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Virotherapy – cancer targeted pharmacology

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Building on their success in vaccination, many groups are now exploring the use of viruses as anticancer agents. In general, viral therapeutics provide the possibility to express anticancer proteins directly at the tumour site, decreasing exposure to normal tissue during delivery and maximising therapeutic index. Some viruses are also 'oncolytic', either naturally or by design, and these agents function to kill cancer cells selectively before spreading to infect adjacent cells and repeat the process. This whole field of cancer 'virotherapy' is moving forward rapidly at the moment, with notable clinical successes demonstrated with a range of oncolytic agents developed as directly oncolytic and also as oncolytic cancer vaccines. Given the versatility of oncolytic viruses to express therapeutic proteins we anticipate this approach will provide the platform for useful application of a broad range of innovative biological therapies.

Introduction

The medicinal use of viruses as vaccines represents the most successful class of agents in the history of medicine, with over 500 million lives saved [1]. Building on this impressive pedigree many groups around the world are developing viral therapeutics for cancer, both as the basis of cancer vaccines and also to exploit the lytic properties of some viruses for direct killing of cancer cells, so-called 'cancer virotherapy' [2].

Virotherapy offers several advantages over the use of conventional small drugs as cancer therapeutics, including selectivity, potency and mechanism of cell kill [3]. Many viruses show intrinsic selectivity for replication in cancer cells, and selectivity can be augmented if required using a range of strategies, including placing essential virus genes under transcriptional control of tumour-associated transcription factors [4,5]. More intriguing is to exploit the similarities between the cellular deregulation processes that occur during malignant transformation and those that occur following virus infection; this is the basis of the paradigm for functional complementation by the tumour phenotype of viruses deleted of key functionalities, such as the ability to activate the cell cycle or to inhibit apoptosis [6–8].

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The potency of virotherapy arises from the ability of the lytic virus to replicate itself within tumour cells before spreading to infect adjacent cells. This provides the highest concentration of therapeutic agent (the virus, and any therapeutic proteins it expresses) actually within the tumour tissue, not in the bloodstream during delivery, and hence should provide a very high Therapeutic Index.

Finally, whereas conventional anticancer agents rely mostly on cellular apoptosis mechanisms for tumour cell kill, viruses express their encoded genes within the cancer cells and might impose their own mechanisms of cell killing. The feasibility of 'arming' lytic viruses by introducing additional transgene cassettes provides the possibility of further programming the precise mechanism of cell kill, to maximise anticancer activity and provide a means to overcome apoptosis-defective pathways in cancer cells that are resistant to drug therapies.

Early clinical trials of virotherapy

In 1910 the Italian physician Nicola De Pace presented findings that one of his female patients suffering from cervical carcinoma had undergone a complete spontaneous remission after receiving an inoculation of the live attenuated Pasteur-Roux rabies virus vaccine [9]. This was the first documented case of a tumour remission that was attributed to the lytic effects of a virus and

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began the field of oncolytic virotherapy. Many virotherapy clinical studies followed, including the testing of a variety of viruses from members of the *Adenoviridae*, *Herpesviridae*, *Poxviridae*, *Reoviridae*, *Paramyxoviridae* and *Flaviviridae* families in humans. In some cases, such as the early studies with wild type strains of both West Nile virus and Ilheus virus, treatment led to substantial pathology and sometimes death, confirming the potency of the approach but emphasising the need for careful control of virus activity [10].

Adenoviruses were found to be particularly well suited to virotherapy because they showed relatively mild pathology, could be replicated to a high-titre *in vitro*, were genetically stable, and could infect and kill a broad range of cell types. An initial clinical study, using 11 different wild type adenovirus serotypes given by a range of delivery routes to treat cervical carcinoma [11], showed acceptable toxicology and enticing hints of anticancer activity. Most obvious was that the virus was able to induce the formation of necrotic cavities within the centre of some injected cervical tumour masses. Therapeutic efficacy was ultimately limited, however, and was lower in patients with pre-existing antibodies against adenovirus.

