GENETIC INNOVATIONS FOR BREAST CANCER TREATMENT: A REVIEW OF GENE THERAPY STRATERIES AND FUTURE IMPLICATIONS

By

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A project submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Approval

The thesis titled "An Overview of Gene Therapy for Breast Cancer Treatment And Future Aspects "submitted by Sakib Hasan (18346078) has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

The study does not involve any kind of animal and human trail.

Abstract

Breast cancer is the widespread form of cancer & the second most frequent reason for female mortality on a global scale. It is resulted from various ecological and genetic elements. As a result, new therapeutic methods are needed to effectively treat these types of cancer. The conventional techniques for treating this type of cancer have limitations, so various researches have been conducted in the past decade to discover fresh strategies to address these challenges. Investigations into the molecular foundations of this illness have resulted in the advancement of genetic treatment as an effective choice for addressing and managing breast cancer. Before genetic material can be used to fix, add, or control a gene, it needs to be initially inserted into the desired cells using a carrier. The focus is on selectively attacking cancer cells without causing harm to normal cells, which is necessary for this approach. Clinical trials have additionally demonstrated that this method is comparatively safer than conventional medication. This research will investigate various aspects of breast cancer, techniques for gene treatment, the difficulties they present, and the latest discoveries in this field.

Keyword: Gene therapy, Clinical trial , tumor cell, genetic factors , anticancer therapy.

Dedication

I dedicate this project to my loving and supportive parents.

Acknowledgement

First and foremost, I would like to express my gratitude to the Almighty for his endless gifts, which have been given to me in an effort to provide me with the strength and determination to complete this project. It is my genuine pleasure to offer my heartfelt appreciation to my academic supervisor, Dr.Sabrina Sharmin (Assistant Professor at Brac University's Department of Pharmacy), for her invaluable guidance and encouragement during this research. Through the course of my education and project writing, she was a true source of advice and support for me. I am quite grateful to her for her valuable comments and ideas during my study, which helped me much in completing my project work in a timely manner.

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List of Acronyms

CAR-T cell therapy - Chimeric Antigen Receptor T-cell therapy

TIL therapy - Tumor-Infiltrating Lymphocyte therapy

TCR-T cell therapy - T-cell Receptor T-cell therapy

AAV - Adeno-Associated Virus

LV - Lentivirus

HD-Ad - Helper-Dependent Adenovirus

CRISPR-Cas9 - Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR associated protein 9

sgRNA - single-guide RNA

mRNA - messenger RNA

DSB - Double-Strand Breaks

HDR - Homology-Directed Repair

NHEJ - Non-Homologous End Joining

PD-L1 - Programmed Death-Ligand 1

CTLA-4 - Cytotoxic T-lymphocyte-associated protein 4

GvHD - Graft-versus-Host Disease (can be relevant in CAR-T therapy)

GTBC: Gene Therapy for Breast Cancer

HER2: Human Epidermal Growth Factor Receptor 2

ER: Estrogen Receptor

PR: Progesterone Receptor

BRCA: Breast Cancer gene

CAR-T: Chimeric Antigen Receptor T-cell Therapy

CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats

TALEN: Transcription Activator-Like Effector Nucleases

ZFN: Zinc Finger Nucleases

HSV: Herpes Simplex Virus

AAV: Adeno-Associated Virus

Chapter 01

Introduction

Cancer, a devastating disease that affects many people worldwide, has always been a difficult adversary for the medical field. Even though there has been significant advancement in standard cancer therapies such as chemotherapy, radiation, and surgical procedures, the battle against cancer continues to be complex and challenging. However, in recent times, a revolutionary approach has emerged, offering new hope and opportunities for considerable progress: genetic therapy. Gene therapy is a revolutionary technique in the battle against cancer, using hereditary information and the study of small-scale biological structures to combat this relentless disease. By directing attention towards and modifying specific genes or genetic pathways associated with the development and progress of cancer, gene therapy presents the possibility of customized and improved treatments that might bring about a significant transformation in the realm of cancer care. The word "cancer" is very broad. This phrase describes the sickness that happens when uncontrolled cell growth and replication are caused by biological changes. Cells undergo growth and replication at varying rates because of diverse types of cancer. Some forms of cancer show a noticeable sign called a tumor, while other diseases like leukemia do not have a visible sign known as a physical manifestation. (Golden, 2022) The majority of the cells in the body have been given instructions to carry out particular jobs and die off at predetermined time intervals. The death of cells is naturally beneficial and a natural process known as apoptosis, despite its unpleasant connotation. The body communicates with aging cells, instructing them to expire and create space for fresher, superior cells. Malignant cells lack the elements that instruct cancerous cells to stop multiplying and expire. As a result, they build up in the body, depriving healthy cells of necessary oxygen and nourishment. The capacity of the body to function effectively is impeded

by formations, decreased immunity, and further changes brought about by unhealthy cells. Once malignant cells develop in a specific area, there is a possibility that they may spread to nearby lymph nodes. These are clusters of cells that battle against illnesses present in every region of the body. Gene editing has the ability to rectify the unevenness of genes within a cell by removing the problematic segment of the gene and altering the DNA of the harmful growth. This form of gene therapy has the potential to repair the modification instead of attempting to eliminate it. In simple terms, gene replacement involves substituting a defective gene with a sound one. Using this form of gene therapy, the objective is to correct the genetic change instead of attempting to eradicate it. Gene addition involves the inclusion of extra genetic material into a cell to support another cell, usually a defense cell in the immune system, in fighting against the protein associated with the faulty gene. CAR T-cell serves as a prominent illustration of gene addition. Rather than inserting another duplicate of an existing gene, this form of gene therapy involves incorporating a completely fresh gene, typically with the purpose of triggering the immune system to eradicate the cancerous cell. Physicians possibly have the ability to directly introduce a novel gene into the cancerous cell which triggers the cancer cell to engage in apoptosis (self-destruction). Gene suppression simply deactivates the troublesome gene. The cell could either die because of this or be stopped from behaving in a cancerous way, like rapidly multiplying and growing. (Golden, lliance for Cancer Gene Therapy, 2022)

