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Phage Oncology:
The Potential of Bacteriophages to Enable Targeted Therapies During the Treatment of Cancer

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Research examining the potential application of bacteriophages as a novel tool of medical oncology has expanded. The biological mechanism of phage display has prospectively enabled selective targeting of cancer tissues. The selectivity of viral bodies coupled with the variety of display proteins observed within viral populations, particularly amongst bacteriophages, has opened the door for the modification and utilization of the viruses for targeted cellular selection. The benefits of phage-based drugs for use in oncological medicine is potentially enormous and perhaps represent the answer to treating a variety of cancers. The research is ongoing and genetic interactions are complex. This paper will seek to provide an explanation of the biological mechanisms of phage targeting and their potential use as genetic modifiers in cancer cells.

Introduction

Viruses and the living genome have a long and complex history. To the benefit and detriment of cellular health, viral bodies have hijacked the genomic replication machinery of living organisms and inserted segments of their own genetic material to be replicated. In the process of the viral insertions the original genetic material is mutated and DNA sequences that previously encoded regulatory mechanisms within a cell can become dysfunctional. Typically, we observe viral bodies and the retroviruses that insert segments of their viral genome into our DNA as pathogens that potentially pose serious human health and epidemiological concerns, and for the most part this tends to be the case. Many retroviruses, including the two Lentivirus subspecies responsible for proliferation of the human immunodeficiency virus (HIV), utilize reverse transcriptase and integrase proteins to convert their RNA genome into DNA that can been inserted into a host's native genome. Retroviruses that cause genetic diseases like HIV are responsible for an immeasurable loss of human life. Furthermore, some retroviruses have been noted to insert mutated gene segments into host chromosomes that have the capacity to disrupt proper regulation of the cell cycle and can potentially lead to the development of cancer. These disruptive gene segments are termed "viral oncogenes" and were first observed by American microbiologists John Michael Bishop and Harold Varmus in the 1970s. However, the relationship between the genetic information of single and multicellular organisms and viral integration is more complex than simply a story of disease. If fact, there is likely a link between the integration of viral genomes and the progression of human evolution. A class of transposons in human genome that serve to regulate the movement of transposable genetic elements between human chromosomes and enable the genetic variations that are observed between parent and offspring operate in a molecular fashion that is not unlike the integration

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mechanisms observed in retroviruses (Arney, 2020). Additionally, researchers have understood that some classes of viruses have unique genetic mechanisms that act specifically to invade and ultimately destroy bacterial cells, making them potentially valuable as a biological medicine or antibiotic. For oncologists the use of phages in the treatment of cancerous diseases could potentially solve the dilemma of non-targeted therapies, allowing cancer cells to be targeted for destruction or arrest without harming healthy cells.

Phage Display

Phage display as a biological mechanism was first observed in the late 1980s by George Smith, a research biologist who had been studying modifications to the outer protein coats of bacteriophages while at the University of Missouri. Smith's original research publications noted that the insertion of a mer peptide into the filamentous phage gene III would lead to the phage's development of a protein fused to the filamentous extensions on the outside of its protein coat. Continued research found that the proteins displayed by the phages could be modified, reliably selected for, and presented in a manner that was immunologically accessible. For the value of his scientific efforts into genetic modification of phage display proteins and the potential for its use as a tool of research/medicine in 2018 Smith was awarded the Nobel Prize in Chemistry. In the decades since its original observation, researcher have greatly expanded on their use of phage display as a tool for the selection of unique protein presentations among large, varied populations of bacteriophage viral bodies, known as phage libraries. M13 phages, a type of filamentous bacteriophage that naturally infects E. Coli bacteria, are among the most researched phages for the purpose of generating a huge variety of peptide libraries and were the same type used in George Smith's original gene modification experiments. The M13 phages have a relatively simple RNA genome and a modifiable series of protein coat genes, making them an ideal viral body for research into the presentation of peptides that are selectively attracted to unique biomarkers. M13 phages have a series of nine genes that encode its protein coat; however, only the major coat protein gene pVIII and minor protein coat gene pIII are typically modified. Modification of the pVIII gene is ideal for the display of smaller proteins in larger quantities, whereas alteration of the pIII gene is an adept means for display of larger, more complex protein sequences but in fewer numbers. Practical application of phage gene modification generally follows along the same lines as George Smith's original research, wherein a ligand-encoding DNA segment is inserted into the designated phage coat protein encoding gene. Once the inserted DNA segment is expressed in the phage phenotype an exogenous display of the desired ligand can be observed as a fusion with the coat polypeptides (Jaroszewicz, et al., 2021). The value of these modifications to the phage peptide displays comes in their ability to be used for the purposes of selection. Once researchers have isolated a display protein that is attracted to the unique binding sequence of a chemical structure or cellular biomarker the selected phage can be easily amplified and reisolated by infecting a population of E. Coli.

