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Review Article

Virotherapy: A new Approach for Cancer Treatment Overview

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Abstract

Oncolytic Virotherapy is an emerging bio-therapeutic platform based on genetic engineering of viruses capable of selectively infecting and replicating within cancer cells. Such viruses have been found to be both safe and to produce antitumour effects in a number of phase I and II clinical trials. Early work in this field has been pioneered with strains of adenovirus which, although well suited to gene therapy approaches, have displayed certain limitations in their ability to directly destroy and spread through tumour tissues, particularly after systemic administration.Vaccines virus is perhaps the most widely administered and successful medical product in history; it displays many of the qualities thought necessary for an effective antitumour agent and is particularly well characterized in people due to its role in the eradication of smallpoxAdvances in gene modification and viral therapy have led to the development of a variety of vectors in several viral families that are capable of replication specifically in tumor cells. The nature of viral delivery, infection, and replication, this technology, oncolytic Virotherapy, may prove valuable for treating cancer patients, especially those with inoperable tumors.Oncolytic viruses (OVs) demonstrate the ability to replicate selectively in cancer cells,resulting in antitumor effects by a variety of mechanisms, including direct cell lysis and indirect celldeath through immune-mediate host responses. Several solutions and strategies already exist, however, to minimize or circumvent many of these limitations, supporting viral oncolytic therapy as a viable option and powerful tool in the fight against cancer.

Keywords: Virotherapy, Censer, viral oncolytic therapy, Adenoviral, Antitumour, Cytotoxic genes, Genome Stability.

Introduction

There are some methods which are useful of treatment for cancer in which Radiation therapy (also called radiotherapy, X-ray therapy, or irradiation) is the use of ionizing radiation to kill cancer cells and shrink tumors. Radiation therapy can be administered externally via external beam radiotherapy (EBRT) or internally via brachytherapy. The effects of radiation therapy are localized and confined to the region being treated. Radiation therapy injures or destroys cells in the area being treated by damaging their genetic material, making it impossible for these cells to continue to grow and divide. Chemotherapy is the treatment of cancer with drugs that can destroy cancer cells. In current usage, the term chemotherapy usually refers to cytotoxic drugs which affect rapidly dividing cells in general, in contrast with targeted therapy. Chemotherapy drugs interfere with cell division in various possible ways, e.g. with the duplication of DNA or the separation of newly formed chromosomes. Most Forms of chemotherapy target all rapidly dividing cells and are not specific for cancer cells, although some degree of specificity may come from the inability of many cancer cells to repair DNA damage, while normal cells generally can. Hence, chemotherapy has the potential to harm healthy tissue, especially those tissues that have a high replacement rate. These cells usually repair themselves after chemotherapy [1].Targeted therapy, which first became available in the late 1990s has had a significant impact in the treatment of some types of cancer, and is currently a very active research area. This constitutes the use of cancer, and currently a very active research area. This constitutes the use of agents specific for the deregulated proteins of cancer cells. Small molecule targeted therapy drugs are generally inhibitors of enzymatic domains on mutated, over expressed or otherwise critical proteins within the cancer cell. Prominent examples are the tyrosine kinase inhibitors imatinib and gefitinib. Virotherapy is an experimental form of cancer treatment using biotechnology to convert viruses into cancer-fighting agents by reprogramming viruses to only attack cancerous cells while healthy cells remained undamaged. The human immunodeficiency virus (HIV), which causes AIDS, is a candidate for this and is currently under investigation. Usually the viruses used are Varicella Zoster viruses (The Herpes simplex and Adenoviruses (First isolated in adenoid tissue).The researchers tested the new Virotherapy on Glioblastoma multiform patients and achieved promising results for the first time. Virotherapy shows promise in treating mesothelioma. Viruses may also be engineered to kill ovarian cancer and glioma cells. Mesothelioma, in particular, is long overdue for advances in treatment. Normally, viruses replicate to increase their number, and by virtue of that process, healthy cells are killed. Virotherapy is about engineering viruses so that they replicate only in tumor cells and kill only tumor cells. The catch is that in order to engineer an effective virus, scientists must first understand the molecular workings of the cancer. An adenovirus-based Virotherapy agent is engineered by incorporating a tumor specific promoter (TSP) into virus genes. The TSP restricts the expression of certain genes and viral replication in tumor cells, while sparing in normal cells. This discovery set the stage for the team to design a Virotherapy agent effective against mesothelioma, a disease that has not seen an improvement in outcomes resulting from new therapies in 20 years, Curiel and the researchers have engineered a virus that replicates in mesothelioma cells and spares normal cell. The adenovirus-based vector has emerged as a leading candidate for in vivo oncolytic Virotherapy [2]. Adenoviruses are attractive vectors because they can be produced in high titers, do not integrate into the host chromosome, and have a broad tropism. In addition, adenoviral vectors infect both dividing and no dividing cells, have high stability in vivo and have a high capacity for gene transfer. Another beneficial attribute contributing to their employment in antitumor therapy is that adenoviruses possess a lytic life cycle that can be exploited for oncolysis. Although adenoviruses do not have a natural tendency to replicate in tumor cells, they can be rendered to do so [3].

