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Oncolytic Viruses

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Edited by

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Preface

Anecdotal clinical reports of tumor remissions after viral infections laid the foundation for the field of oncolytic virotherapy. Advances in molecular virology, tumor biology, and immunology have enabled more refined studies of tumor-selective viruses. Concomitant with the resurgence of cancer immunotherapy and after the approval of Talimogene laherparepvec by the FDA and EMA, oncolytic virotherapy has gained unprecedented momentum. The field has flourished in recent years, yielding many notable preclinical studies and clinical trials. This book aims to provide a guide for basic virologists, translational researchers, and clinician scientists in the field by providing reference protocols from vector development to clinical translation.

The initial chapter provides an introductory review of the field, followed by a series of chapters describing virus modifications to enhance tumor specificity and anti-tumor efficacy. Reflecting the increasing interest in immunotherapeutic effects of oncolysis, a number of chapters address different strategies for immunomodulation and immunomonitoring. The third section of the book covers methodologies for different model systems to study oncolytic viruses, including mouse tumor models, patient-derived samples, and also mathematical modeling.

A number of virus platforms and approaches are represented, providing a survey of state-of-the-art methods for study of this unique treatment approach. Therefore, I would like to take this opportunity to thank all authors who have made this possible with their contributions. Hopefully this book will serve the research community as a useful resource to further enhance progress in the field of oncolytic virotherapy.

Heidelberg, Germany *Christine E. Engeland*

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Chapter 1

Introduction to Oncolytic Virotherapy

Christine E. Engeland and John C. Bell

Abstract

Oncolytic viruses exploit key hallmarks of cancer for replication in malignant cells, leading to tumor cell lysis, modulation of the tumor microenvironment and in situ vaccination effects. Diverse virus platforms have been developed as oncolytic vectors and designed for improved tumor specificity, intratumoral spread, therapeutic gene delivery and especially as targeted cancer immunotherapeutics. This chapter provides a concise overview of the basic principles as well as current progress in preclinical and clinical studies of oncolytic virotherapy.

Key words Oncolytic viruses, Viral vectors, Cancer immunotherapy, Tumor targeting, Cancer gene therapy

1 Principles of Oncolytic Virotherapy: Exploiting Hallmarks of Cancer and Turning Cold Tumors Hot

Treating cancer patients with replicating viruses may seem an outrageous idea—which was actually inspired by clinical observations of tumor remissions after natural virus infections [1]. Indeed, these experiments of nature were followed up by clinicians and researchers, who deduced the following principles of oncolytic virotherapy (Fig. 1):

On a cellular level, viruses with oncolytic properties show tumor-selective infection, replication, and spread—supported by inherent characteristics of cancer cells, the "hallmarks of cancer." As such, cancer cells show many properties conducive to viral replication including sustained proliferation, resistance to apoptosis, and immune evasion [2, 3]. Malignant transformation can include upregulation of viral entry receptors (e.g., CD46, a complement regulator) and proliferative signaling pathways usurped by viruses (e.g., Wnt/ß-Catenin and EGFR) as well as downregulation of antiproliferative and antiviral signaling (especially interferon) [4].

