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HPMA copolymers for masking and retargeting of therapeutic viruses ☆

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ABSTRACT

Hydrophilic polymers are widely used already for steric stabilisation of bioactive proteins, changing their pharmacokinetics and modifying their interactions with the biological environment. Polymers may also be conjugated to biological surfaces, such as viruses, bacteria and mammalian cells, also to endow steric protection and changed properties. Reactive polymers based on N-[2-hydroxypropyl]methacrylamide have shown particular promise for surface coating of viruses, particularly adenovirus, and here we describe the important observations and innovations arising from this combination of chemical and genetic engineering. Adenovirus is a versatile agent that already finds important experimental applications as a recombinant vaccine, and also for cancer therapy, although its activity in both settings is often limited by a potent antibody-neutralising response in humans that is generally not seen in experimental animals. Coating with HPMA copolymers provides protection against neutralisation by antibodies and complement, and covalent linkage of novel ligands to the surface of the polymer can endow new infectious tropisms, mediated through different receptors, that can expand the potential applications of this versatile technology for a range of settings.

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1. Introduction

The use of genetic material for therapeutic applications is now an established field of research involving a variety of different approaches. Viruses are particularly useful for this purpose because they are readily modified by cloning techniques and can efficiently transfer DNA payloads into target cells. Already employed as vaccines, these versatile nanoparticles have contributed to major health care milestones including the world wide eradication of smallpox. In recent times viruses have been developed for additional medical uses, including therapeutic gene transfer and direct killing of tumour cells [1,38]. Some viruses are capable of expressing therapeutic genes inside mammalian cells for very long duration, opening up the possibility of lifelong correction of genetic disorders. For example, herpesvirus, lentivirus and parvoviruses can all enter cells and express transgenes for months or even years, either by integrating their genetic material into the host genome or by maintaining it as an episome within the host cell nucleus [41]. Other types of virus have rapid replication cycles following cell entry, resulting in very high protein expression levels and cell lysis. These viruses, such as adenovirus, reovirus and alphaviruses, are particularly useful for

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delivering vaccine antigens or for direct lysis of tumour cells [38]. All of these applications are considered in more detail below.

2. Viral therapeutics as vaccines

In the context of vaccination, one successful approach is to employ viral vaccines that are attenuated compared to the disease-causing organism, in order to provoke a protective immune response with very little risk of infection from the vaccine. Well known examples of this approach include the use of live Vaccinia virus as a vaccine for smallpox [13], or the use of attenuated polio virus in the Sabine polio vaccine [14]. Because many viruses tend to produce a robust immune response to not only the particle itself but also expressed genes, there is a clear possibility to use them in a 'Trojan Horse' format whereby a 'safe' non replicating or attenuated vector such as adenovirus or Vaccinia virus is used to encode and deliver protein components of a more virulent pathogen such as Ebola, HIV or malaria [5,31,32].

The main advantage of this approach is that it produces very high immune responses against the pathogen proteins, without exposing recipients to any risk from the disease-causing pathogen. Acting as both an adjuvant and a delivery vector, viruses can provide a very authentic means of presenting antigens to the immune system. They also have advantages in scale up because the production methods developed same vector which can be used to generate a wide variety of vaccines.

However, while recombinant adenovirus 'Trojan Horse' vaccines are very effective agents in mice, their activity is usually limited in humans by the presence of anti-adenovirus antibodies that bind the virus and neutralise it before it can infect target cells [36]. Most people have antibodies recognising common serotypes of adenovirus due to their lifelong exposure to the virus in the environment [39]. This often renders the initial injection of vaccine not very effective, and individuals who receive just a single injection quickly generate very high levels of virus-neutralising antibodies that effectively preclude any attempts to readminister the vaccine to 'boost' the immune response against the pathogen transgene. The most high profile adenovirus vaccine trial is Merck's recent 'STEP' trial [21], where the virus was used to express a combination of antigens from the human immunodeficiency virus (HIV-1), where the trial was eventually abandoned because of suggestions of increased HIV infection in patients who were receiving the vaccine [26]. A common feature of many such trials is the difficulty of obtaining effective anti-HIV responses in people with pre-existing antibodies against the adenovirus vector, and particularly of effectively boosting any such response.