Desirable Features For Oncolytic Virotherapy Agents

When considering a virus species for development as an oncolytic therapy, a number of efficacies, safety, and manufacturing issues

need to be as acessed. The virus should infect, replicate in, and destroy human tumor cells, ideally including non-cycling cancer cells. The parental virus should preferably cause only mild, well-characterized human diseases. Alternatively, deletion mutants that are themselves no virulent should be considered. Non-integrating viruses have potential safety advantages in that unpredicted events caused by genomic integration are avoided.A genetically stable virus is desirable from both safety and manufacturing standpoints. Genetic approaches to prevent viral replication in essential normal tissues are critical and a secondary mechanism to inactivate the virus should ideally be available. Finally, the virus must be amenable to high-titer production and purification under Good Manufacturing Practices (GMP) guidelines for clinical studies [4] (Figure 1).

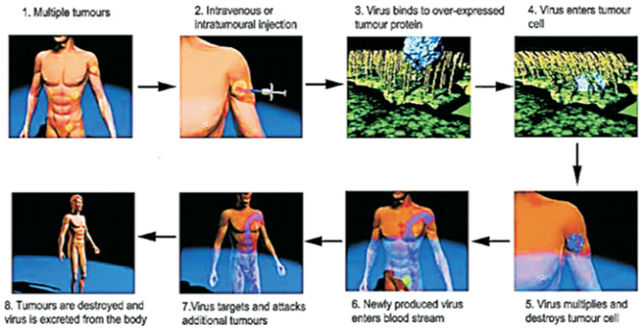

Figure1: Schematic Representation of Oncolytic Virotherapy

Mechanisms of Tumor Selectivity

Viruses have evolved to dramatically alter the phenotype of the infected cell to maximize their own replication and survival. The cellular changes induced by viral infection are often strikingly similar to the cellular changes acquired during carcinogenesis. Given this genetic convergence, it is not surprising that many viruses inherently grow preferentially in tumor cells and/or that viruses can be engineered for tumor selectivity. Five general mechanistic approaches to tumor-selective replication have been described to date. These include, the use of viruses with inherent tumor selectivity. deletion of entire genes or functional gene regions that are necessary for efficient replication and/or toxicity in normal cells but are expendable in tumor cells; engineering of tumor/tissue-specific promoters into viruses to limit expression of gene(s) necessary for replication to cancer cells and modification of the viral coat to target uptake selectively to tumor cells. Each of these approaches has potential advantages and disadvantages [5] (Figure 2).

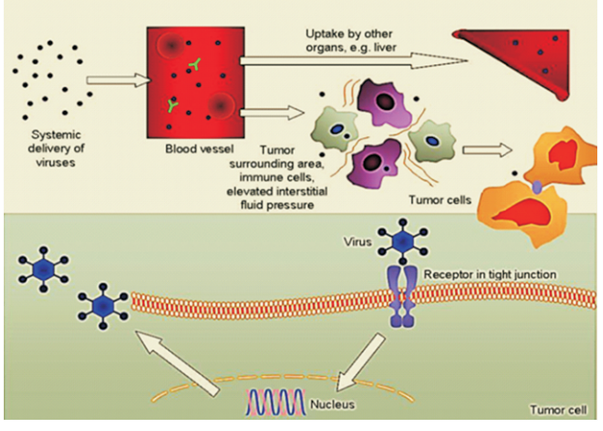

Figure 2: Mechanism of action of oncolytic virus

Tumour Selective Promoters

A slightly different approach to achieving tumor-selective replication involves linking viral genes to promoter that are only functional in tumor cells. This strategy has been used primarily with adenovirus and HSV1. One tumor specific promoter is derived from the gene that encodes 1alpha-fetoprotein (AFP). AFP is expressed in several tissues during development, but in adult tissues its expression is limited to tumors of hepatic and intestinal origin. In an adenoviral vector, this promoter can be used to regulate the expression of both E1A and E1B55kD.There is a 10,000-fold increase in the replication of this virus in AFP expressing cells, compared with AFP-negative cells. Intravenous administration in mice causes regression of AFP-positive tumors, such as hepato-cellular carcinomas, with minimal toxicity to normal cells [6].

