Review

Virus chimeras for gene therapy, vaccination, and oncolysis: adenoviruses and beyond

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Several challenges need to be addressed when developing viruses for clinical applications in gene therapy, vaccination, or viral oncolysis, including specific and efficient target cell transduction, virus delivery via the blood stream, and evasion of pre-existing immunity. With rising frequency, these goals are tackled by generating chimeric viruses containing nucleic acid fragments or proteins from two or more different viruses, thus combining different beneficial features of the parental viruses. These chimeras have boosted the development of virus-based treatment regimens for major inherited and acquired diseases, including cancer. Using adenoviruses as the paradigm and prominent examples from other virus families, we review the technological and functional advances in therapeutic virus chimera development and recent successful applications that can pave the way for future therapies.

Viral chimerism as a tool to generate effective virus-based therapies

Virus chimeras contain genetic and/or structural components from different virus serotypes, species, or families. They can be generated either by exchanging genetic regulatory sequences, whole coding sequences for proteins or parts thereof, or by inserting the entire genome of small viruses into those of larger viruses. Chimeric virus generation has been used in basic research to contribute to our understanding of viral life cycles, virulence factors, and transformation mechanisms (see [1] for discussion and references). Current efforts also encourage the use of viral chimerism in translational research as a tool to address the ample challenges in this field.

Viruses are widely used to repair gene defects or transfer therapeutic genes (gene therapy), to generate modern vaccines, or to develop replicative therapies for cancer (virotherapy/oncolysis; Figure 1a–c). All of these approaches rely on efficient and specific delivery of the virus to a certain target cell or tissue for safe and potent application. Clearly, this *in vivo* targeting is not solely influenced by receptor usage in the tissue itself. Blood components, such as coagulation factors and pre-existing antibodies, affect viral biodistribution after systemic

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Glossary

AAV: adeno-associated virus, a helper-dependent parvovirus.
Ad: adenovirus, a family of nonenveloped, icosahedral viruses with a double-stranded DNA genome. Their nomenclature first denotes the host species (B for bovine, C for canine, H for human, O for ovine, and S for simian adenoviruses) and includes a serial number for each serotype infecting the respective species (e.g. HAdV-5 for human adenovirus serotype 5). An exception to this rule in this review is the abbreviation Ad5FB4 describing a HAdV-5-based vector with a chimeric fiber composed of a HAdV-2 tail and BAdV-4 shaft and knob.
Adenovirus early regions: E1A encodes the first proteins to be expressed during adenoviral infection and is essential for viral replication. E2B encodes the viral DNA polymerase and pre-terminal protein. E3 encodes proteins mostly involved in modulation of host response to viral infection, and E4 encodes various proteins involved in viral gene expression, replication, and interaction with host cell components.
Arming: introduction of a coding sequence into the genome of an oncolytic virus. The encoded RNA or protein mediates enhanced killing of the infected cell or bystander killing of neighboring cells in addition to viral oncolysis.
B7 proteins: a family of proteins providing T cell co-stimulatory or co-inhibitory signals. B7 proteins are overexpressed on tumor cells mediating immunoevasion and suppression of T cell-mediated immunity by binding to inhibitory receptors.
C4BP: C4b-binding protein, a part of the complement system. It is an inhibitor of the complement factor C4 in the classical and lectin pathways of complement activation.
CAD: pyrimidine biosynthesis enzyme combining three enzymatic functions: (i) carbamyl phosphate synthetase (EC 6.3.5.5); (ii) aspartate transcarbamylase (EC 2.1.2.3); (iii) dihydroorotase (EC 3.5.2.3). It directly controls pyrimidine synthesis, thus being essential for cell proliferation.
Capsid chimerism: umbrella term for chimerism and exchange of capsid proteins, as in the case of adenoviruses fiber, hexon, and/or penton proteins.
Chimeric virus: a virus containing nucleic acid fragments or proteins from two or more different viruses. Strictly, each of the nucleic acid fragments contain genes essential for replication, but here the term is more generally used to describe the fusion of genomic fragments from two viruses independent of the function encoded by these fragments. Proteins can be part of the virus without being encoded in the virus genome when complemented during virus production.
Complex chimera: a chimeric virus with genomic fragments or proteins from more than two parental viruses.
Detargeting: ablation of natural viral tropism.
eIF4: eukaryotic translation initiation factor 4. This protein binds to both the 40S subunit of the ribosome and the methionine tRNA and recruits them to the 5′ cap of an mRNA. It then supports translation initiation by promoting ribosomal scanning, start codon recognition, and recruitment of the 60S ribosome subunit.
Fiber: one of the major adenovirus capsid proteins. It comprises an N-terminal tail domain for incorporation into the viral capsid, a shaft domain, and a knob domain mediating homotrimerization and attachment to the host cell. After attachment, virus internalization is triggered by binding of the viral penton base to integrins.
Fiber/hexon chimerism: parts of the respective protein are exchanged with those of a different adenovirus serotype or virus.
Fiber/hexon exchange: the whole protein is exchanged with that of a different adenovirus serotype.
FIX: coagulation factor IX, a serine protease (EC 3.4.21.22).
FX: coagulation factor X, also called prothrombinase (EC 3.4.21.6).
Gene therapy: insertion, alteration, or removal of genes to repair a gene defect responsible for disease or to introduce a therapeutic activity (e.g. direct or