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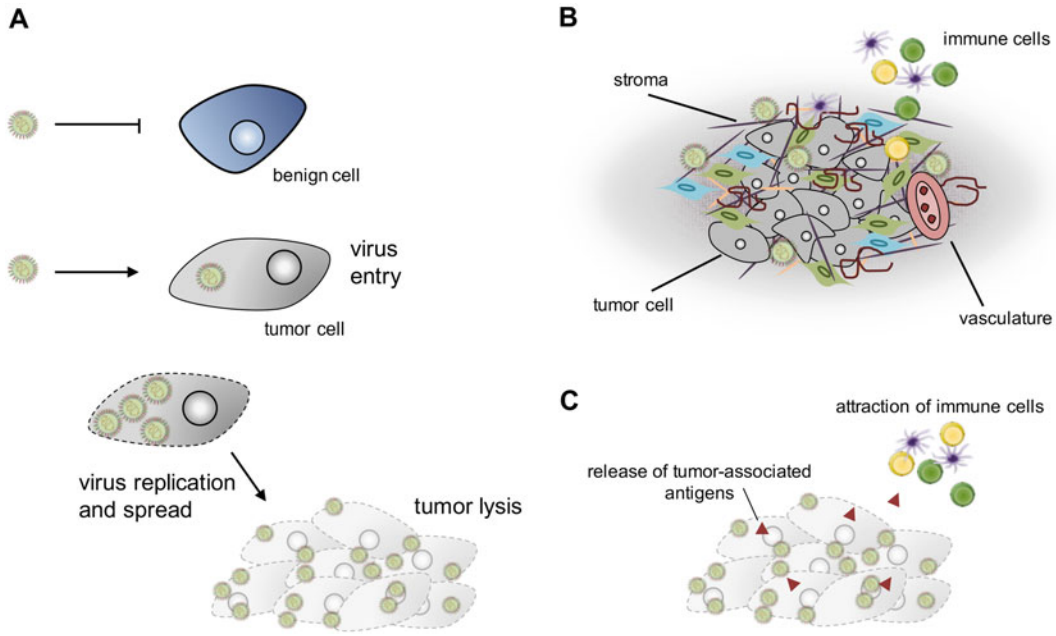

Fig. 1 Principles of oncolytic virotherapy. (**a**) Oncolytic viruses replicate selectively in malignant cells. (**b**) Oncolysis reshapes the tumor microenvironment. (**c**) Exposure of tumor antigens in the context of oncolysis can elicit tumor vaccination effects

A tumor comprises not only individual malignant cells but a complex microenvironment composed of stroma, vasculature, and leukocytes, typically characterized by immunosuppression. Oncolytic virotherapy can act to reshape the local milieu. An acute viral infection serves as a potent stimulus for the immune system. Local inflammation, innate immune activation, and danger signals (DAMPs and PAMPs) arise during viral replication which can change the immune contexture, thereby "turning cold tumors hot" [5].

During oncolysis, tumor-associated antigens are released in this context, which provides adjuvants for induction of adaptive antitumor immune responses. Thus, on a systemic level, oncolytic virotherapy can act as an in situ tumor vaccine, inducing therapeutic and protective antitumor immunity [6].

Preclinical and clinical data have provided proof of these principles. However, the role and contribution of these mechanisms of action to efficacy of oncolytic virotherapy has been a subject of debate. Moreover, this may depend on the specific oncolytic vector and the therapeutic setting.

2 Oncolytic Vector Platforms: From Adeno to Zika

These principle mechanisms of action outlined above are common to a diverse set of viruses which have been developed as oncolytic vector platforms (Fig. 2). These include the following:

- Small (e.g., parvovirus, approximately 25 nm and 5 kb), large (Vaccinia virus, 300 nm and 200 kb).
- Enveloped (herpes) and nonenveloped (PVSRIPO, derived from polio).
- DNA (adeno), RNA positive (Coxsackie) and negative (Maraba), and double-stranded (reovirus) RNA viruses as well as retroviruses (Toca 511, derived from amphotropic murine leukemia virus).
- Human (mumps), animal (Newcastle disease, vesicular stomatitis, myxoma).
- Pathogenic (influenza, Zika) and live-attenuated (measles) viruses.

These diverse viruses have been tested in preclinical studies and many have advanced to clinical trials. Overall, the clinical data have