Current clinical status of virotherapy

The first engineered replication-selective virus to be used in human trials was the adenovirus dl1520 [ONYX-015, Onyx Pharmaceuticals (http://www.onyx-pharm.com/), Emeryville, CA, USA], a hybrid of serotypes 2/5 with deletions in both the E1B-55K and E3B genome region [12]. It was originally hypothesised that the deletion of E1B-55K would facilitate replication exclusively in cells with a defective p53 pathway, a common phenotype in cancer cells. However, recent findings have implicated that other genetic components influence its selectivity [13].

The results from over 15 clinical trials with dl1520 have been published to date [14] with encouraging antitumour activity observed in patients treated with dl1520 in combination with the chemotherapeutic agents, cisplatin and 5-fluorouracil [15]. Although dl1520 never entered Phase III trial in the USA, a related E1B-55K gene deleted serotype 5 adenovirus (Ad5) (H101) has shown impressive efficacy for local treatment of head and neck cancer in China, and a product licence was granted to Shanghai Sunway Biotech (http://www.sunwaybio.com.cn/) in 2007 by the Chinese State Food and Drug Administration [16,17].

A range of other oncolytic Ad5-based adenoviruses have been trialled in the West, such as transcriptionally targeted prostate specific CG7870 and CG7060 [Cell Genesys (http://www.cellgenesys.com), San Francisco, CA, USA] [18], and combination therapies [19,20] with no serious toxicity but also no measurable efficacy particularly when given intravenously. This might reflect the strong Ad5 neutralising status of most patients even before treatment. The first systemic trial of a different serotype oncolytic adenovirus is scheduled to start in the UK in 2012, an Ad11/Ad3 hybrid virus known as ColoAd1 [PsiOxus Ltd (http://www.psioxus.com/), Oxford, UK]. This virus was selected from a library of recombinants for its powerful ability to kill colorectal cancer cells selectively, and hence was not produced by rational design [21]. Most patients have lower levels of pre-existing antibodies against Ad11 than against Ad5, hence the virus might show better performance following intravenous injection and assessment of its clinical performance is awaited with interest.

Other viruses that have advanced into clinical trials include the oncolytic herpes simplex virus (HSV) constructs G207 [22], 1716 [23], NV1020 [24] and OncoVex$^{GM\text{-}CSF}$ [BioVex (http://www.biovex.com/), Abingdon, UK] [25]. These have all been engineered with deletions in the virulence factor γ-34.5 gene resulting in neuroattenuation. The most clinically advanced HSV oncolytic, OncoVex$^{GM\text{-}CSF}$ carries an additional deletion in ICP47 gene which restores major histocompatibility complex I (MHC I) presentation on the host cell and also incorporates the granulocyte–macrophage colony-stimulation factor (GM-CSF) as a transgene to enhance the immune response [26]. Herpes virus is rapidly inactivated in human blood, although a Phase I study involving intratumoural injections of cutaneous melanoma metastases demonstrated HSV antigen-associated tumour necrosis with low levels of toxicity [27]. In Phase II clinical trials, OncoVex$^{GM\text{-}CSF}$ was injected intratumourally into metastatic melanomas and achieved an objective response rate of 26% including responses of non-injected tumours [25]. OncoVex$^{GM\text{-}CSF}$ has now entered an international Phase III trial studying patients with both metastatic melanoma and squamous cell carcinoma of the head and neck [28].

An oncolytic poxvirus, JX-594 [Jennerex Biotherapeutics (http://www.jennerex.com/), San Francisco, CA, USA] has been developed from the Wyeth vaccine strain of vaccinia virus and engineered with a deletion in the thymidine kinase gene to improve cancer selectivity and also carries GM-CSF. Phase I clinical trials in which JX-594 was administered intratumourally into patients with metastatic melanoma revealed no dose limiting toxicity and a 71% objective tumour response rate including non-injected tumours [29]. More recently, one trial has included intravenous administration of a single dose of JX-594 to 23 patients in a dose-escalation schedule. Delivery and replication of the virus within tumours was reported in patients who received the highest administered dose, however, there was no consistent effect on clinical outcome [30].

