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A Statistical Analysis of CRISPR/Cas9 in Breast Cancer Treatments

Authors
Williams, Etain
Chiang, Lauren
Giang, Allie
et al.

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A Statistical Analysis of CRISPR/Cas9 in Breast Cancer Treatments

Lauren Chiang, Allie Giang, Amber Wei, Julia Zhang

Mentor: Etain Williams

Public Health and Health Sciences Undergraduate Lab at Berkeley

University of California, Berkeley

Contents

Abstract

Despite an increased understanding of the pathophysiology of breast cancer in recent years, the disease remains largely prevalent in the female population due to its disordered process. Breast cancer is known to affect 1 in 8 women and is the second leading cause of cancer deaths in women as well. Treatments are currently being administered mostly in surgical procedures, chemotherapy, and hormone therapy, but current research indicates that there are potential remedies that could alter the metastasis of breast cancer on the DNA level.

The usage of CRISPR(Clustered Regularly Interspaced Palindromic Repeats)/Cas9 within breast cancer treatments has grown in popularity immensely in the last decade. CRISPR/Cas9 is a system that utilizes a guide RNA to target and cut specific DNA sequences with the intention of genetically altering the genome. In cancer treatments, the knockout of specific genes utilizing the CRISPR/Cas9 system has proven variably successful in apoptosis by indirectly regulating tumor suppressor genes and causing gene knockouts. However, there are serious limitations and consequences that are being investigated within these therapies, as discussed in this paper.

Utilization of CRISPR-Cas9 with Nanolipogel Systems in Lipocalin 2 Knockout to Analyze Tumor Suppression

Introduction

CRISPR-Cas9, a technology utilized to edit genes, is revolutionizing various fields, including cancer research. CRISPR-Cas9 involves a synthetic guide RNA that breaks a double-stranded DNA at a precise location, allowing DNA to be replaced, inserted, or removed[1]. Breast cancer is the second most common type of cancer that causes death in women, and even though there has been improving progress in breast cancer research, there has been no cure found and patients experience harmful side effects with the current treatments[2]. One breast cancer subtype is triple-negative breast cancer, which represents 12% of all breast cancers and is where there is the loss of estrogen and progesterone receptors, and the loss of the human epidermal growth factor receptor 2[3]. By using CRISPR-Cas9 in breast cancer research, more solutions can be discovered to treat breast cancer. Lipocalin 2 is a gene that has been analyzed through the utilization of CRISPR-Cas9 and is a gene that is overexpressed in triple-negative breast cancer and promotes the progression of breast cancer[3].

Data Analysis

In the Lipocalin 2 research study, knocking out Lipocalin 2 using CRISPR-Cas9 showed significant suppression of tumor growth in triple negative breast cancer tumors[3]. There was a nanolipogel system made that targeted tumors, was able to be deformed, and was not cationic[3]. These nanolipogel systems consist of lipid bilayers and alginate hydrogel[3]. They then encapsulated 3 CRISPR plasmids, where each encodes a Cas9 nuclease and 20-nt guide RNA[3]. Each of these nucleases guide RNAs will work together to identify and disrupt Lipocalin 2 in the targeted triple-negative breast cancer cells found in humans[3]. This

occurs because the nanolipogel systems are able to identify and enter triple negative breast cancer cells over normal breast cancer cells, where they release the plasmids into the triple-negative breast cancer cells' cytosol[3]. After knocking out Lipocalin 2, this in vivo CRISPR genome editing efficiency is measured through its gene expression loss through utilizing qRT-PCR[3]. qRT-PCR, also known as Real-Time Quantitative Reverse Transcription PCR, utilizes PCR technology to measure the products during each PCR cycle[4]. The efficiency was about 81% for the triple-negative breast cancer-specific nanolipogel system with Lipocalin 2 CRISPR knockout plasmid, in reference to the sham group (phosphate-buffered saline-treated triple-negative breast cancer cells)[3]. In comparison, the efficiency was about 53% for the triple-negative breast cancer non-specific nanolipogel system with Lipocalin 2 CRISPR knockout plasmid, in reference to the sham group[3]. After in vivo genome editing, the treatment group's Lipocalin 2 protein levels significantly decreased compared to the sham group[3]. The Ki67-positive cell numbers in the triple-negative breast cancer tumors also reduced significantly due to the high efficiency of the Lipocalin 2 knockout[3]. Tumor volume was suppressed by 77% and tumor weight was suppressed by 69%[3].