1.1 Cancer

One of the most feared illnesses of the twentieth century, cancer is one that continues to grow and is becoming more common. Every fourth individual has a lifelong risk of developing cancer because of the dire condition. (Roy & Saikia, 2016). The cancer is a broad collection of illnesses that can begin in practically any organ or tissue of the body. These illnesses are brought on if cells that are abnormal develop out of control, cross boundaries that are normal to infect parts of the body nearby as well as spread across organs. The latter process, known as

spreading, is a significant contributor to cancer-related mortality. The terms "neoplasm" & "malignant tumor" are also used to describe cancer. (World Health Organization, n.d.) With an anticipated 9.6 million fatalities, or one in every six deaths, from cancer in 2018, it is the second largest cause of death worldwide.

1.2 Classification of cancer

Our body is made up of many hundred billions of cells. Due to their potential small size, it is possible to most clearly observe the cells under a microscope. Organs and tissues in our body are composed of group able cells. They've got many things in common. These framing organs do perform a lot of different roles, though, thus they vary in many ways. For example, because the nerves and muscles serve a variety of purposes, their cells have unique structures. There are about 200 different types of cancer, and we may also classify them according to wherein the body's cellular growth begins, such as breast cancers or lung cancers. (Mingozzi, F., & High, K. A. (2013) Additionally, we can group malignancies entirely according to the type of cell they first appear in. There are five main groups. Below is a discussion of them:

Carcinoma: It begins in the skin or in the tissues that surround or border the organs inside the body. This kind of cancer develops in the epithelial layer of cells that lines the interior of the body's organs or the outer layers of the tissues in the body. Although epithelial cells are found all throughout the body, from the skin to the covering and lining of organs and internal passages like the gastrointestinal system, carcinomas, or malignancies of epithelial tissue, represent 80 to 90% of all cases of cancer. The breast, lungs, bladder, colon, & prostate are among the glands or organs that are most frequently affected by cancer.

Leukemia: It is a type of cancer that affects white blood cells. It begins inside the bone marrow and other blood-producing organs. These cancers are a subset of blood cancer. These cancers attack bone the bone marrow which produces blood cells. When the marrow in the bones turns

cancerous, it creates an excess of immature white blood cells that are unable to perform their jobs, putting the affected individual at danger of infections.

Lymphoma: Lymphatic systems tumors are known as lymphhocytic malignancies. While leukemias are "liquid tumors" that affect the blood, lymphomas are "strong malignancies." Along with the stomach, brain, and intestines, they can also affect certain lymph nodes. A type of lymphoma known as extranodal lymphomas develops outside of the lymph nodes.

Myeloma: These are produced by the plasma cells of the bone marrow. Myeloma. Plasma cells can generate a range of antibodies in response to infections. A type of cancer that affects the blood is myeloma.

1.2 Breast Cancer

Worldwide, women are frequently affected by the cancers known as breast cancer. Abnormal cells in the breast multiply and propagate without control, resulting in a formation. These cancerous cells can eventually infiltrate the tissues around them and disperse throughout the body via the blood or lymphatic systems.(Kolak et al. (2017) Typical methods for treating breast cancer include a surgical operation, chemical treatment, radiology, and therapy using hormones. Even though these techniques have shown considerable benefits in many cases, researchers are persistently aiming for more accurate and effective treatments. Gene therapy is a sophisticated technique in the field of medical research that provides optimism for treating various diseases, such as breast cancer.There are two primary methods for addressing breast cancer through genetic therapy.Tumor Suppressor Genes & Oncogene Targeting Genes. (Kolak et al. (2017)

1.3 Gene Therapy

In comparison to chemotherapy, which frequently lacks sensitivity and may result in generic harm, gene therapy involves replacing a damaged gene with a functional, healthy copy of that gene. Gene therapy is a potentially effective cancer treatment method. Several barriers, such as non-specific expression, low-efficiency the delivery process, and biological safety still stand in the way of clinic effectiveness after tremendous pre-clinical development with regard to both increased targeting and expression in a tumor-selective way. Gene expression can now be targeted to any particular cell or organs using advanced delivery technology. Using these developments, gene therapy is positioned to be used as a first-line treatment for neoplasia illnesses and potentially become a regular part of cancer treatment. Gene therapy is a sophisticated technique in the field of medical research that provides optimism for treating various diseases, such as breast cancer. Gene therapy's core concept is to alter cells' genetic material (also known as DNA or RNA) in order to replace or correct damaged genes that contribute to the development and spread of diseases. (Das et al.,2014) Concerning breast cancer, gene therapy aims to pinpoint specific genetic abnormalities that promote the growth and survival of tumors. There are two primary methods for addressing breast cancer through genetic therapy. Tumor Suppressor Genes & Oncogene Targeting Genes. (Das et al.,2014)

