IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

Engineered Vaccinia Virus Vaccine To Treat Multiple Hypoxic Condition Cancers

Oncolytic Vaccinia Virus Vaccine

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The development of targeted cancer therapies has revolutionized the treatment landscape, offering more precise and effective solutions with minimal side effects. This thesis explores the innovative approach of using a genetically modified vaccinia virus to treat hypoxic conditions in cancer. Hypoxic cancer cells are typically more resistant to conventional therapies and pose a significant challenge in oncology. By manipulating the DDRP (DNA-dependent RNA polymerase) region of the vaccinia virus and replacing it with the promoter region of the PHD2 (Prolyl Hydroxylase Domain-containing protein 2), a novel engineered virus capable of selectively targeting and killing hypoxic cancer cells is developed. Bioinformatics-driven methodology involved a series of computational tools and databases to identify and validate the genetic modifications necessary for this transformation. The PHD2 promoter, known for its role in the cellular response to hypoxia, directs the mutated virus to specifically recognize hypoxic environments typical of certain cancer cells. This specificity ensures that the engineered virus selectively infects and lyses cancer cells while sparing normal, healthy cells. The thesis results indicate a promising new direction for cancer treatment, offering a potential breakthrough in targeting hypoxia-driven tumor resistance. This research underscores the critical role of bioinformatics in the development of next-generation viral therapies for cancer and highlights the potential of genetically modified viruses as precision medicine tools.

I. INTRODUCTION

Vaccinia virus

Vaccinia virus (VACV) is a large, complex, enveloped DNA virus which belongs to the poxvirus family. It has a linear, double-stranded DNA genome approximately 190 kb in length, encoding approximately 250 genes. Vaccinia virus is a naturally oncolytic virus which was found to have a natural tropism for tumor cells due to its sensitivity to type I interferon. Moreover, Vaccinia virus entry does not have a specific receptor which makes it a potential candidate for the treatment of all tumor types. Vaccinia virus has many inherent characteristics that make it an ideal choice for oncolytic virotherapy, including the ability to infect a wide range of tumor types with efficient infection and gene expression, a large amount of foreign DNA to be incorporated and potent lytic activity, selectively target and infect cancer cells *in vivo*.

Historical background of vaccinia virus

The history of the vaccinia virus is that of smallpox, a serious illness characterized by the eruption of small pock like lesions throughout the skin and internal organs.

The variola virus causes smallpox and may have begun infecting humans approximately 10,000 years ago. The characteristic pockmarks on 8000-year-old mummies possibly indicate smallpox infection. Smallpox was known in China during the 11th century BC. The disease spread to Europe between the fifth and seventh centuries BC. Epidemics in Europe during the 17th and 18th centuries were associated with a 25%-30% mortality rate.

In 1967, the World Health Organization (WHO), in an unprecedented effort, targeted smallpox for eradication from the planet by the end of the 20th century. The WHO achieved this goal, with the last endemic case of smallpox reported in Somalia in 1977 and eradication declared in 1980. This effort was successful for several reasons, including the lack of any natural reservoir for variola virus and the ease of identifying infected individuals.

Modified versions of vaccinia virus have been developed for use as recombinant vaccines. These vectors are less pathogenic than vaccinia virus and may induce a less potent neutralizing antibody response, allowing multiple immunizations.

Oncolytic vaccinia virus

Vaccinia virus is no longer necessary to prevent smallpox in the general population, vaccinia is now used to generate live recombinant vaccines for the treatment of other illnesses. Vaccinia virus can accept as much as 25 kb of foreign DNA, making it useful for expressing large eukaryotic and prokaryotic genes.

Vaccinia virus is a mystery in virology. Whether vaccinia virus is the product of genetic recombination, a species derived from cowpox virus or variola virus by prolonged serial passage, or the living representative of a now extinct virus is unknown. Different strains of vaccinia virus were generated in different cities throughout Europe and Asia, complicating efforts to track the origins of the virus.

PHD2 and its promoter

The promoter region of the PHD2 gene (also known as EGLN1) contains hypoxia response elements (HREs) that are essential for the regulation of PHD2 expression under hypoxic conditions The PHD2 (Prolyl Hydroxylase Domain 2) gene, also known as EGLN1 (Egl-9 Family Hypoxia Inducible Factor 1), is a crucial regulator of cellular response to hypoxia (low oxygen levels). The transcription of PHD2 is regulated by various factors, including hypoxia-inducible factors (HIFs) and other transcription factors.