Generation of clone viral bodies, each displaying a unique ligand, is an important process in the creation of new phage libraries. Cumulatively, each phage library has the potential to contain viral bodies that present billions of different peptides. Phage libraries are categorized by the modification to viral gene structure and their potential for biological effect. Phage peptide libraries are noted by the viral expression of peptide groups on the outside of their protein coats that have typically been selected for. Phage deletion libraries are comprised of varied viral bodies that can potentially effect or modify unique segments of host DNA; these types of libraries are particularly useful in genetics research of human CRISPR. Random libraries, as the name suggests, are made up of populations of bacteriophages that display a random selection of mutations. These are by no means the only categorize of useful phage libraries, and given the potential for modification and amplification of phage populations that possibilities for generation of new phage libraries are practically endless.

The screening methodology used by researchers to observe phage interactions and amplify phages that display peptides with the desired targeting to generate larger libraries is known as Biopanning. The experimental process requires multiple steps and potentially a series of repetitions. Biopanning begins with a microwell plate being covered with the desired target, potentially a purified protein, cultured cells, or tumor tissue that had been collected during a biopsy. The next step involves exposing the target-containing microwell plate to a phage library that contains a population uniquely modified phages with the potential that one or several of the phages may bind to the target. After washing, target-bound phages can then be observed. If the desired interactions are achieved target-bound phages can be enzymatically unbound and introduced into a bacterial colony for amplification (Bakhshinejad, et al., 2014). All in all, the relatively simple and repeatable process of Biopanning makes the process of isolating phages with selective properties efficient and affordable for researcher laboratories and drug-manufactures alike. The ease of phage modification, isolation and amplification has already yield positive results in the development novel antibiotic and antibody recognition drugs, and their potential for use in the field of oncology has only just begun to be explored.

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Targeted Cancer Therapy

The expansion and ease of Biopanning procedures has enabled researchers to isolate phage peptide presentations with a high degree of specificity towards a target of interest. Although phages libraries also have the potential to present and introduce antibodies into a target substrate with the same degree of specificity, more recent examinations have observed smaller peptide presentations as having a greater of tissue penetration and a higher likelihood of reaching intracellular targets (Almagro, et al., 2019). A pair of researchers investigating the affinity of select peptide displays to target malignant tumor tissues at Sun Yat-sen University in Guangzhou, China, observed positive results in whole target-cell capture by the phage peptides when tumor cells had been isolated from laboratory mice and studied in vitro. The team described the in vitro phage screenings as a high throughput approach that could identify multiple peptide displays that specifically bind to the target tumor cells; furthermore, random peptide screenings have the potential to reveal previously unidentified cell surface receptors present on the tumor cells (Saw & Song, 2019). The identification of these unique tumor biomarkers provides researchers the opportunity to examine cell receptor changes on the surface of mutated tumor cells compared to those observed in healthy, non-cancerous tissues. Disappointingly, examinations into specific binding of phage display peptides targeting tumor cells in vivo did not yield the same positive, replicable results as were observed in the Petri dish isolations. Although in vivo observations still showed an inclination for the introduced phages to isolate their intended tumor cell targets, the lack of a single target introduced the possibility for drug interference via non-specific binding. Additionally, in vivo experimentation has shown a downside to peptide half-life durations, meaning that the peptide-presenting phages must reach their intended target quickly if positive results are to be expected. Lastly, researchers have been limited in their ability to translate phage peptides that have been effective at targeting tumor cells in mice into humans because of present difference in cellular peptide binding between the species. Despite the need to overcome these scientific roadblocks, sustained research into the effectiveness of phage peptide libraries as a means to present nonimmunogenic specific-binding ligands that can penetrate tumor cells continues to yield promising results.