1.4 Aim of the project

To alter the appearance of a gene's output or modify the biological composition of a cell for medical intentions, gene therapy necessitates introducing external genetic material into the recipient's tissue. Even though the initial goal of gene therapy was to address inherited issues, it is now utilized to treat a broad variety of ailments that involve both acquired and inherited abnormalities. In the last thirty years, improvements in genome manipulation technology have enabled the application of genetic treatment to address and avert life-threatening illnesses. With careful positivity, scientists are progressing with the expectation that individuals with

complicated acquired diseases and genetic mutations will be provided with secure and effective treatment. Currently, more than 3000 genes have been linked to genetic mutations that result in illnesses, and there are currently 2600 experimental trials underway for gene therapy, aiming to address a wide range of medical conditions. The processes behind various genome-editing techniques, including CRISPR/Cas9, nucleases using zinc fingers and nucleases involving transcription activator-like actions. Physically (DNA bombardments, electroporation), chemical-based (cationic lipids, charged polymeric), and biologically (adenoviruses, adeno-associated viruses, the herpes simplex virus) techniques have been utilized means of delivering genes are discussed in this survey. In the final section of this research, gene therapy drugs currently on the market for treating breast cancer, including their brand names, approved uses, vectors, and gene therapy delivery modality.

1.5 Objectives of this study

The objective of this study are-

- To recognize how gene therapy works.

- To offer insight into the use of this in therapy strategies.

- To improve judgment while altering gene therapy for the treatment of breast cancer.

Chapter 02

Methodology

The first step was to gather 60 papers that may have been relevant from a number of sources, like PubMed, and Research Gate, Science Direct and different types of scientific journal. After that 30 most recent relevant articles were selected in order to obtain the most recent data. Each of these articles underwent a thorough assessment. The content was arranged and rewritten with the goal of highlighting the key passages. The references were attached using Mandalay and Scribbr library, and the review paper afterwards contained them. Finally, a bibliography containing all the sources already cited was created.

Chapter 03

Breast Cancer

Breast cancer arises from genetic changes that occur in healthy breast cells and cause uncontrolled growth and tumor development. These tumors can be divided into invasive and non-invasive (in situ) kinds. Ductal carcinoma in situ (DCIS), for example, is a non-invasive tumor that is contained within the milk ducts and has not spread to the surrounding tissues (Li et al., 2020). The risk of metastasis is increased by invasive tumors, such as invasive ductal carcinoma and invasive lobular carcinoma, which have invaded the surrounding tissues (Weigelt et al., 2010). The intricate interplay of genetic, hormonal, and environmental factors plays a role in the multifactorial etiology of breast cancer. A well-known risk factor is family history, especially for those who have BRCA1 or BRCA2 gene mutations (Kuchenbaecker et al., 2017). By increasing lifelong estrogen exposure, hormonal factors such early menarche and late menopause raise the risk of breast cancer (Key et al., 2019).

3.1 Breast cancer prevalence worldwide

Globally, 2.3 a million of women were diagnosed with breast cancer in 2020, and 685 thousand people lost their lives to the disease. Breast cancer is the most common cancer worldwide, with 7.8 million women alive at the end of 2020 having been diagnosed within the previous five years. As with other cancers, the risk of developing breast cancer rises with age, and it affects women of all ages worldwide after puberty. From the 1930s through the 1970s, although radical mastectomy was the only option for treating breast cancer, death rates remained relatively stable. Prevention programs for breast cancer, in conjunction with more extensive treatment strategies that included very successful medicinal medicines, led to a rise in survival rates beginning in the 1990s in many nations.

3.2 Pathogenesis of breast cancer

Natural inflammasome activation enables progress. Breast cancer cells produce sCD44 and WNT ligands into the microbial environment of tumors in the breast, stimulating the release of from macrophages. The ATP released by dying tumor cells and the TGF- produced by breast carcinoma cells both stimulate secretion from cells called dendritic cells, which in turn promotes synthesis in CD4+ T cells. In a nutshell, this helps breast cancer progress and spread.

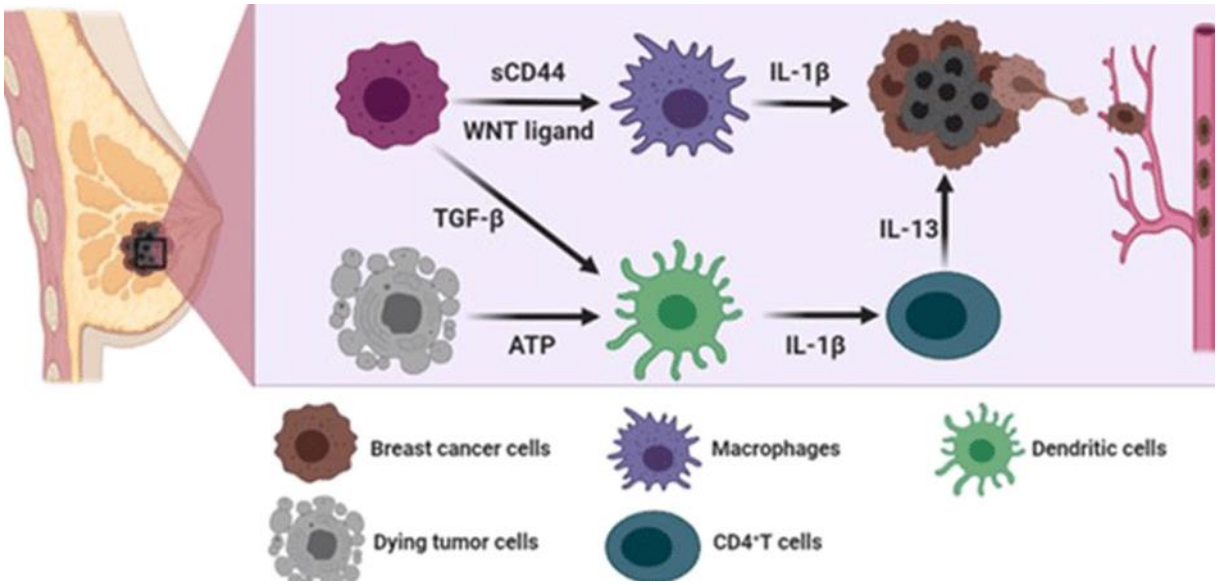

Fig: Mechanisms of breast cancer (Sun et al., 2019)