By controlling the stability of HIF, it influences a wide range of physiological processes, including angiogenesis, erythropoiesis, and metabolic adaptation. Understanding and manipulating this pathway has significant implications for treating various diseases, including cancer, anaemia, and cardiovascular disorders. It's important to note that the regulation of gene expression is complex and involves interactions between multiple regulatory elements, transcription factors, and signalling pathways. Therefore, the specific promoters and regulatory mechanisms controlling PHD2 expression in different tissues and physiological contexts may require further research for a comprehensive understanding.

Gene Name: EGLN1 (Egl-9 Family Hypoxia Inducible Factor 1)

Protein Product: Prolyl hydroxylase domain-containing protein 2 (PHD2)

Mutated vaccinia virus in Hypoxia-Cancer treatment

PHD2 protien in human cells is upregulated by HIFα. Under normoxia condition HIFα subunits degrades the prolyl hydroxylase enzymes (PHD2). HIFα in normoxia is continually produced and degrades. If PHD2 proteins are inhibited by any means, HIFα subunits are stabilized and are translocated in the nucleus. If any protien is not needed or misfolded it is destructed by Ubiquitin Proteosome Pathway. These HIFα subunits forms an active complex with HIF-1β (HIFα + HIF-1β). Now this complex is binds to hypoxia responsive elements (HREs). To induce the transcription process in the cell the major complex (HREs+HIFα + HIF-1β) binds to the promoter region of PHD2 called as EGLN1 gene. Hence after binding these complex PHD2 is upregulated in cell causing oxygen deficiency in the cell.

It is identified that when a cell has low level of oxygen it will continue to produce PHD2 protien.

The promoter region of PHD2 protien is placed in the DNA Dependent RNA Polymerase region of vaccinia virus by the process of gene manipulation, when virus enters the hypoxic-cancer cell it will get activated due to the activation of promoter region (EGLN1 gene) because of the presence of PHD2 in hypoxic cancer cell. As a result, virus will start its growth and development process ultimately killing that cancer cell.

Replication process, protein production and cytotoxicity of mutated vaccinia virus is not affected by hypoxic condition in the cell. For cell entry vaccinia virus are not dependent on any specific surface receptors as they are dependent to membrane fusion pathways.

Biggest challenge for cancer vaccine production is delivering antigen to antigen presenting cells in-vivo. As this mutated vaccinia virus will kill the cancer cells via a combined effect of necrosis and immunogenic apoptosis. This process leads to a strong inflammatory response that can overcome the immune suspension within the tumor microenvironment.

II. MATERIALS AND METHODS

GenBank

The GenBank database is designed to provide and encourage access within the scientific community to the most up-to-date and comprehensive DNA sequence information. Therefore, NCBI places no restrictions on the use or distribution of the GenBank data. However, some submitters may claim patent, copyright, or other intellectual property rights in all or a portion of the data they have submitted. NCBI is not in a position to assess the validity of such claims, and therefore cannot provide comment or unrestricted permission concerning the use, copying, or distribution of the information contained in GenBank.

GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences. GenBank is part of the International Nucleotide Sequence Database Collaboration, which comprises the DNA Data Bank of Japan (DDBJ), the European Nucleotide Archive (ENA), and GenBank at NCBI. These three organizations exchange data on a daily basis.

BLAST

The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance.

Promoter 2.0

Promoter2.0 predicts transcription start sites of vertebrate PolII promoters in DNA sequences. It has been developed as an evolution of simulated transcription factors that interact with sequences in promoter regions. It builds on principles that are common to neural networks and genetic algorithms. A new approach to the prediction of eukaryotic PolII promoters from DNA sequence takes advantage of a combination of elements similar to neural networks and genetic algorithms to recognize a set of discrete sub patterns with variable separation as one pattern: a promoter. The neural networks use as input a small window of DNA sequence, as well as the output of other neural networks. Through the use of genetic algorithms, the weights in the neural networks are optimized to discriminate maximally between promoters and non-promoters.