In addition to oncolytic DNA viruses, several RNA viruses have also entered the clinic. These include Newcastle Disease Virus [31] and Seneca Valley Virus [32]. Probably the most extensive studies have been performed using Reolysin [Oncolytics Biotech Inc. Corporation (http://www.oncolyticsbiotech.com/), Canada, Calgary, AB], an oncolytic reovirus type 3 Dearing strain, which have been ongoing for more than a decade. Reolysin is capable of selectively targeting cells with an activated Ras pathway enabling specific replication in tumour cells. A variety of Phase I clinical trials have been designed to test both safety and efficacy, including multidose intravenous administration of Reolysin to patients with advanced cancer resulting in intratumoural localisation of reovirus with little toxicity [33]. Approval has now been granted for a Phase III trial testing the co-administration of Reolysin with paclitaxel and carboplatin in patients with head and neck cancer [34].

Barriers to systemic virotherapy

Subsequent clinical trials both of wild type and genetically modified viruses, designed to combine potency with cancer-selectivity, have confirmed the considerable anticancer activity of virotherapy given locally but have also shown the presence of significant barriers to delivery and spread of most therapeutic

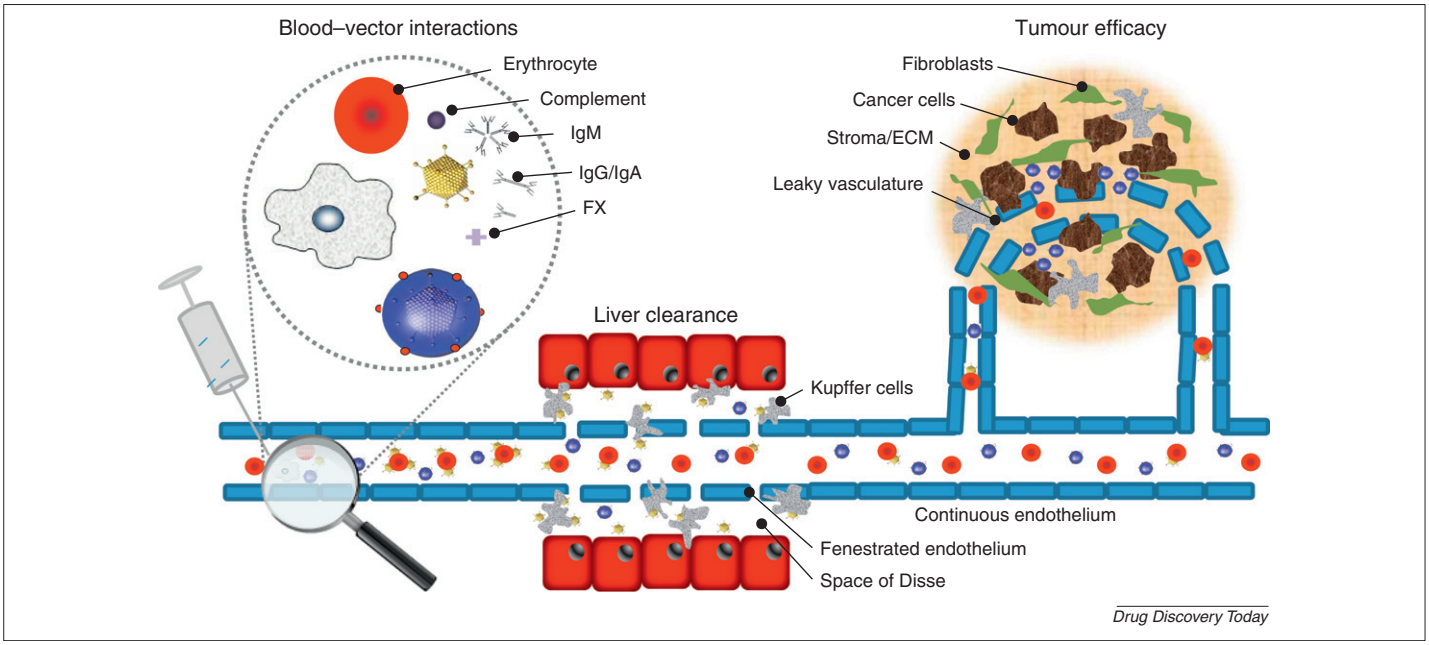

FIGURE 1

Barriers to systemic delivery of oncolytic viruses. There are three main areas that must be addressed to enable systemic delivery of virus particles, namely (i) avoiding neutralisation by components of the bloodstream including complement, Factor X, leukocytes, erythrocytes and antibodies, (ii) minimising unwanted infection of irrelevant cells, notably hepatocytes, which are usually present in vast excess, and avoiding premature scavenging by phagocytes, such as hepatic Kupffer cells, (iii) maximising extravasation within tumour vasculature and penetration to infect all viable tumour cells whilst avoiding other components of the interstitium. Polymer coated viruses present one promising approach to address these barriers, although several other technologies are also under development. *Abbreviations:* ECM: extracellular matrix, IgA: Immunoglobulin A, IgG: Immunoglobulin G, IgM: Immunoglobulin M, FX: Factor X.