3.3 Modern breast cancer therapies

3.3.1 Surgery

Breast cancer treatment strategies are based on a number of factors, such as the cancer's subtype, its stage of development, and the patient's unique characteristics.The following drug categories are frequently used in the treatment of breast cancer:Removing the breast cancer (lumpectomy) is a common operation where the tumor and some healthy tissue are removed.A

lumpectomy may be recommended for smaller tumors, while larger tumors may require chemotherapy before surgery.Removing the entire breast (mastectomy) involves removing all breast tissue, including the nipple and areola.Skin-sparing mastectomy and nipple-sparing mastectomy are newer techniques to improve breast appearance. Lymph node dissection may be done in conjunction with surgical procedures like mastectomy and lumpectomies to remove tumors (Veronesi et al., 2010).

3.3.2 Chemotherapy

Chemotherapy which targets rapidly proliferating cancer cells all over the body, is crucial in the treatment of breast cancer. Chemotherapy is a systemic treatment strategy that employs potent medications to stop or kill cancer cells. Chemotherapy is used in the treatment of breast cancer at several phases, such as neo adjuvant therapy before surgery, adjuvant therapy after surgery, and in situations of advanced or metastatic disease. Chemotherapy medications function by interfering with several cell cycle stages, thereby preventing the growth and division of cancer cells that are rapidly proliferating. In order to give healthy cells time to recuperate in between treatments, these medications are frequently given in cycles. The kind, size, stage, and general health of the patient are all important considerations when selecting a chemotherapy plan. Systemic administration of cytotoxic drugs targets rapidly dividing cancer cells, reducing tumor size and preventing metastasis (Sparano et al., 2018).

3.3.3 Medicines used in targeted therapy

Targeted medical therapy are aimed directly at the defects that cause cancer in the first place. Most tumor cells in breast cancer overproduce a protein called human epidermal growth factor receptor 2 (HER2), which is the target of a variety of drugs used in targeted therapy. Breast cells with cancer benefit from the the protein's ability to proliferate and survive. By selectively targeting cells with elevated HER2 expression, the drugs can effectively kill cancer cells while

sparing healthy ones.Medicines used in particular treatments can kill cancer cells that have additional defects. Particular treatment is also a major area of research in the field of cancer. Trastuzumab, a type of targeted medication, has been shown to be successful in treating breast cancer that is HER2- positive by blocking off certain molecular pathways (Slamon et al., 2001).

3.3.4 Immunotherapy

Modern immunotherapeutic methods use the immune system to detect and destroy cancer cells.Cutting-edge immunotherapeutic drugs use the immune system to identify and kill cancer cells, and they are showing promise in some subtypes of breast cancer (Emens et al., 2020). Anti-HER2 monoclonal antibody (mAb) therapy is one passive immunotherapeutic strategy that has significantly enhanced the prognosis for breast tumors that over express HER2, which is Circuit block modifications and mAb treatment are two novel effective immunotherapeutic approaches. Research studies for checkpoint blockade modifiers have shown tremendous promise despite their lack of specificity. Tumors vaccines, a type of targeted, functional the immunotherapy procedure, can be employed on their own or alongside the mAb treatments discussed above. Although the topic of cancer immunology has shown a lot of early promise, more research is still needed due to the complexity of the relationship between the immune system of the host and the tumor as well as the enormous variety of prospective immunological responses. Emens (2018)

3.3.5 Hormone Therapy

Hormone therapy, also known as hormonal therapy, hormone treatment, or endocrine therapy, works by preventing the body from producing hormone or by dealing with the way hormones affect cancer cells in the breast. It slows or even completely prevents the development of hormonal-sensitive tumors. Hormone-insensitive tumors do not possess receptors for hormones and may not accept treatment with hormones. (Márton and Kiss,2000).Selective estrogen

receptor modulators (SERMs) and aromatase inhibitors are effective treatments for hormone receptor-positive malignancies in endocrine therapy (Jordan, 2017).Selective estrogen receptor modulators are medications that prevent hormones from adhering to cancer cells.

Chapter 04

Gene therapy

Cancer, a set of diverse diseases characterized by unchecked cell growth and proliferation, continues to pose a serious threat to global health. The effectiveness of traditional cancer therapies, such as surgery, chemotherapy, and radiation therapy, has significantly advanced. The drawbacks and possible side effects of these methods, however, highlight the demand for newer, more focused therapeutic approaches. Various strategies that target particular biological pathways involved in carcinogenesis are used by gene therapy to treat cancer. This novel strategy tries to either improve the immune system's capability to identify and destroy cancer cells or fix the genetic defects responsible for cancer growth. Gene therapy for the treatment of cancer treatment.

CAR T-cell treatment steps include: Chimeric antigens receptors cells are a promising new frontier in immunotherapy for cancer. It has achieved notable clinical effects in patients with leukemia or B-cell lymphoma. Eliminating T cells from the body is the first step in the CAR T-cell treatment procedure.

1.Look for a protein biomarker (an overexpressed protein) on cancer cells that have genetic damage.

