ID: 779

Anticancer Viruses and Their In Vitro Applications: An Updated Study

Samra Mededovic[1*], Zeliha Selamoglu[2,3]

[1]*Heath care study, "Dzemal Bijedic" University of Mostar, Mostar, Bosnia and Herzegovina*
[2]*Department of Medical Biology, Medicine Faculty, Nigde Omer Halisdemir University, Nigde, Türkiye*
[3]*Department of Biology, Faculty of Sciences, Khoja Akhmet Yassawi International Kazakh-Turkish University, Turkestan, Kazakhstan*

Abstract

Oncolytic viruses have emerged as a transformative class of therapeutic agents in cancer research, offering selective targeting and destruction of malignant cells while sparing normal tissues. Advances in molecular virology, genetic engineering, and tumor immunology have significantly expanded the scope of anticancer viruses, particularly in controlled in vitro systems that enable mechanistic exploration and therapeutic optimization. This study provides an updated and comprehensive analysis of anticancer viruses, focusing on their mechanisms of action, engineering strategies, and in vitro applications. Emphasis is placed on viral platforms such as adenoviruses, herpes simplex viruses, reoviruses, and vaccinia viruses, as well as emerging approaches integrating immunotherapy and gene editing technologies. The review highlights both the promise and limitations of current methodologies and outlines future directions for translational cancer research.

Keywords: *Anti-cancer, Cancer, Therapy, Viruses*

Introduction

Cancer remains one of the leading causes of mortality worldwide, necessitating the continuous development of innovative therapeutic strategies that overcome the limitations of conventional treatments such as chemotherapy, radiotherapy, and surgery (Ali et al., 2025; Naqvi et al., 2025). In this context, oncolytic virotherapy has gained significant attention due to its dual capacity to directly lyse tumor cells and stimulate antitumor immune responses. Anticancer viruses, also referred to as oncolytic viruses (OVs), are either naturally occurring or genetically engineered viruses that preferentially infect and replicate within cancer cells. The concept of using viruses as anticancer agents dates back to early clinical observations in the mid-20th century, where viral infections were occasionally associated with tumor regression. However, the lack of molecular understanding and safety concerns limited their early development. With the advent of recombinant DNA technology and improved understanding of tumor biology, oncolytic virotherapy has re-emerged as a promising therapeutic modality. In vitro studies have played a crucial role in elucidating the mechanisms underlying viral selectivity, replication, and cytotoxicity, thereby providing the foundation for clinical translation (Fukuhara et al., 2016; Twumasi-Boateng et al., 2018).

Mechanisms of Action of Anticancer Viruses

Anticancer viruses exert their therapeutic effects through multiple interconnected mechanisms, which are often studied extensively in vitro to dissect their molecular basis. One of the primary mechanisms is selective viral replication within tumor cells. Cancer cells frequently exhibit defects in antiviral defense pathways, such as interferon signaling, which render them more susceptible to viral infection. This selective permissiveness allows viruses to replicate efficiently within malignant cells, leading to cell lysis and the release of progeny virions that can infect neighboring tumor cells. In addition to direct oncolysis, anticancer viruses induce immunogenic cell death characterized by the release of tumor-associated antigens, damage-associated molecular patterns (DAMPs), and pro-inflammatory cytokines. These molecules activate innate and adaptive immune responses, transforming the tumor microenvironment from immunosuppressive to immunostimulatory. In vitro co-culture systems involving cancer cells and immune cells have been instrumental in demonstrating these immunomodulatory effects. Furthermore, many engineered oncolytic viruses are designed to express therapeutic transgenes, such as cytokines, immune checkpoint inhibitors, or prodrug-converting enzymes. These modifications enhance the antitumor efficacy of viral therapy and enable combination strategies with other treatments. In vitro models provide a controlled environment to evaluate the expression and function of these transgenes, as well as their impact on cancer cell viability and immune activation (Chiocca and Rabkin, 2014; Fukuhara et al., 2016).

Types of Anticancer Viruses

A wide range of viral platforms have been explored for oncolytic therapy, each with unique biological properties and advantages. Adenoviruses are among the most extensively studied oncolytic viruses due to their well-characterized genome and ease of genetic manipulation. Modified adenoviruses can selectively replicate in tumor cells and have been engineered to express various therapeutic genes. Herpes simplex virus type 1 (HSV-1) represents another prominent platform, particularly due to its large genome, which allows for the insertion of

multiple transgenes. The FDA-approved oncolytic virus talimogene laherparepvec (T-VEC) is based on HSV-1 and has demonstrated clinical efficacy in melanoma treatment. In vitro studies of HSV-based vectors have revealed their ability to induce robust cytolytic and immunogenic responses. Reoviruses are naturally occurring viruses that preferentially replicate in cancer cells with activated Ras signaling pathways. Their inherent tumor selectivity makes them attractive candidates for oncolytic therapy without extensive genetic modification. Similarly, vaccinia viruses offer advantages such as rapid replication and high transgene capacity, making them suitable for combination therapies. Emerging viral platforms, including measles virus, vesicular stomatitis virus (VSV), and Newcastle disease virus, are also gaining attention due to their unique targeting mechanisms and immunogenic properties. In vitro investigations of these viruses have provided valuable insights into their replication kinetics, cytotoxic effects, and interactions with cancer cells (Kaufman et al., 2015; Ribas and Dummer, 2017; Marelli, et al., 2018).