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indirect cytotoxicity in cancer treatment). It can make use of mostly nonreplicative viral vectors, or of physical or chemical methods.
H1-PV: autonomously replicating, rodent parvovirus H1. This virus is under investigation as an oncolytic agent.
Hexon: the main structural protein of the adenovirus capsid.
Hybrid: strictly speaking, a heterozygous organism with two alleles from two different organisms. As viruses are monoallelic, the term is used here for a virus with genome parts originating from two different organisms (c.f. chimeric virus).
HVRs: hypervariable regions, domains of the adenovirus hexon protein that differ greatly between the different serotypes. They represent the major immunogenic epitopes of adenoviruses.
IRES: internal ribosomal entry site, a sequence within an mRNA that allows translation initiation independent of a 5′-terminal mRNA cap.
Morbillivirus: a genus of paramyxoviruses. Prominent members include measles virus, canine distemper virus, and rinderpest virus.
Mosaic adenovirus: an adenovirus with two different types of fibers.
Neutralizing antibody: an antibody that prevents a cell from being infected by an infectious agent; binding to a virus is not necessarily equivalent to neutralization.
Oncolysis: destruction of a tumor by an oncolytic virus that specifically infects and replicates within the tumor. Viral spread is associated with host cell lysis and, hence, tumor cell killing. At the same time, the therapeutic agent is amplified.
Orthotopic transplant: a transplant of a living cell, tissue, or organ located at the same anatomical position as the native tissue or organ.
PKR: protein kinase R. This protein is induced by interferon and activated by double-stranded RNA. It plays an important role in innate immunity against viral infections as it inhibits translation of both cellular and viral proteins.
Pseudotype: virus or viral vector with membranous envelope containing envelope proteins of another virus. Classically, the foreign proteins are not encoded in the viral genome, although the term is often used independent of coding status.
pTP: pre-terminal protein of adenovirus functioning as a protein primer for viral DNA replication.
PVS: poliovirus type 1 vaccine strain Sabin.
Retargeting: redirection of viral tropism to a new host cell type or to a new receptor that is not infected or utilized naturally. It is commonly used in combination with detargeting.
Seroprevalence: fraction of people in a population whose blood is tested positive for previous exposition to a particular pathogen or immunogen.
Serotype: subspecies of an infectious agent classified based on its surface antigens.
TRAIL: tumor necrosis factor-related apoptosis-inducing ligand.
Tropism: defines an organism, tissue, or cell type that supports replication of a particular virus. It is influenced by host cell surface molecules permitting viral attachment and entry and the presence of host factors necessary for viral replication.
Virotherapy: administration of a virus for infectious tumor treatment (c.f. oncolysis).
Virus strain: a genetic variant or subtype of a virus.
VSV: vesicular stomatitis virus. Its surface glycoprotein is abbreviated as VSV-G.
Xenograft: living cells, tissue, or organ transplanted from one species to another.
Xenotype: a species of an infectious agent that infects nonhumans.