2. Create a CAR RNA strand that has been programmed to look for the protein biomarker.

3. Connect the purified T cells to the chimeric antigen receptors.

4. Boost the quantity of newly created CAR T cells in a lab.

5. Replenish the body with the CAR T cells that have just been activated.

A process comparable to this is used for CAR NK-cell therapy. Chimeric antigen receptors are being developed by researchers to increase natural killer (NK) cells, a different kind of immune system white blood cell.

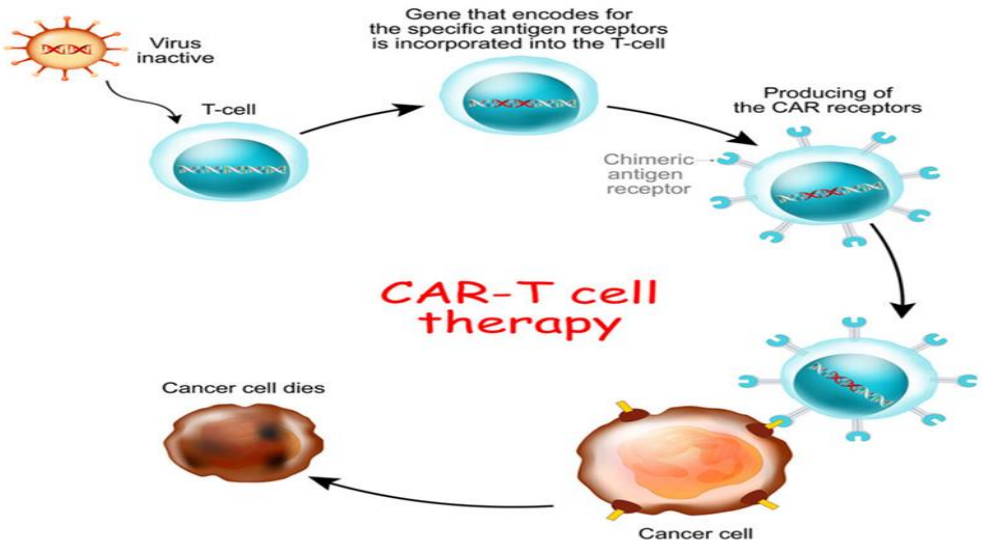

Fig: CAR-T cell therapy (Schoemaker et al. (2020)

4.1 Replacement of a gene

Cancer can occasionally develop as a result of deletions or mutations in vital genes that control cell proliferation and death. The goal of gene replacement therapy is to treat cancer by replacing a damaged or missing gene with a functional copy. Viral vectors and other methods of delivering genes can be used to accomplish this. For instance, it is possible to reactivate the tumor suppressor gene p53 in cancer cells in order to restore its function. This gene is essential for regulating cell division and inhibiting tumor growth (Yin et al., 2020).

4.2 Silencing of genes

In cancer cells, several genes are overexpressed, which aids in the survival and growth of the cells. The expression of these oncogenes is intended to be downregulated using gene silencing approaches like RNA interference (RNAi). Small interfering RNA (siRNA) molecules can be used to specifically target and degrade the mRNA of overexpressed genes in cancer cells, which inhibits the growth of tumors by reducing protein synthesis (Bouchie, 2013).

4.3 Immunomodulation

Gene therapy can strengthen the immune system's defenses against cancerous cells. Antibodies against PD-1 or CTLA-4 are examples of immune checkpoint inhibitors that can be designed to be produced by the patient's own cells at the tumor location. As a result, the immune system can generate a more potent anti-tumor response by blocking the inhibitory signals that cancer cells use to avoid immune identification (Gajewski et al., 2013).

4.4 Inhibitory Viro therapy

Oncolytic viruses are a class of genetically altered viruses that reproduce only inside cancer cells and kill them while leaving healthy cells unharmed. The expression of therapeutic genes by these viruses can be manipulated to increase their anti-tumor effects. For example, the virus may carry genes that cause the death of cancer cells or activate the body's immune system to attack the tumor. In addition to directly killing cancer cells, oncolytic viro therapy also causes the immune system to attack the tumor (Russell et al., 2012).

4.5 Gene modification

The precise editing of the genome is made possible by cutting-edge gene editing tools like CRISPR-Cas9. CRISPR-Cas9 can be used to target and silence genes necessary for tumor

survival or growth in the treatment of cancer. Additionally, it can be used to modify immune cells so they can detect and combat cancer cells more effectively. With ongoing studies and clinical trials, the promise of CRISPR-Cas9 in cancer gene therapy is still a developing field (Zhang & Wang, 2020).

4.6 Gene Delivery Using Viral Vectors

Gene delivery utilizing viral vectors has emerged as a potential area of research for efficient cancer treatment methods.Adenoviruses, adeno-associated viruses (AAVs), retroviruses, and lentiviruses are examples of viral vectors used to deliver genetic material to host cells.Adenoviral vectors exhibit effective gene transfer and can deliver tumor-suppressing genes or immunomodulatory drugs.(Montaño-Samaniego et al., 2020)AAVs provide long-term transgene expression and have low immunogenicity, making them useful for long-lasting therapeutic benefits.Retroviral and lentiviral vectors can incorporate their genetic information into the host genome, resulting in long-lasting therapeutic benefit.Early-phase trials using viral vector-mediated gene treatments for various cancer types have yielded encouraging results.Gene delivery using viral vectors continues to be an exciting area of research for precise cancer therapy. (Montaño-Samaniego et al., 2020)

4.7 Gene Delivery Using Non-Viral Vectors

It has been established that viruses are efficient vectors for transposing genetic material. Scientists have been forced to develop newer synthesized genome delivery systems due to issues like the rapid clearance of viral vectors from the bloodstream (when administered systemically) and the fact that they make people sick and have the propensity to create inflammation. There haven't been many successful methods for administering gene therapy. Non-viral vectors have not undergone as much evolutionary change as viruses, which they

transport. Figure 1 shows some of the agents used as delivery systems for nonverbal nucleic acid cargo as well as some of the methods created to enhance delivery. With advancements in nonverbal gene therapy techniques, there has been little success in translating promising research from laboratories treatment of breast cancer.