virus particles around the body when used to treat metastatic disease. Some of these barriers are illustrated in Fig. 1 and described briefly below.

The immune system

Viruses are regarded as pathogens by the body, and are targeted by a range of immune defences. These include innate immune mechanisms (neutralisation and/or opsonisation by low affinity natural antibodies and complement, phagocytosis by neutrophils and macrophages, sequestration on erythrocytes, among others) and adaptive immune mechanisms for viruses that have been encountered by the body previously, either through environmental exposure or in earlier rounds of treatment [35]. These defences severely restrict bloodstream kinetics of virus particles and make systemic delivery particularly difficult. A range of approaches have been explored to improve systemic delivery kinetics, such as using non-human viruses (to minimise likelihood of prior exposure) or loading virus particles into cells to protect them during the delivery phase. The most impressive circulation kinetics currently are achieved using polymer-stealthed adenoviruses, because these lose their tendency to infect non-target cells (which are present in excess) in addition to being protected from rapid clearance by the immune system [36]. They also have the possibility for chemical reprogramming to achieve infection through cancer-associated receptors [37,38].

Extravasation into tumour nodules

The second major hurdle to successful use of systemic virotherapy is inefficient delivery of virus particles into tumour nodules. Although leaky tumour-associated vasculature can sometimes enable limited extravasation of virus particles, it is difficult to exploit receptor-mediated targeting of tumour cells when poor extravasation limits access to target cells. Some approaches to this problem include strategies to increase the permeability of tumour-associated vasculature [39], whereas others prefer to target viruses to infect tumour-associated (activated) endothelial cells [40]. This latter approach can be powerful for lytic viruses that replicate in activated normal cells, although for true cancer-selective viruses additional mechanisms (such as the formation of heterocellular syncytia [41]) might be necessary to enable access of viruses to the permissive intracellular environment of tumour cells.