EXPASY-Translate Tool

The EXPASY-Translate tool is a web-based application provided by the Swiss Institute of Bioinformatics (SIB) that allows users to translate nucleotide sequences (DNA or RNA) into protein sequences. This tool is widely used in bioinformatics for analysing genetic data and understanding the protein products encoded by specific genes. Here's a detailed explanation of how the tool works and its features:

Applications: -

Gene Prediction: Helps in predicting the protein-coding regions within a genomic sequence.
Molecular Biology Research: Facilitates the study of gene expression and protein function.
Biotechnology: Used in designing and optimizing synthetic genes for protein production.

Protein Data Bank (PDB)

The **Protein Data Bank** (**PDB**) is a database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography, NMR spectroscopy, or, increasingly, cryo-electron microscopy, and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organisations (PDBe, PDBj, RCSB, and BMRB[1]). The PDB is overseen by an organization called the Worldwide Protein Data Bank.

EXPASY-ProtParam

ProtParam calculates the physio-chemical parameters of protien sequence such as the amino acid composition, the pl, the atomic composition, the extinction coefficient, etc. ProtScale computes and represents the profile produced by any amino acid scale on a selected protein.

Swiss Model

SWISS-MODEL is a web-based integrated service dedicated to protein structure homology modelling. It guides the user in building protein homology models at different levels of complexity. Building a homology model comprises four main steps:
(i) identification of structural template(s),
(ii) alignment of target sequence and template structure(s),
(iii) model-building, and
(iv) model quality evaluation.

These steps require specialised software and integrate up-to-date protein sequence and structure databases. Each of the above steps can be repeated interactively until a satisfying modelling result is achieved.

III. Result And Conclusion

Hypoxic conditions, characterized by low oxygen levels in tissues, can contribute to the development and progression of various types of cancer, such as Breast Cancer, Lung Cancer, Prostate Cancer, Colorectal Cancer, Pancreatic Cancer. PHD2 (a protien) can cause oxygen deficiency condition in the cell. HIFα factors function in hydroxylation of PHD2, which are degraded in hypoxic condition. If the Oncogenic virus (Recombinant Virus) is infected in cell it will detect the activation of PHD2 protien because of the promoter in its gene to that of PHD2.

As a result, it will activate the killing gene in virus and ultimately killing the cancer cell.

II. **Acknowledgment**

I place on record my indebtedness and profound sense of gratitude to all other faculty, staff members and lab technicians for their kind support.

In the end I would like to express my deep love and respect to my parents, family and friends for their encouraging support.

References

1. Bailar, John C., and Heather L. Gornik. "Cancer undefeated." *New England Journal of Medicine* 336.22 (1997): 1569-1574.

2. Cooper GM. The Cell: A Molecular Approach. 2nd edition. Sunderland (MA): Sinauer Associates; 2000. The Development and Causes of Cancer. Available from: https://www.ncbi.nlm.nih.gov/books/NBK9963/

3. Cancer Research UK. (2023, October 2023). Cancer. Retrieved from https://www.cancerresearchuk.org/about-cancer/what-is-cancer/stages-of-cancer

4. Open Government Data [OGD], Goverment of INDIA. (2024, May 2024). Cancer. Retrieved from https://data.gov.in/keywords/Cancer

5. Malkin AJ, McPherson A, Gershon PD. Structure of intracellular mature vaccinia virus visualized by in situ atomic force microscopy. J Virol. 2003 Jun;77(11):6332-40. doi: 10.1128/jvi.77.11.6332-6340.2003. PMID: 12743290; PMCID: PMC155008.

6. Jacobs BL, Langland JO, Kibler KV, Denzler KL, White SD, Holechek SA, Wong S, Huynh T, Baskin CR. Vaccinia virus vaccines: past, present and future. Antiviral Res. 2009 Oct;84(1):1-13. doi: 10.1016/j.antiviral.2009.06.006. Epub 2009 Jun 26. PMID: 19563829; PMCID: PMC2742674.

7. Al Yaghchi C, Zhang Z, Alusi G, Lemoine NR, Wang Y. Vaccinia virus, a promising new therapeutic agent for pancreatic cancer. Immunotherapy. 2015;7(12):1249-58. doi: 10.2217/imt.15.90. Epub 2015 Nov 23. PMID: 26595180; PMCID: PMC4976866.

8. Meneses AM, Wielockx B. PHD2: from hypoxia regulation to disease progression. Hypoxia (Auckl). 2016 Apr 11;4:53-67. doi: 10.2147/HP.S53576. PMID: 27800508; PMCID: PMC5085284.