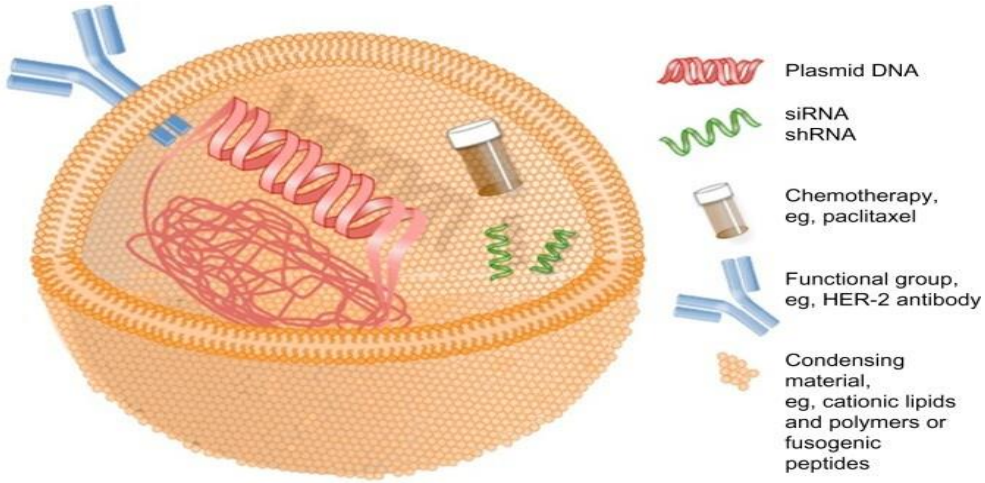

Fig: Simplified schematic of nonviral gene therapy delivery vehicle (Mc Crudden and McCarthy (2014)

Chapter 5

Gene therapy for breast cancer treatment

The prevalence and complexity of breast cancer continue to be major obstacles for the world's healthcare systems. The complexity and variety of breast cancer call for novel therapeutic methods despite significant advancements in early detection and traditional treatment modalities including surgery, chemotherapy, and radiation. A viable path forward for improving the accuracy and efficacy of breast cancer treatment is gene therapy, a cutting-edge and developing science. **(**Kolak et al. (2017)

5.1 Targeting Specific Genetic Alterations

Focusing on particular genetic changes is a hopeful method in treating breast cancer.The initial phase involves recognizing gene mutations or irregularities in a patient's breast cancer cells.Tumor suppressor genes help regulate cell development and prevent cancer, but changes can lead to unchecked cell multiplication.Oncogenes can lead to cancer if they are excessively active or altered. Gene treatment can hinder the production or function of cancer-causing genes.Targeted therapies like Trastuzumab and Pertuzumab focus on overproduced proteins like HER2.Blocking the PI3K/AKT pathway can prevent abnormal cell survival and proliferation.PARP blockers have shown potential in treating breast cancer with BRCA mutations.Immunotherapy can be effective in targeting breast cancer cells with certain genetic changes. (Tharmapalan et al. (2019)

5.2 Tumor-suppressing genes

One of the most common kinds of genes that halt the growth of cancerous cells is known as a tumor inhibitor gene.When there is a mutation in genes that suppress tumors, it is like the brakes

suddenly being pressed instead of the accelerator, causing an accelerated growth of cells.Restoring the activity of genes that suppress tumor growth using viral and non-viral carriers.Tumor-blocking genes comprise approximately 80% of the identified mutations associated with cancer.Oncolytic viruses (OVs) have displayed significant potential in the management of cancer.The application of the genetically modified herpes virus talimogene laherparepvec as a treatment for skin cancer called melanoma marked the initial occurrence of the triumph of OV-derived drugs.Several OV's are undergoing examination in clinical trials as potential therapies for cancer.(Arabi et al. (2022)

5.3 Personalized Medicine

Personalized medical treatments using gene therapy is a pioneering method in the treatment of breast cancer. (DeVita & Rosenberg, 2012)This approach aims to customize treatments for each patient according to their distinct genetic attributes. Gene therapy come together in the treatment of breast cancer. Personalized gene treatment starts with a thorough analysis of a patient's breast cancer cells. This requires examining the tumor's genetic material to detect particular changes, enhancements, removals, and other genetic modifications that stimulate tumor development. Genetic investigation assists in pinpointing prospective therapeutic objectives, like altered cancer-causing genes or deactivated tumor inhibitor genes. (DeVita & Rosenberg, 2012)

5.4 Using the CRISPR/Cas9 system to treat breast cancer

In both fundamental and applied studies of the biology of cancer, CRISPR/Cas9 has been extensively employed. In order to slow the spread of cancer by a number of ways, the technology can be used to target oncogenes & tumor suppressor genes (TSG). knocking out, modifying genes, suppression, and epigenetic modifications can all be used to produce the desired result. Non-homologous end-joining (NHEJ) or repair directed by homology (HDR)

routes are used in the CRISPR/Cas9 tool's editing of genes process. Double-strand break (DSBs) occur more frequently. They involve an unforeseen insertion or deletion of nucleotide bases at the point of breaking.