Improving preclinical test systems

Many of these obstacles to successful virotherapy have become apparent from clinical studies and were not predicted from animal models, reflecting the problems of working with agents that are species-specific, such as replicating human adenoviruses, in non-human model systems. For example, although human tumour xenografts in nude mice can provide a 'permissive' preclinical model for adenovirus, the inability of the virus to replicate in normal mouse tissues is likely to prevent observation of any relevant off-target effects in such systems. These models are particularly deficient when working with immune modulatory viruses because xenograft models are by definition immune-compromised. There is considerable interest in developing permissive murine models to study adenovirus activity in immune-competent mice, and some murine tumours do seem to be at least partly permissive [42]. Superior alternative models for study of oncolytic group C adenoviruses are the immune-competent

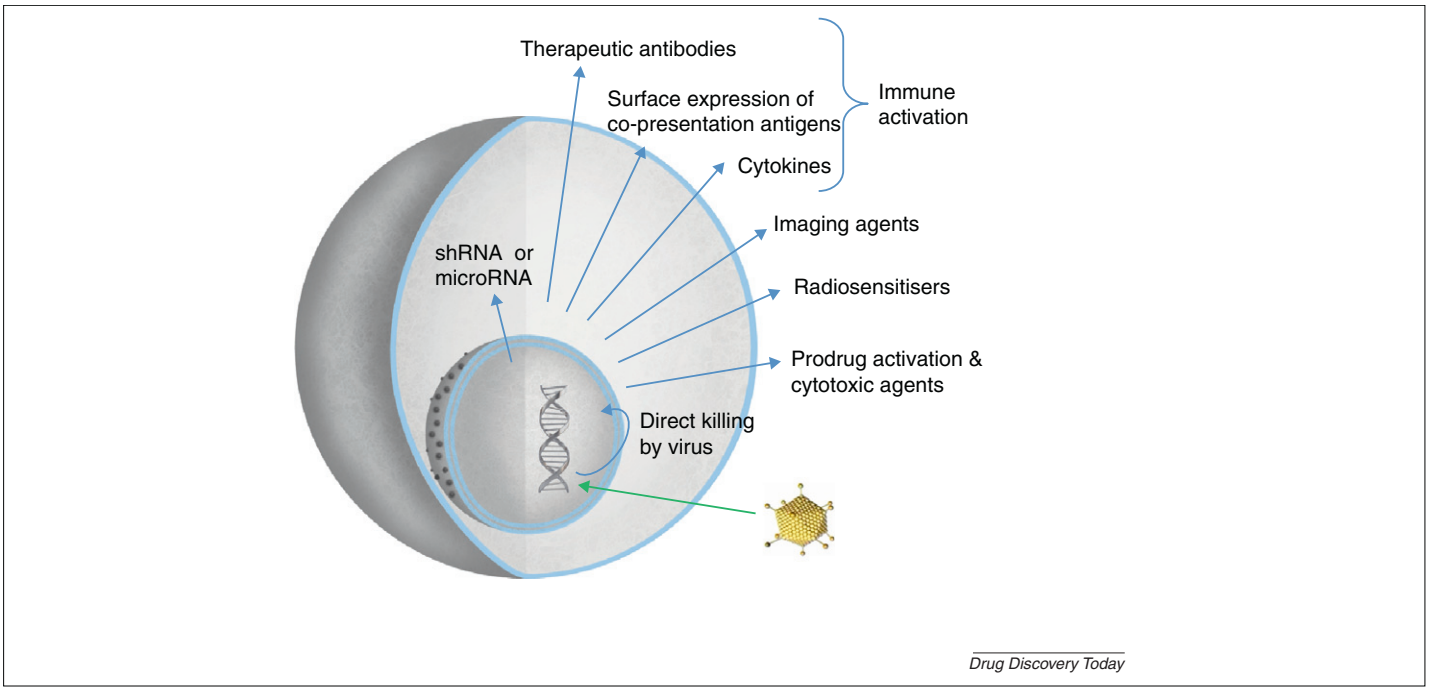

FIGURE 2

Oncolytic viruses producing biologics *in situ*. Biological agents can be encoded within viruses and expressed only within tumour cells, perhaps as a function of oncolytic virus replication. This provides diverse strategies to enhance and complement the direct killing by virotherapy, for example by expressing monoclonal antibodies to be secreted by the tumour cell and mediate paracrine effects on adjacent cells, including stimulation of the immune system. Viruses provide the only means to achieve high concentrations of biologics within tumour tissue without exposing the patient to high, potentially toxic, levels in the bloodstream during delivery. *Abbreviation*: shRNA: small hairpin ribonucleic acid.

