand, have complete complementarity with their target mRNA because they are usually generated from a transcribed gene and act in the regulation of these same genes, which is why they are referred to as cis regulators. Because siRNAs have high specificity for the target mRNA, when they bind to it, they induce its degradation, thereby inhibiting protein synthesis. Thus, RNAi mechanisms seek to preserve genomic integrity by regulating gene expression (BOBBIN; ROSSI, 2016; MAIA, 2020; TIAN et al., 2021;). Given this property, siRNA and miRNA have been widely investigated, aiming their use in the therapy of various types of diseases, including cancer (FRANÇA et al., 2010).
One serious challenge for the effective implementation of cancer treatment via RNAi, however, is the delivery of these molecules to the tissues where tumors are located. The difficulty is associated with transport across cell membranes and the easy degradation of this material, with delivery methods classified as viral or non-viral (XIN et al., 2017). Viral vectors have high delivery efficiency, particularly in terms of stability and resistance to high temperatures, with lentiviruses, adenoviruses, retroviruses, and adeno-associated viruses being the most commonly used vectors. However, because they are pathogenic agents, they increase the likelihood of an immune response due to their immunogenicity and cytotoxicity, in addition to the possibility of causing insertional mutagenesis (GOSWAMI et al., 2019; KANVINDE et al., 2022;). As a result, non-viral delivery systems have been investigated, highlighting the use of ionizable cationic lipids, which pack the siRNA into stable nanoparticles capable of translocating through cell membranes (KULKARNI et al., 2018), and the use of exosomes, which easily fuse to the plasma membrane without becoming trapped in endosomes (UDDIN et al., 2022).

3.2 APPLICATION OF RNAi IN CANCER TREATMENT

Overall, the use of RNAi as a strategy against cancer involves the silencing of genes involved in tumor apoptosis inhibition, the silencing of oncogenes, the inhibition of tumor angiogenesis factors, and the reduction of chemoresistance. In lung cancer, RNAi therapies are based on regulatory molecules of cellular pathways, such as cell proliferation, migration, and apoptosis, that effectively target and deliver therapeutic genes to cancer cells via nanocarriers and known biomarkers for this type of cancer (TIAN et al., 2021). Zhu et al. (2015) used hybrid lipid/polymer nanoparticles for siRNA delivery to silence the PHB1 gene, which is responsible for reducing apoptosis and chemotherapy resistance in lung cancer cells, so that its silencing significantly inhibited the growth of tumor cells. The potential of nanoparticles as a strategy for the treatment of stomach cancer and skin cancer has also been evidenced (ABREU et al., 2022; SOARES et al., 2022).

The successful expression of recombinant siRNA against the Nrf2 gene, responsible for inhibiting cellular oxidation pathways, resulted in the reduction of tumorigenic progression in osteosarcoma and even increased cellular sensitivity to the chemotherapy drugs doxorubicin, cisplatin, and sorafenib, indicating a promising strategy to overcome chemoresistance (LI et al., 2018). RNAi has shown a high potential for treatment in pancreatic cancer, which is very invasive, lethal, and drug-resistant, in
addition to reducing cancer cell resistance to radiotherapy and chemotherapy when combined with these. As a result, a miRNA-based therapeutic agent against pancreatic cancer has recently been developed (TIAN et al., 2021).

The use of RNAi in the treatment of other types of cancer, such as breast, colorectal, ovarian, gastric, and cervical cancer, has received a lot of attention (TIAN et al., 2021). The silencing of the mutant tumor suppressor gene p53 by RNA interference inhibited cell proliferation and viability in bladder cancer, resulting in cell cycle arrest in the G2 phase and inducing apoptosis, demonstrating that RNAi application can be a promising therapeutic strategy for the treatment of this type of cancer (ZHU et al., 2013).

Kase et al. (2021) demonstrated promising results in gene therapy for oral squamous cell carcinoma. The authors used exosomes from fibroblasts engineered to deliver siRNA to cancer cells in their experiment. As a result, the exosomes were stable and efficient at transferring specific siRNA to oral squamous cell carcinoma cells, resulting in a significant tumor suppressor effect both in vitro and in vivo, slowing cancer progression. Lin et al. (2022) also demonstrated the efficacy and low toxicity of exosome-mediated siRNA delivery for tumor-directed gene therapy.

In a study conducted by Carvalho (2021), it was possible to develop a device for screening breast, prostate, and cervical cancer that detects and quantifies miRNA, showing promise in screening patients and reducing costs in the healthcare field. RNAi-based therapies, on the other hand, are unstable and difficult to deliver to affected tissues. Nonetheless, the search for new methods, such as those based on plasmonic nanoparticles, has been critical for accurate and sensitive diagnosis, as well as having a powerful therapeutic effect on cancers (YOON et al., 2022). In breast cancer, Valle et al. (2020) demonstrated high efficiency in the delivery of siRNA for the silencing of the HOXB7 gene, whose overexpression is generally associated with resistance to treatment drugs such as tamoxifen. The use of RNAi in the fight against cancer has proven to be extremely promising, but its success as a therapeutic approach depends on efficient targeting and delivery.

4 FINAL CONSIDERATIONS

Although recent and facing some challenges, the use of RNAi in the diagnosis and treatment of cancer is extremely promising and advantageous, as such molecules, in addition to allowing the silencing of faulty genes, are also effective in reducing resistance to drugs used to combat the disease. As a result, while the success of using RNAi in the
fight against cancer is dependent on the efficiency with which they are delivered to the target tissue, research in this area has been advancing, bringing the possibility of more efficient therapies with fewer side effects closer and closer.
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