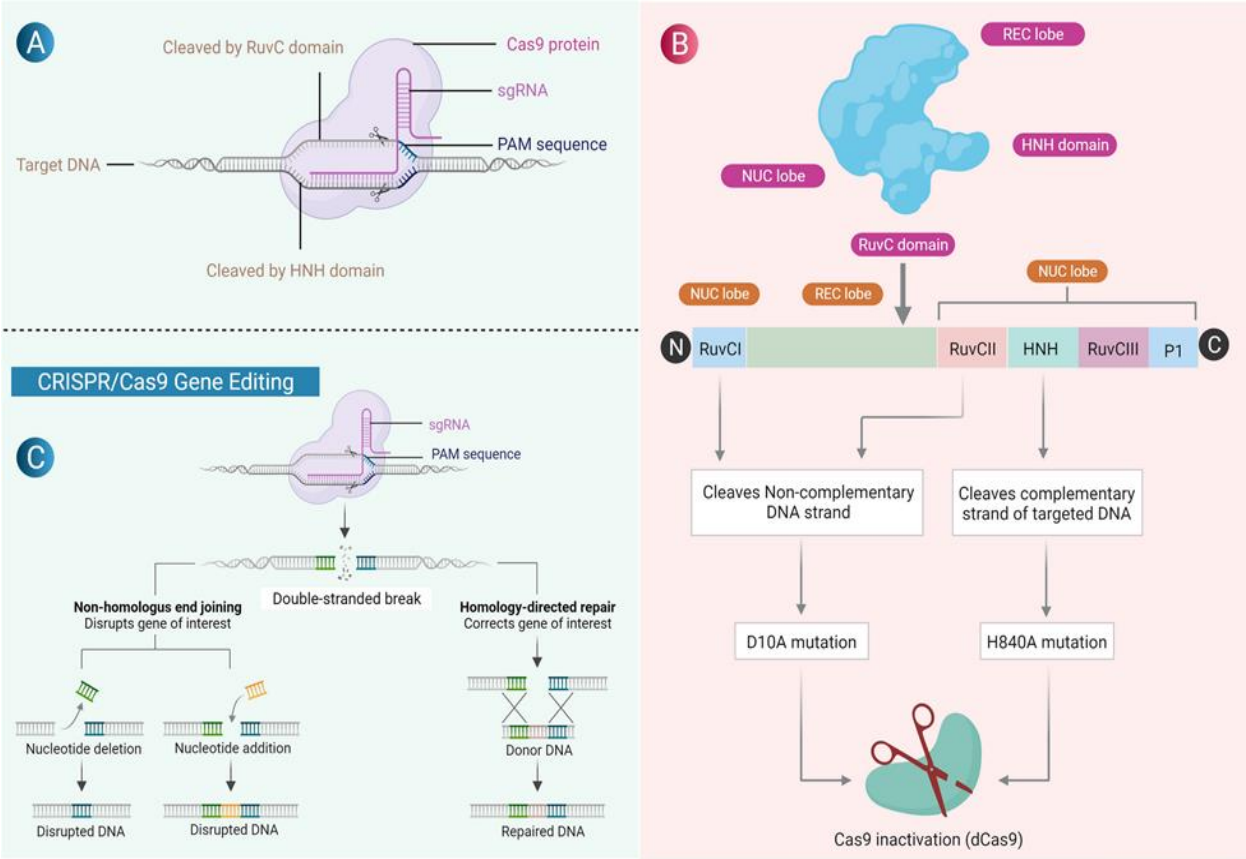

Fig: CRISPR/Cas9 system in breast cancer (sKarn et al. (2022)

A set of elements for CRISPR/Cas9: The targeted DNA sequence is cut by the Cas9 endonuclease, one guide RNA created by the combination of crRNA and tra-crRNA, and a protospacer adjacent motif (PAM) sequence that is necessary for Cas binding that exists in the targeted sequence of DNA. B The alpha & nuclease lobes of the protein Cas9 form a bi-lobed form. The HNH region and RuvC region of the nuclease lobes cut both the complementary and non-complementary strand of genetic material, correspondingly. Cas9 (dCas9) becomes inactive by mutations at D10A in the RuvC region and H840A in the HNH protein.

Chapter 06

Limitations & Future Opportunities

Gene therapy, a groundbreaking medical technique, has great potential in the battle against cancer. This process entails the insertion of genetic material into a patient's cells with the aim of fixing or substituting faulty genes. Consequently, this leads to specific and individualized therapy for cancer. Despite the potential it holds, gene therapy encounters multiple obstacles and restrictions that impede its extensive implementation. Difficulties in Gene Editing Advancing gene-editing methods, like CRISPR-Cas9, provide new opportunities for gene therapy in the treatment of cancer. However, guaranteeing the precision and security of genetic modification in a medical environment is intricate. Concerns are raised regarding the potential long-term effects of gene editing therapies due to off-target effects and unintended genetic modifications. Scientists are continuously striving to enhance the effectiveness and security of gene transportation systems. Improving viral and non-viral carriers will be essential for the effective application of gene therapy in the treatment of breast cancer. With advancements in our comprehension of breast cancer genetics, the utilization of gene therapy can enhance in terms of accuracy and focused approach. Identifying new healing targets and merging them with current treatments could result in improved results for patients.