In Vitro Applications of Anticancer Viruses

In vitro systems play a central role in the development and optimization of anticancer viruses. Traditional two-dimensional (2D) cell culture models have been widely used to assess viral infectivity, replication, and cytotoxicity. These models allow for high-throughput screening of viral constructs and facilitate the study of molecular pathways involved in viral oncolysis. However, 2D cultures often fail to capture the complexity of the tumor microenvironment. To address this limitation, three-dimensional (3D) culture systems, including spheroids and organoids, have been developed. These models better mimic the structural and functional characteristics of tumors, providing more physiologically relevant insights into viral behavior. Co-culture systems incorporating immune cells have further advanced the study of oncolytic viruses by enabling the investigation of immune-mediated effects. These systems are particularly valuable for evaluating the immunostimulatory properties of engineered viruses and their potential synergy with immunotherapies. Recent advancements in microfluidic technologies and organ-on-a-chip platforms have introduced novel in vitro models that replicate tissue architecture and fluid dynamics. These systems allow for precise control of experimental conditions and enable the study of virus-host interactions in a more realistic context (Russell et al., 2012; Lichty et al., 2014).

Genetic Engineering and Optimization Strategies

The success of anticancer viruses largely depends on their ability to selectively target tumor cells while minimizing toxicity to normal tissues. Genetic engineering plays a crucial role in achieving this balance. Strategies such as gene deletion, promoter modification, and transgene insertion are commonly employed to enhance tumor specificity and therapeutic efficacy. For example, deletion of viral genes required for replication in normal cells but dispensable in cancer cells can improve selectivity. Similarly, the use of tumor-specific promoters ensures that viral gene expression is restricted to malignant cells. Advances in genome editing technologies, including CRISPR/Cas systems, have further expanded the possibilities for precise viral engineering. In vitro studies are essential for validating these modifications, as they allow for detailed analysis of viral replication, gene expression, and cytotoxicity. These studies also facilitate the identification of potential safety concerns and off-target effects (Ribas and Dummer, 2017; Twumasi-Boateng et al., 2018). Thus, it was synthesized the most recent advances in molecular virology, immunogen design, and biotechnology that will propel the next generation of prevention and treatment tools. We begin with the genomic and structural characteristics of high-consequence zoonotic viruses, highlighting the molecular determinants for virulence, host switching, and immune evasion (Ebadi et al., 2025).

Conclusion

The future of anticancer virotherapy lies in the integration of multidisciplinary approaches, combining virology, immunology, bioengineering, and computational modeling. Personalized virotherapy, tailored to the genetic and immunological profile of individual patients, represents a promising direction. The combination of oncolytic viruses with immune checkpoint inhibitors, targeted therapies, and conventional treatments is expected to enhance therapeutic outcomes. Advances in artificial intelligence and machine learning will further facilitate the design and optimization of viral constructs. Despite significant progress, several challenges hinder the widespread application of anticancer viruses. One major limitation is the discrepancy between in vitro and in vivo results. While in vitro models provide valuable mechanistic insights, they often fail to fully replicate the complexity of tumor biology and host immune responses. Another challenge is the development of antiviral immunity, which can limit the efficacy of repeated viral administration. Additionally, the heterogeneity of tumors poses a significant obstacle, as different cancer types and even individual tumors may respond differently to viral therapy.

Anticancer viruses represent a rapidly evolving field with significant potential to transform cancer therapy. In vitro applications have been instrumental in advancing our understanding of viral mechanisms and optimizing therapeutic strategies. Despite existing challenges, continued research and innovation are expected to unlock new opportunities for the clinical application of oncolytic viruses. Safety concerns, including the risk of unintended

viral spread and toxicity, also need to be carefully addressed. These challenges underscore the importance of developing more sophisticated in vitro models and integrating them with in vivo studies.

References

Ali, A., Naeem, M. Y., Selamoglu, Z., & Naqvi, M. R. (2025). Exploring Quantum in Cancer Biology: A Comprehensive Review of Nontrivial Quantum Events. *Archives of Razi Institute*, *80*(2), 295.

Chiocca, E. A., & Rabkin, S. D. (2014). Oncolytic viruses and their application to cancer immunotherapy. *Cancer Immunology Research*, 2(4), 295–300.

Ebadi, A. G., Selamoglu, Z., Issa, H. Y., Arici, E. C. A., & Abbas, S. (2025). Next-generation vaccines and antiviral platforms: Molecular advancements in the struggle against emerging zoonotic and viral diseases. *Archives of Razi Institute*, *80*(3), 555.

Fukuhara, H., Ino, Y., & Todo, T. (2016). Oncolytic virus therapy: A new era of cancer treatment. *Cancer Science*, 107(10), 1373–1379.

Kaufman, H. L., Kohlhapp, F. J., & Zloza, A. (2015). Oncolytic viruses: A new class of immunotherapy drugs. *Nature Reviews Drug Discovery*, 14(9), 642–662.

Lichty, B. D., Breitbach, C. J., Stojdl, D. F., & Bell, J. C. (2014). Going viral with cancer immunotherapy. *Nature Reviews Cancer*, 14(8), 559–567.

Marelli, G., Howells, A., Lemoine, N. R., & Wang, Y. (2018). Oncolytic viral therapy and the immune system: A double-edged sword. *Frontiers in Immunology*, 9, 866.

Naqvi, M. R., Abbas, S., Abbas, M., Batool, A., Mansoor, G., Bashir, S., ... & Selamoglu, Z. (2025). Immune boosters and immunotherapy in allergic diseases and in cancer management. *Archives of Razi Institute*, *80*(1), 271.

Ribas, A., & Dummer, R. (2017). Oncolytic virotherapy for melanoma and other cancers. *Nature Medicine*, 23(6), 645–650.

Russell, S. J., Peng, K. W., & Bell, J. C. (2012). Oncolytic virotherapy. *Nature Biotechnology*, 30(7), 658–670.

Twumasi-Boateng, K. et al. (2018). Oncolytic viruses as engineering platforms for combination immunotherapy. *Nature Reviews Cancer*, 18, 419–432.