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Viral Transduction for Targeted Delivery of CRISPR-Cas9 Gene Editing in Tumor Cells

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Abstract
The use of gene editing techniques such as CRISPR-Cas9 (the so-called "molecular cut-and-paste" system) to manipulate DNA and treat cancer represents one of the most promising areas of research. However, it is not currently applied in a widespread or generalized manner to directly eliminate tumors due to significant technical, safety, and biological complexity challenges.
A major difficulty lies in accurately directing this 'cut-and-paste' mechanism to every cancer cell. To effectively eradicate cancer, it would be necessary to edit the DNA of each and every tumor cell, including those that have spread throughout the body (metastases). Delivering CRISPR precisely to billions of diseased cells without affecting healthy cells remains an अत्य challenging task at present.
The objective of this work is to simplify antiviral tumor therapy and make it more accessible by proposing the deliberate and benign infection of the entire cancer-affected organ. This is achieved through viruses engineered using 'cut-and-paste' gene editing techniques. Through the mechanism of viral transduction, the DNA of all cells within the infected organ—both healthy and cancerous—is modified. The aim is to ensure that tumor cell DNA is also altered, specifically targeting and normalizing their uncontrolled proliferation mechanisms. At the conclusion of the process, the subject would experience only a mild, benign flu-like infection.

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1. Introduction

Viral infections, including influenza, are a common part of human life and involve processes in which host cells incorporate viral genetic material into affected tissues. This natural mechanism can be harnessed for therapeutic purposes, particularly in the control and regression of cancer cells.
The proposed approach consists of infecting a subject with a specific virus engineered using gene-editing techniques, designed to target the organ affected by cancer. Through this process, cells within the infected organ would incorporate the viral genetic payload. As a result, the DNA of both healthy and cancerous cells in the affected organ would be modified. In tumor cells, this modification is intended to disrupt and alter the intrinsic mechanisms responsible for uncontrolled proliferation encoded in their DNA.
By inducing a controlled and benign viral infection, it may be possible for cancer cells, following genetic modification, to regain regulation over their previously dysregulated proliferative mechanisms.

2. Current Status of Antitumor Therapies

The main known mechanisms of gene transfer include:
"Genetic transduction" is a process in which viruses (especially bacteriophages) transfer DNA from a donor bacterium to a recipient bacterium, facilitating horizontal gene transfer. It occurs when errors during the lytic or lysogenic cycle result in

bacterial DNA being packaged instead of viral DNA, thereby introducing new genetic material into the next infected cell.
"Viral transduction":
Viral transduction is a technique in which a modified virus is used as a vehicle to introduce a gene into a cell.
Currently, research is focused on personalized therapeutic vaccines, which help the immune system recognize and attack existing cancer cells. This approach is at an advanced stage of development and represents a rapidly expanding field.
mRNA vaccines provide the body with the instructions to produce a cancer-specific protein, enabling the immune system to learn to recognize and target it.
Among therapies involving viruses that directly attack tumors (oncolytic viruses), we find:
Oncolytic virus therapy is a cancer treatment that uses genetically modified viruses to infect and destroy cancer cells while minimizing damage to healthy cells.
How does it work?
The virus enters the tumor: a modified virus is introduced into the tumor or into the patient's body.
The virus infects cancer cells.

3. Limitations of Current Oncolytic Virus Therapies

They specifically target tumor cells as their primary objective.
They aim to destroy tumor cells through viral infection while attempting to avoid damage to healthy cells in the body.
They treat the virus as an invasive and destructive agent rather than as a potential collaborative therapeutic tool.
They rely on viruses to identify tumor cells in order to selectively attack them.
There is significant difficulty and complexity in identifying or engineering viruses that exclusively target tumor cells without affecting healthy cells.

4. Limitations of Molecular "Cut-and-Paste" Gene Editing

Risk of off-target effects

CRISPR may introduce unintended cuts in the DNA at undesired locations. If these molecular "scissors" alter healthy or critical genes (such as tumor suppressor genes), they could potentially induce new cancers rather than cure them.

Genetic instability of cancer

Cancer cells are highly heterogeneous; they continuously mutate and evolve to survive and resist treatments. A genetic modification that is effective at one point in time may become ineffective as the tumor evolves.

Unpredictable DNA repair mechanisms

When CRISPR induces a cut in the DNA, the cell attempts to repair it. However, this repair process can introduce new errors (insertions or deletions), potentially disrupting functional genes and leading to unwanted genomic rearrangements.

Complexity of cancer

Cancer is not a single disease but a collection of hundreds of distinct diseases with diverse genetic alterations. There is no single "DNA sequence" that can be universally targeted to eliminate all cancer types.

Primary limiting factor preventing widespread clinical application

The main challenge remains the efficient delivery of gene-editing tools to every individual tumor cell.

5. Tumor Cells as Self-Recognized Cells

Tumor cells are, in essence, cells of the host organism in which the mechanisms regulating cell proliferation have become impaired. As a result, the body continues to recognize them as self-cells. Targeting these cells may therefore imply acting against the body's own cells, whether healthy or tumorigenic.
Consequently, the immune system often fails to mount an effective response, as it does not readily identify tumor cells as foreign or harmful.

6. Proposed Solution

Gene editing technologies such as CRISPR are currently employed primarily in research and advanced therapies (e.g., CAR-T cell therapy), where immune cells are extracted from the patient, genetically modified ex vivo, and reinfused to target cancer cells. However, direct *in vivo* editing of tumor cells remains a major challenge due to issues of safety and precision.
It is essential to consider that tumors arise from cells whose DNA has been altered, leading to uncontrolled proliferation due to impaired regulatory mechanisms.
In this context, viruses should be regarded not solely as harmful agents, but as potential collaborators capable of delivering genetic material into infected cells.
By introducing genetic material into all infected cells, both healthy and cancerous, a genetically modified virus using gene-editing techniques could alter the genetic instructions of tumor cells, including those that govern uncontrolled proliferation.
Rather than focusing exclusively on identifying viruses that selectively target tumor cells while sparing healthy ones, an alternative approach would be to identify viruses capable of infecting the full affected organ. This strategy may offer a faster, more efficient, and potentially less costly pathway for therapeutic intervention.

7. Strategy

- Development of viral libraries targeting different cell types in human organs (e.g., respiratory epithelial cells, lung cells, epidermal cells, neuronal cells). In the presence of a tumor in a specific organ, the corresponding virus that affects that organ would be selected.
- Use of genetic engineering in these viruses through gene editing techniques to incorporate the desired DNA modifications into the cells of the affected organism.

8. Conclusion

Cancer cells are healthy cells in our body that have a malfunctioning replication control mechanism. Our immune system is unable to detect and attack tumor cells because it mistakes them for healthy, self-regulating cells. The therapy proposed here circumvents this autoimmune problem, allowing viruses treated with CRISPR and molecular "cut and paste" technology to target cancer cells, modifying their DNA so they regain their controlled replication mechanism and continue replicating normally,

resulting in tumor reduction and a cure. Meanwhile, the treated patient experiences only a mild flu-like illness.

Cancer cells are derived from normal host cells with disrupted regulatory mechanisms controlling their replication. The immune system often fails to detect and eliminate these cells because they are recognized as self.

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