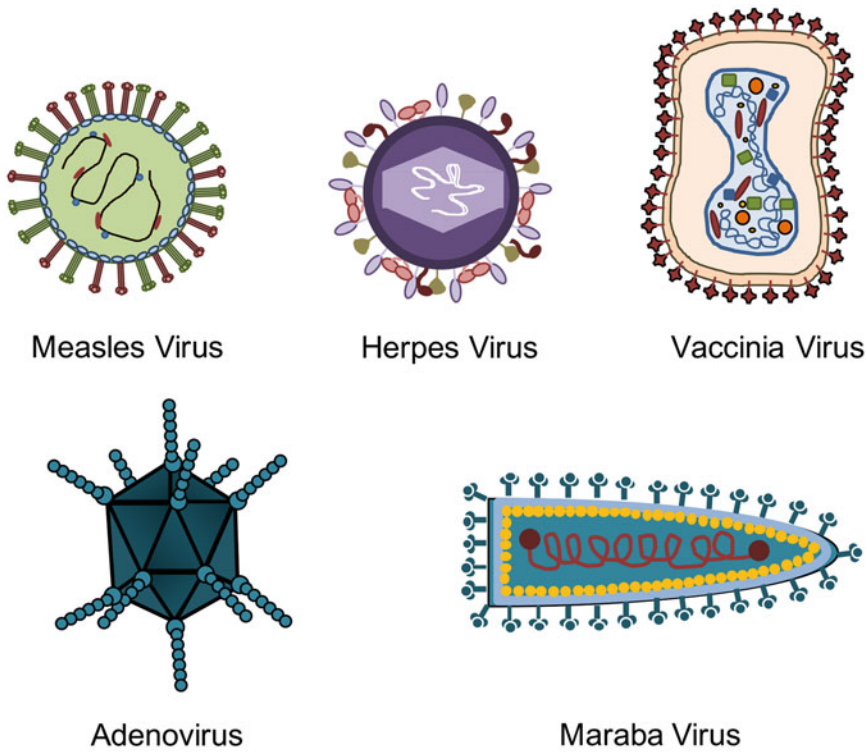

Fig. 2 Schematic depictions of five representative oncolytic viruses

demonstrated safety and typically mild, often flu-like symptoms as adverse events as well as some promising results in terms of anti-tumor efficacy [7]. While the adenovirus Oncorine H101 has been licensed for treatment of nasopharyngeal cancer in China since 2005, 2015 marked the approval of the herpes virus talimogene laherparepvec for treatment of advanced melanoma in the USA and Europe. Thus, the paradigm of using replicating viral vectors for cancer treatment has entered clinical practice.

To date, systematic head-to-head comparisons of these diverse viruses have not been performed. Viruses which have evolved a specific tissue tropism, conceivably, may be especially adapted to replicate in tumors originating from these tissues. In addition to the range of naturally occurring oncolytic viruses, the possibilities opened by genetic engineering offer a plethora of treatment options with vectors designed for specific therapeutic purposes.

3 Vector Design: Tumor Targeting and Spread, Tracers, Therapeutic Genes

Progress in molecular biology including the development of reverse genetics systems has enabled the design of oncolytic therapeutics with improved properties (Fig. 3) [8]. Main arenas of vector design include *tumor targeting* to increase specificity, which can be achieved on the entry level by modifying receptor tropism or incorporating matrix metalloproteinase cleavage sites into viral surface proteins. Targeting on the post-entry level can be achieved by placing viral genes under transcriptional control of a tumor-specific promoter, inserting target sites for microRNAs with differential expression in healthy and malignant cells or deletion of virulence

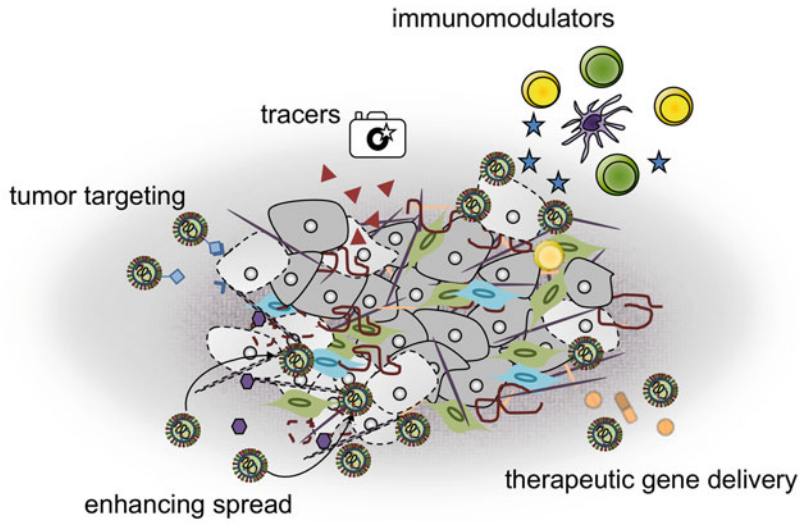

Fig. 3 Strategies to improve efficacy of oncolytic viruses by rational vector design