The value of phage peptide displays that bind specifically with unique biomarkers extends beyond their ability to identify novel extracellular binding sites on tumor cells. The effectiveness of peptide penetration into the intracellular regions of tumor cells makes the displaying phages an ideal means of delivery for targeted therapy drugs. Already, phage display peptides have their benefit in the delivery of imaging molecules into a target cell. Some pharmaceutical companies and biotherapeutic engineering firms have taken the usefulness of modified phage displays as a medium for the delivery of bioactive molecules a step further. Rather than using the phages a therapeutic agent themselves, drug development researchers have utilized the phage peptide specificity to facilitate the entry of gene carries and cytotoxic drugs (Bakhshinejad, et al., 2014). Presently, the majority of widely used cancer treatments attempt to eradicate cancerous cells by introducing non-selective drugs with cytotoxic effects. Problems with this methodology emerge because the introduction of non-targeted drugs into the entire organic system will inevitably damage healthy cells, leading to further health complication while not always succeeding in the elimination of cancer. The possibility for phage-based drugs to selectively target cancer cells in the delivery of cytotoxic agents without negatively impacting healthy erythrocytes or immunological cells would be a monumental development in the treatment of cancerous disease.

Genetic Vectors

The hunt for a means of cancer treatment that not only eliminates the continued proliferation of cancerous cells, but rather genetically corrects the mutations that overrode the regulatory mechanisms of cell growth/division that led to the initial development of cancer remains one of the most important yet complex areas of oncological research. Although the scientific understanding of the genetic disruptions that trigger cancer has increased dramatically over the past several decades, the majority of current cancer treatment methods ignore the genetic distinctions of cancer cells and simply act to destroy cells that are undergoing rapid division. The obvious problems with that lie in our current understanding that not all tumor cells, i.e., cancer stem cells, undergo rapid division; furthermore, functional cells that are present in developing biological systems naturally undertake accelerations in their division, this makes current treatments of childhood cancers particularly tricky. Hence there is a need for the development of novel therapy methods that address the genetic malformations at the root of cancer development. Bacteriophages, not unlike the retroviruses observed has human pathogens, act as genetic vectors in bacteria by hijacking their cellular replication machinery in order to generate new viral clones that can proliferate into other cells. The benefit of this to drug developmental researchers is that the phages still have the potential to act as genetic vectors in eukaryotic cells, but without the ability to produce new viral bodies. Already bacteriophages have been shown to be safe and effective for the treatment of bacterial infection in humans. Either alone or when coupled with antibiotics, the narrow spectrum of phage activity succeeds in elimination of a population of bacterial pathogens without causing harm to or further invasion of the host microbiome (Principi, et al., 2019). In the area of

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cancer treatment, uses of phage bodies to act as targeting mechanisms or genetic vectors for cancer cells likewise does not show signs of causing harm to healthy cells nor do the cancer cells enable to phages to replicate themselves. Overall investigations into the use of engineered phages as therapeutic agents have shown that they are overwhelmingly harmless and are relatively easy to administer.

In 2019 a group of researchers working at the Imperial College in London investigated the use of adeno-associated virus phages (AAVP) to deliver therapeutic genes at the site of glioblastoma brain tumors in laboratory mice. The research team had modified the phage outer coat protein genes to display a specific RDG4C ligand that would interact with an overexpressed αvβ3 integrin receptor, one of the primary causative mutations in the formation of the brain tumors. The binding of the phage presented ligand enabled the viral insertion of a corrective AAV genome that encoded a thymidine kinase (HSVtk) under a specific gene promoter (Grp78). When exposed to a combination of trimetazidine and ganciclovir therapeutic drugs the latter drug, GCV, could be integrated into the tumor cell genome. Subsequently, this gene integration inhibits tumor cell DNA polymerization and triggers cell death via apoptosis. The results of the phage mediated genetic modifications showed an 80% reduction in the mortality of the experimental group over a 60-day observation period, compared to the control group of mice who had all succumb to the cancer after 38 days (Przystal, et al., 2019). The profoundly positive results for the treatment of the glioblastoma tumors highlights the potential for application of phage genetic vectors to be used in the elimination of tumor forming cancers.

On a practical level, phage therapies offer one of the most promising research avenues towards the development cancer-curing drugs. Bacteriophage viruses are some of the most abundant microorganisms on the planet. They are found in nearly all soil samples and can exist in extreme conditions at varying temperatures and oxygen levels, making them ideal candidates for collection and laboratory modification. The stability of the phage structures makes it pragmatic for scientists to modify the virions that can been inserted into cancer cell genomes as part of targeted treatment strategy. The low cost of modification and synthesis of phage-based drugs can go a long way toward incentivizing pharmaceutical companies to pursue their development once the appropriate foreground research has been conducted. Ultimately, there isn't much standing in the way of bacteriophages being utilized as an effective treatment for cancer. As our scientific understanding of cancerous diseases continues to progress, the potential for one of earth's simplest microorganisms to be the solution to one of humanities' most complex health issues cannot be ignored.

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