Chapter 7

Clinical Trial

Clinical trials play a crucial role in assessing the safety and effectiveness of gene therapy for breast cancer and obtaining regulatory approval. As additional information is collected, governing bodies might authorize particular gene treatments for medical application, thereby enhancing their availability to patients. Gene therapy encounters difficulties such as possible unintended consequences on other targets, immune reactions, and restricted gene transport to specific tumor areas. Scientists are actively engaged in tackling these problems and enhancing gene therapy methods. To sum up, gene therapy for the treatment of breast cancer displays immense promise as a focused and individualized therapeutic alternative. Although it is still in the initial phases, continuous investigations, improvements in technology, and medical experiments show potential for a future where gene treatment plays a vital part in fighting against breast cancer and enhancing the results for patients. Worldwide Research Areas for Gene Therapy. (Cornetta et al., 2022)

Country	1994-1998	1999-2003	2004-2008	2009-2013	1994-2013
United States of America	133 (59.91)	309 (57.01)	281 (49.38)	258 (36.34)	981 (48.02)
People Republic China	0	19 (3.5)	88 (15.47)	225 (31.69)	332 (16.25)
Germany	21 (9.46)	42 (7.75)	45 (7.91)	50 (7.04)	158 (7.73)
Japan	10 (4.5)	50 (9.22)	47 (8.26)	37 (5.21)	144 (7.05)
Canada	20 (9)	36 (6.64)	19 (3.34)	32 (4.51)	107 (5.24)
Australia	2 (0.9)	3 (0.55)	5 (0.88)	14 (1.97)	24 (1.17)
France	8 (3.6)	28 (5.16)	29 (5.1)	16 (2.25)	81 (3.96)

Worldwide Publications Activity and the Parenthetical Share of the Top 8 Productive Nations in "Gene Therapies for Breast Cancer," 1994–2013 (Anaya-Ruiz1, 2015)

Chapter 8

Discussion and Finding

The field of cancer treatment has seen the emergence of gene therapy as a promising strategy with the potential to fundamentally alter how we treat this multifaceted and varied condition. Gene therapy provides a variety of cutting-edge methods to identify and destroy cancer cells while sparing healthy tissues by utilizing the power of genetic alteration. Gene transfer can introduce a range of molecular processes that, in theory, can stop tumor growth, gene therapy presents a potentially beneficial strategy for the treatment of breast cancer. However, for these strategies to be effective, a number of significant challenges must be solved. First, we don't fully comprehend the molecular processes that cause breast cancer. Therefore, a lack of knowledge about the basic molecular and cellular mechanisms may limit the effectiveness of medicines targeting the molecular events known to promote tumor growth. Second, because most breast cancers develop slowly, it may be challenging to employ some vectors, like retroviruses, to transfer genes into breast cancer tissues. Lastly, The frequently slow-growing nature of breast cancer cells may necessitate a longer duration of gene expression than would otherwise be necessary for those gene therapies that are not instantly lethal. The development of gene therapy methods for breast cancer has advanced significantly despite these obvious issues.

Chapter 9

Conclusion

When no other medications have been successful in treating a condition, gene therapy is a cutting-edge option. In the past 30 years, gene therapy has advanced significantly, with some treatments now being sold under license and others still undergoing clinical trials. Compared to chemotherapy, gene therapy has a lower risk of causing serious side effects in cancer patients. The selection of future candidates for gene therapy will take into account tumor genetic information as well as host humoral and cellular immunity. Thanks to recent advances in the creation of risk-free and effective gene delivery vectors and the comprehension of nuclease action, genome editing has the potential to be employed as a new treatment method for untreatable disorders like cancer. Adoptive immunotherapy, which employs both autologous and allogenic chimeric antigen receptor integrated T cells, has improved the safety and efficacy of gene therapy. Gene therapy will become more affordable to a bigger group of cancer patients as a result of increased biological research and the availability of less expensive gene vectors. (Kolak et al., 2017) As a result, the current trend in cancer treatment is towards personalized care that is tailored to each patient based on their genome, immune system, and tumor's genetic profile. It is anticipated that patients who receive gene therapy will recover faster and have a higher probability of being cured. As of November 2017, there were approximately 2,597 clinical studies that were in progress throughout numerous nations. As of August of last year, 22 gene therapies had received approval from several drug regulatory organizations. Since the 1980s, gene therapy has been largely embraced by the public and government agencies, and in recent years, it has grown in importance as a therapeutic option. In the future, gene therapy medications that use secure vectors and cutting-edge biological technologies will be more highly valued for both cancer prevention and therapy. The quick

development of cancer genetic therapy is encouraging for its potential inclusion in upcoming cancer treatments. Several prospective cancer vaccines are now undergoing research as a result of genetic engineering advancements. Combining current chemotherapy regimens with gene transfer technology for treatment of cancer may considerably help cancer patients. With significant advancements, oncolytic biotherapy is now being tested in treating both malignancies and precancerous diseases. Many of the previous obstacles to therapy are actively being removed, and second and third generation medications are currently being tested. Even if not every ongoing study yields a viable therapy medicine, there is a great deal of hope that these discoveries will contribute to making cancer a tolerable chronic illness without excruciating agony and mortality. Gene therapy needs to be closely monitored if we don't want it to be utilized for genetic enhancement and to support irrational prejudices regarding others. Even Nevertheless, the speed of translating the remarkable bench-generated information has not been as imagined. The clinical trials of gene treatments (viral and nonverbal) used to treat breast cancer patients described here were generally well-tolerated, with few significant adverse effects. The development of clinical trials of genetic treatments for women with breast cancer is expected to result from the ongoing generation of useful preclinical data and delivery vector improvement.

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