3. Viral therapeutics as anticancer agents

Viruses are also being developed as agents for cancer treatment. At one level they can be designed to express therapeutic proteins within cancer cells, such as wild type copies of tumour suppressor genes [9] or enzymes capable of activating innocuous prodrugs to yield cytotoxic species selectively within the tumour [37]. At another level they can be designed to replicate selectively within cancer cells and lyse them before spreading to infect adjacent cells, a new approach known as 'virotherapy' [4,15]. Even wild type viruses often show selectivity for replication within tumour cells, thought to reflect the modified tumour phenotype [18], although viruses can also be genetically modified to take advantage of specific tumour-associated changes [2]. These agents show great promise when they are injected directly into individual tumour nodules, often leading to significant shrinkage [25], they show very little promise for treatment of metastatic cancer, where tumour nodules are spread widely around the body. The vast majority of deaths from cancer are caused by metastatic disease, and to access these disseminated tumour growths the therapeutic viruses must be administered intravenously. Unfortunately adenoviruses do not generally survive the harsh environment of the human bloodstream, which usually contains high levels of adenovirus-neutralising antibodies, and there are few signs of activity of viruses administered via this route [33].

In both these settings – recombinant viral vaccination and cancer therapy – the intrinsic infectious tropisms of adenoviruses may also be a problem, as well as their susceptibility to neutralisation by the host immune system. For effective T cell vaccination it is thought to be desirable for the virus to infect selectively the antigen-presenting cells, while for cancer treatment best results are likely to be achieved if the virus infects selectively cancer cells. Unfortunately, following administration into the living body, adenoviruses usually face a variety of opportunities to infect a range of non-target cells, and the overwhelming majority of virus particles are thought to distribute to sites where they can provide no significant therapeutic effect.

Some of the major challenges confronting therapeutic viral particles intended for systemic (intravenous) delivery to metastatic cancer are overviewed in Fig. 1. The first challenge is the complex and often neutralising environment of the bloodstream [19], designed to inactivate invading pathogenic viruses; the second challenge is infection or capture by non-target cells, notably cells in the liver, where therapeutic viruses may actively infect irrelevant cells or be captured by phagocytes [30]; the third challenge is in entering and destroying the tumour following arrival of the therapeutic virus at the tumour site. Without underestimating the significance of the third challenge, this review will focus predominantly on the first two challenges, and will assess engineering strategies to overcome them.

4. Engineering adenovirus to improve tropism and prevent neutralisation by the immune system

To realise the exciting potential of these therapeutic approaches, it is desirable to protect adenoviruses from premature neutralisation by components of the innate immune system and also to target viral tropism towards cells where it can mediate a useful outcome. These goals are both important, and both represent significant technological challenges. Generally there are two approaches, based respectively on genetic and on chemical engineering.

Genetic engineering adenovirus to evade immune-recognition is very difficult, as each component protein would need to be re-engineered to remove presentable epitopes [24]; indeed this approach, even if feasible, is arguably rather dangerous in the context of replication-competent viruses and would require very careful assessment and development. Engineering viral tropism at the genetic level is relatively straight forward to achieve in vitro, with several examples now published [12,17], however the range of adenovirus infection pathways makes it difficult to achieve receptor-specific infection, indeed new pathways of adenovirus infection are still being discovered even now [40].

5. Coating of adenovirus with multivalent reactive HPMA copolymers

Chemical engineering of virus particles with hydrophilic polymers provides an opportunity to mask surface epitopes and prevent antibody neutralisation or other unwanted interactions. The first approach to this goal made use of reactive poly(ethylene glycol) (PEG) polymers, building on many years' experience using PEG in the field of polymer-drug conjugates. PEG-modification is able to solubilise drugs and also provides stealth coats for liposomes, enabling extended circulation and tumour targeting [45]. Typically PEG molecules are attached to amino groups on the active drug component or liposome via reactive esters on the PEG termini. The presence of the PEG can create a dynamic barrier that restricts access to surfaces or to other large molecules, in a process called steric interference [46]. Using PEG to coat virus particles is possible, although certain problems need to be addressed; for example the reaction of polymers in solution with particles is slow, necessitating