Cotton Rat [43] and Syrian Hamster [44] models, which are widely used as preclinical models for some adenovirus serotypes. However, the innate immune system also differs between species, for example human erythrocytes carry complement receptor 1 in addition to the Coxsackie and adenovirus receptor (CAR), forming an effective trap for adenovirus in humans and rats, whereas this does not occur in mice [45]. Some other types of virus, notably herpes simplex and vaccinia, are less species-specific and can be usefully explored in immune competent animal models providing more robust data on both efficacy and possible toxicity. A general feature of preclinical animal models where the animal is not a natural host to the viral test agent is the probable absence of pre-existing antiviral antibodies at the start of study. It is worth assessing whether the patient population will have pre-existing antivirus antibodies, and the model adjusted accordingly. For those viruses where no representative animal model exists, we anticipate that advances in tissue engineering and biopsy/explant technologies might well provide more relevant preclinical test systems that can supplement the use of animal models in the future.

'Arming' oncolytic viruses

During the process of oncolysis there is an enticing opportunity to encode additional therapeutic proteins within the virus genome so that they are produced selectively within tumour cells following infection (Fig. 2). For example, oncolytic viruses could be modified to produce immune modulators [46,47] a variety of prodrug-activating enzymes [48,49] to maximise synergy between virotherapy and chemotherapy [50]. Alternatively oncolytic viruses could be used to express reporter proteins or the dual purpose imagining/therapeutic iodide symporter [51] for use with positron emission tomography (PET) scanning or ^{131}I radiotherapy. One intriguing possibility is to exploit their ability to express proteins *in situ* to manipulate the local immune environment [46,47]. Although the presence of the virus itself should already begin to counter the chronic state of sterile inflammation [52] that often enables tumours to escape immune recognition, programming the virus also to express cytokines capable of reversing immune suppression at the disease site could be a powerful approach. Immune modulatory cytokine produced from within the tumour could include GM-CSF (as already included in OncoVex^{GM-CSF} and JX-594, see above), IL-2, IL-12, TNF, among others and would provide a concentration gradient suitable to encourage chemotaxis of immune cells into the tumour in a way that conventional systemic use of those agents would not enable.

Concluding remarks

Compared *in vitro*, virotherapy shows cytotoxicity to cancer cells at concentrations far lower than conventional chemotherapeutic agents, whether measured in molarities or as mass of therapeutic agent. They also show much greater selectivity than most conventional drugs for killing cancer cells rather than normal cells. As a therapeutic modality, oncolytic viruses offer a step change in cancer treatment [53]. However current agents (with the possible exception of Reolysin) show much better activity when applied locally than when given intravenously, largely because of the ability of the innate and adaptive immune system rapidly to inactivate virus particles. Accordingly, the

major factor currently limiting clinical utility for treatment of metastatic cancer is poor delivery of virus particles to tumour cells. Current approaches are focussed on addressing this limitation, and might soon be able to unleash the considerable anticancer potency of the approach.

Oncolytic viruses represent the ultimate customisable drug capable of producing a variety of biological agents, or combination of biological agents, selectively within the diseased area [54]. Enabling a plethora of therapeutic modalities simply by modifying the genomic sequence is commercially very attractive because the drug substance (the virus particle) will have the same pharmacokinetic properties and use similar manufacturing protocols independent of the transgene encoded. Given the rapid development of this field we anticipate a range of potent agents to be reaching the clinics in the next few years.

Conflict of interest

Len W. Seymour and Kerry D. Fisher have a financial interest in PsiOxus Ltd.

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