application (Figure 1d). In the case of oncolytic viruses, the environment in the target cell/tissue contributes to their local replication potential. These challenges are addressed by a growing number of virus chimeras, and several are being prepared for or have recently proceeded to clinical trials in humans to treat or protect against detrimental diseases.

Here, we review how recent progress in virus chimera technology offers solutions for major medical needs in gene therapy, vaccination, and viral oncolysis according to the challenges being addressed by virus engineering, complemented by figures depicting the technical approaches. In particular, we will discuss (i) improving transduction specificity and efficacy by exchanging entry-mediating proteins on the viral surface; (ii) evasion of pre-existing immunity by interchanging major neutralizing determinants; (iii) changes to biodistribution after systemic application by modifying the viral interaction with various blood factors other than antibodies; (iv) replication regulation after substitution of regulatory sequences; and (v) the use of viral chimerism for delivering heterologous virus/vector genomes. Advances in virus chimera technology and their applications will be discussed extensively in the context of adenoviruses (Ads; Ad chimera illustrated in Figure 2) because a remarkable variety of hybrids have been generated for these species and all issues mentioned above have been addressed owing to extensive knowledge of their structure and life cycle and the numerous serotypes in the Ad family. Moreover, Ads are among the most popular viruses used for gene therapy, vaccination, and oncolysis with Ad serotype 5 (HAdV-5) being the most commonly used serotype with a superior safety profile after systemic application [2]. Additionally, we will give selected examples of chimeras based on other virus families to draw a broader picture of the field (paragons depicted in Figure 3).

(i) Improving transduction efficiency and/or specificity at the level of direct cell binding

Having demonstrated promising utility as therapeutic vectors, it was revealed for HAdV-5 and other viruses that their natural cellular attachment receptors represent a major limitation for their applications. Low or absent receptor expression on target tissues results in insufficient infection efficiency. Furthermore, widespread receptor expression in healthy tissues triggers virus sequestration and toxic side effects. Consequently, ways to modify the natural virus tropism were sought to create effective virus-based therapies.

Ads attach to their target cell via the knob domain of the trimeric capsid protein fiber. This domain is structurally conserved between different Ad serotypes, but several different receptors are used. This notion was verified by analysis of the first chimeric fiber protein constructed in 1995 by replacement of the HAdV-5 knob with that of HAdV-3 [3]. The corresponding virus HAdV-5/3 was generated a year later [4] proving that viral tropism can indeed be altered by fiber knob exchange: not only was the receptor specificity switched but there was also a remarkable enhancement of binding and entry, especially into primary cells. For example, transduction levels of primary melanoma cells with HAdV-5/3 increased up to three orders of magnitude when compared to the HAdV-5 control [5]. In combination with transcriptional control elements, such fiber-chimeric viruses are endowed with precise target cell specificity, as exemplified for a melanoma-targeted oncolytic virus [6]. In addition to HAdV-5/3 chimeras, fiber chimeras with knob domains of HAdV-35 (first developed in [7]) and other serotypes have become popular.

In addition to exchange of the knob domain alone, fiber chimeras have been generated by exchanging knob and shaft domains (first developed in [8]). Indeed, Ad infectivity depends on both shaft length and flexibility [8–10]. For example, shaft length influenced *in vivo* biodistribution in ovarian cancer mouse models, increasing the tumor:liver transduction ratio approximately 10-fold when using an