Potential hurdles-overcome

Potential limitations to Virotherapy have been identified. As the majority of researches to date have been with adenoviruses, more is known about the limitations with this virus species than for others. First, although viruses rapidly spread in cell culture monolayer, viral spread within a solid tumor mass can be limiting. In fact, mathematical modeling of the race between viral oncolysis and spread versus tumor cell proliferation and outgrowth demonstrates that the infection of the tumor must be diffuse throughout the tumor in order to control it injection of the tumor core or periphery only results in tumor escape. The relative inefficiency of viral spread may relate to their relatively large sizes (e.g. 90mm for adenovirus), dwarfing anti-tumor chemicals, peptides, and even antibodies. Potential physical limitations to viral spread include fibrosis, intermixed normal cells (up to half of the cells within some tumors), basement membranes, cell-to-cell barriers, and necrotic regions. Viral mutants that are more efficiently released and spread in tumors have been identified; these include E1A-CR2 mutant and E1B-19 kDa mutant adenoviruses and mutants that over express the E311.6 gene product. In addition, certain virus species spread more efficiently that other. Adenovirus, for example, spreads slowly because it remains primarily intracellular and appears to spread through apoptotic bodies; its replication cycle typically lasts for 48-72 hours. In contrast, some viruses are actively pumped out of the infected cell and other kill the infected cell faster. In particular, viruses with extra cellular forms may have distinct advantages in intra-tumor spread [7].

Risk Management

Virotherapy agents raise new biosafety and risk management issues. The risk assessment for trials with these agents must not only take into account potential risks to the treated patient but to patient contacts and the general public. For cancer patients with refractory, end-stage disease, the risk-benefit ratio has supported the development of extremely toxic treatment approaches, some of which routinely result in severe morbidity and mortality this is acceptable because long-term remissions are possible. Nevertheless, there will always be a risk of toxicity during clinical trial testing, and this may be acceptable in patients with terminal cancer. Once an acceptable safety profile has been demonstrated in end-stage, refractory patients, it may be ethical to move into earlier-stage patient populations. The treatment of earlier-state patients may require previous use of the agent in end-stage patients and/or localized administration initially. Finally, use in combination with standard chemotherapy and/or radiotherapy will generally require prior experience with the virus as a single agent. With a great diversity of viral families now described with some degree of tumor selectivity, at some point in time an important question must surely raise its controversial head above the parapet. If so many viruses are potentially useful for targeting cancer cells, whether genetically altered or not, which one is the best? This is not simply a question designed to satisfy intellectual curiosity. It has obvious relevance to

patient care, clearly influences the number of clinical trials that will be carried out, and, perhaps, will avoid the investigation of experimental therapies that turn out to have some negative toxicity. However, although the literature continues to amass descriptions of new and improved versions of different replicating viral systems, the lack of any meaningful head-to-head comparisons between these systems is distinctly notable by its absence. Therefore, it becomes genuinely difficult to compare the safety and efficacy of different viruses. However, a somewhat more cynical view can also be aired. Thus, it may be that, predominantly for commercial reasons, comparisons of potential products are not in the best interests of shareholders [8].