Discussion

The p-value was less than 0.05 when testing the significance of the protein levels and Ki-67 positive cell numbers[3]. This means that the result is statistically significant and that any difference in the data is due to chance. The results of the protein levels between the treatment group and sham group correlate with the in vivo CRISPR genome editing efficiency[3]. The reduction of Ki67-positive cell numbers shows the triple-negative breast cancer-specific nanolipogel system with Lipocalin 2 CRISPR knockout plasmid effectively inhibiting triple-negative breast cancer cells' rapid growth[3]. This also shows that in the end, there was

tumor growth suppression. This methodology can be further explored by utilizing nanolipogel for in vivo CRISPR genome editing in other breast cancer tumors and evaluating its effect in suppressing tumor growth.

PTEN & RB1 Genes Targeted to in Breast Cancer Treatment with the Use of CRISPR/Cas9

Introduction

Breast cancer is the most common cancer among women and annually, 264,000 cases of breast cancer are diagnosed. With a disease that is the second leading cause of death in women, researchers have introduced a new technology within the realm of breast cancer treatment. CRISPR/Cas9 allowed targeted DNA to be mutated and allows repair in cells to happen naturally. To further understand the mechanism behind how these genes can be edited to prevent the development of breast cancer we will focus on the specific genes edited: PTEN and RB1.

Results

We have found that through CRISPR/Cas 9 we can mediated gene editing in human breast organoids was next used to mimic neoplasia[5]. In past studies, it has been shown since the genes PTEN and RB1 reflected a highly efficient process, they had been chosen to be mutated within breast cancer cells. These two genes provide promising results therefore, they should be able to help produce more accurate results in the study of the growth of cancer cells[5]. In healthy cancer-free beast cells, the proliferation was limited however, PTEN and RB1 mutated organoids showed increased proliferation causing them to grow and divide[5]. To further investigate, triple and quadruple mutations were tested[5]. The findings suggest that at least three tumor suppressor genes need to be inactivated to transform normal breast cells into cancerous cells[5]. The results of including the fourth gene enhance the frequency of cancer transformation.

Discussion

Although we were able to grasp insight into the critical drivers of breast cancer through CRISPR/Cas9 gene editing, there are still limitations that need to be addressed for future research. In the future, it would be helpful and beneficial to investigate the role of other tumor suppressor genes in breast cancer and identify combinations of mutations that are most effective in preventing the proliferation of cancerous cells[5]. By improving the efficiency and accuracy of our studies and developing new methods to study cancerous cells, we can make better use of genetic mutations to our advantage[5]. In addition, it may be worthwhile to explore other ways to use CRISPR/Cas9 to genetically modify cells, such as targeting oncogenes or hormones.

Other limitations include the risk of the relapse of cancer through editing the gene and additional toxicity introduced into the cells[6]. CRISPR/Cas9 is still a new system which has its imperfections however, through gene editing PTEN and RB1, it allows our knowledge of how this technology works to expand.

CRISPR/Cas9-Mediated Apoptosis

Introduction

Tumor suppressor genes normally halt the formation of tumors in the human body–P53, for example[7]. When mutations occur in genes such as P53, cancer cells may begin to form and spread as suppressors are weakened. In triple-negative breast cancer specifically, mutations in the P53 genes occur in 70-80% of cases[8,9]. To combat this, gene knockout can be mediated by CRISPR-Cas9 technology, which is cutting a specific gene in an organism's DNA, causing permanent loss of expression.

Mechanism

The two essential components of CRISPR Cas9 technology are Cas9 and gRNA. gRNA binds to a specific sequence in the DNA–meaning that it will solely bind to the target sequence. Cas9, an enzyme, then follows this guide and cuts across both strands of DNA. Naturally, a cell will then realize that the DNA is damaged and try to fix it[14]. This technology can be used for gene knockout. For example, cutting out the genes that downregulate tumor suppressor genes causes apoptosis of mutant cells. In p53, MDM2 and MDM4 are the downregulation of p53. Losing both of these genes could lead to apoptosis of TP53 cells as p53 genes are overexpressed[11]. CRISPR may also target CXCR7 and CXCR4, genes that mediate cancer cell development[12]. This could cause the knockout of MDA-MB231 cells, significantly suppressing triple-negative breast cancer by increasing their rate of apoptosis as it affects the functions of CXCL12, a protein-coding gene that induces migration of cells[13, 15].

Discussion

CRISPR Cas9 genome editing isn't perfect. It has its limitations as the method isn't fully developed. CRISPR has the potential to cause mutations that are carcinogenic as the human body repairs cut genes. It could also cause off-target alterations that would permanently damage DNA. Essentially, it has a significant lack of specificity that is filled with dangerous possibilities. Furthermore, the current delivery method of CRISPR also has the potential to be toxic. Even though there is potential for CRISPR-Cas9 technology to be used to mediate apoptosis of cancer cells, there is much work to be done on the practicality of the treatment[13].

Delivery Methods of CRISPR-Cas9 and Their Corresponding Off-target Effects

Introduction

With its straightforward nature and exceptional specificity across various life forms CRISPR/Cas9 has fundamentally changed genomic research; however apprehensions exist concerning off-target effects that accrue largely because of ineffective delivery procedures leading to unintended interactions with nontarget genes. This paper assesses two different modes of gene transport namely viral vectors and lipid nanoparticles along with their benefits and limitations while highlighting associated off-target costs.

Methods

CRISPR/Cas9 technology has transformed genetic research but key challenges remain such as concerns over undesirable consequences when modifications occur at unintended sites. These issues may arise from poor delivery methods leading to interaction with other nearby genomic sites not intentionally targeted. The advantages and limitations regarding CRISPR/Cas9 delivery using viral vectors versus lipid nanoparticles are discussed in this paper.[18] Off-target issues relating to both techniques are also evaluated through a classification system that distinguishes between specific or nonspecific events based on similarities between genomic sites involved or lack thereof respectively. Viral vector usage is widespread as it facilitates effective gene transfer processes efficiently.[19]

Discussion

These rewrites focus on keeping technical terms while making sure that sentences flow better based on reading level appropriateness for someone who has higher education. Viral

vectors are a highly efficient and flexible tool for delivering genetic material with various types available for different applications. However, they also have some limitations, such as the potential for an immune response and the risk of oncogenesis due to integration into the host genome. Additionally, viral vectors can induce off-target effects when used for CRISPR Cas9 delivery.

Whilst there are some limitations associated with the use of lipid nanoparticles - including instability in biological fluids, size restrictions, and limited cargo capacity- these vectors still possess useful properties for transporting the CRISPR Cas9 system into cells;[16] yet undue care must be taken to avoid off-target effects caused by nonspecific uptake or poorly characterized delivery mechanisms giving incomplete biodistribution resulting thereby. It's nonetheless important to recognize that both viral vectors- which can offer greater efficacy- and lipid nanoparticles- which excel at providing specificity while being far safer also possess inherent advantages alongside limitations as delivery vehicles for this gene editing technique.[17] Ultimately your choice of carrier hinges on weighing up the balance between efficacy and safety when it comes down to each unique application you're approaching; with viral vectors potentially being more ideal where maximal efficacy is sought after but conversely where specificity or safety is required then we should look towards Lipid nanoparticles- despite their drawbacks such as a limited capacity to carry CRISPR Cas9 and being less stable.

Nevertheless, lipid nanoparticles have demonstrated significant promise in terms of delivering the CRISPR Cas9 system with only minimal off-target effects. While viral vectors

offer improved transduction efficiencies in gene editing techniques they are also associated with higher off-target effects due to their ability for integration within a host genome.[19]

As such specific applications and corresponding delivery systems need to be considered when performing gene editing in order for it to be both safe and effective. To achieve greater safety along with efficacy outcomes researchers must focus on developing better delivery methods along with improving existing strategies even more to overcome limitations and meet challenges related to gene editing processes. With continued advancements in these areas, we can ensure broader adoption of CRISPR Cas9 technology across many different fields such as agriculture, medicine amongst others.

Conclusion

Breast cancer treatment research is a field that is being much further investigated in recent years, especially through the usage of CRISPR/Cas9 system. The specific upregulation and downregulation of certain genes have allowed for tumor-suppressing genes to undergo apoptosis, which could prove effective potentially in clinical trials once further perfected. CRISPR/Cas-9 is currently an important tool to edit genes, allowing more explorations to be made in modern-day research, including breast cancer research. Overall, the triple-negative breast cancer-specific nanolipogel system with Lipocalin 2 CRISPR knockout plasmid successfully suppresses tumor growth in triple-negative breast cancer[3]. This shows the revolutionary possibility of using in vivo CRISPR/Cas-9 genome editing with nanolipogel systems in not only other breast cancer research, but in all cancer research. Once off-target effects have been properly addressed, the realm of breast cancer treatments will expand

exponentially using this technology.

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