History of Oncolytic Vitrotherapy

The adenovirus-based vector has emerged as a leading candidate for *in vivo* oncolytic Virotherapy. Adenoviruses are attractive vectors because they can be produced in high titers, do not integrate into the host chromosome, and have a broad tropism. In addition, adenoviral vectors infect both dividing and no dividing cells, have high stability in vivo and have a high capacity for gene transfer. Another beneficial attribute contributing to their employment in antitumor therapy is that adenoviruses possess a lytic life cycle that can be exploited for oncolysis. Although adenoviruses do not have a natural tendency to replicate in tumor cells, they can be rendered to do so. The idea of plain-old oncolytic Virotherapy is more than 50 years old though most real progress has been made in the last dozen years. Therapeutic oncolytic viruses (virotherapeutics) constitute a novel class of targeted anticancer agents that have unique mechanisms of action compared with other cancer therapeutics. The development of viro therapeutics has evolved from the use of in vitro-passaged strains (first generation), to genetically engineered selectivity-enhanced viruses (second generation) and finally to genetically engineered Transgene-expressing armed oncolytic viruses (third generation). Descriptions of cancer remissions following virus infections date back to a century ago. Initial patient treatment publications, written up to 50 years ago, consisted of case reports or case series of treatment with first-generation, non-engineered viruses. Over the past decade, hundreds of patients with cancer have been treated on prospectively designed clinical trials (including phase III), evaluating over 10 different agents, including engineered second-generation and third-generation viruses. This Review summarizes and interprets the data from clinical reports over the last century, including safety, efficacy and biological end points (viral and immunologic). By 2008 much progress had been made as indicated in the publication Oncolytic Virotherapy is molecular targets in tumor-selective replication and carrier cell-mediated delivery of oncolytic viruses. Tremendous advances have been made in developing oncolytic viruses (OVs) in the last few years. By taking advantage of current knowledge in cancer biology and virology, specific OVs have been genetically engineered to target specific molecules or signal transduction pathways in cancer cells in order to achieve efficient and selective replication. The viral infection and amplification eventually induce cancer cells into cell death pathways and elicit host antitumor immune responses to further help eliminate cancer cells. Specifically targeted molecules or signalling pathways (such as RB/E2F/p16, p53, IFN, PKR, EGFR, Ras, Wnt, anti-apoptosis or hypoxia) in cancer cells or tumor microenvironment have been studied and dissected with a variety of OVs such as adenovirus, herpes simplex virus, poxvirus, vesicular stomatitis virus, measles virus, Newcastle disease virus, influenza virus and reo-virus, setting the molecular basis for further improvements in the near future. Another exciting new area of research has been the harnessing of naturally tumor-homing cells as carrier cells (or cellular vehicles) to deliver OVs to tumors.The trafficking of these tumor-homing cells (stem cells, immune cells and cancer cells), which support proliferation of the viruses, is mediated by specific chemokines and cell adhesion molecules and we are just beginning to understand the roles of these molecules." So, with all that great history and the discovery of many powerful anti-cancer viruses, why are oncolytic Virotherapy not now in widespread use? A large part of the answer appears to be that usually a) there is a problem getting the virus specifically to the cancer cells and b) the human immune system detects and wipes out the virus before it can get to the cancer cells and do its job. The immune system in such a case is just doing its job. The 2008 publication Cell carriers to deliver oncolytic viruses to sites of myeloma tumor growthreports several studies have illustrated the potential of utilizing oncolytic viruses (measles, vaccine, Vesicular Stomatitis Virus and cox sickie virus A21) for the treatment of MM (multiple myeloma), but there are significant barriers that prevent the viruses from reaching sites of myeloma tumor growth after intravenous delivery. The most important barriers are failure to extravasate from tumor blood vessels, mislocalization of the viruses in liver and spleen and neutralization by antiviral antibodies. These problems have led to approaches using Trojan horse cells that hide the viruses from the immune system and that can home-in to the cancer cells [9-11].

Virotherapy

Virotherapy is an experimental form of cancer treatment using biotechnology to convert viruses into cancer-fighting agents by reprogramming viruses to only attack cancerous cells while healthy cells remained undamaged. The human immunodeficiency virus (HIV), which causes AIDS, is a candidate for this and is currently under investigation. Usually the viruses used are Varicella Zoster Viruses (The Herpes simplex) and Adenoviruses (First isolated in adenoid tissue).To uses viruses as treatment against various diseases, most commonly as a vector used to specifically target cells and DNA in particular. It is not a new idea - as early as the 1950 doctors were noticing that cancer patients who suffered a non-related viral infection, or who had been vaccinated recently, showed signs of improvement: this has been largely attributed to the production of interferon and tumour necrosis factors in response to viral infection, but oncolytic viruses are being designed that selectively target and lyse only cancerous cells. In the 1940 and 1950, studies were conducted in animal models to evaluate the use of viruses in the treatment of tumors. In 1956 some of the earliest human clinical trials with oncolytic viruses for the treatment of advanced-stage cervical cancer were started. However, for several years research in this field was delayed due to the inadequate technology available. Research has now started to move forward more quickly in finding ways to use viruses therapeutically. In 2006 researchers from the Hebrew University succeeded in isolating a variant of the Newcastle disease Virus (NDV-HUJ), which usually affects birds, in order to specifically target cancer cells. The researchers tested the new Virotherapy on Glioblastoma multiform patients and achieved promising results for the first time [12-14].

Oncolytic Virotherapy

Oncolytic Virotherapy involves the treatment of cancer by using a virus specifically tailored to infect cancer cells while leaving normal cells unharmed. The engineering of such viruses involves ensuring that the viruses can only replicate inside cancer cells, lysing the cells when they exit and ensuring a higher dosage at the site of the tumors. Oncolytic Virotherapy is a science still in its infancy. Only a few cancers have had this method of treatment applied to them, with varying success.The most critical task in oncolytic Virotherapy is ensuring the virus chooses the right cells to destroy. In trans-ductional targeting, the virus's protein coat is modified so that it targets cancer cells rather than non-cancerous cells; this has been used especially with adenoviruses. In non-trans-ductional targeting, the virus can enter other cells, but has been genetically modified so that it can only reproduce inside the cancer cells. Cox-2, because its expression is elevated in many cancers, is used in non-trans-ductional targeting to promote the reproduction of these viruses. A double-targeting virus, using both trans-ductional and non-trans-

ductional methods to target the virus, is the safest and most effective method for oncolytic Virotherapy [15] Figure 3.

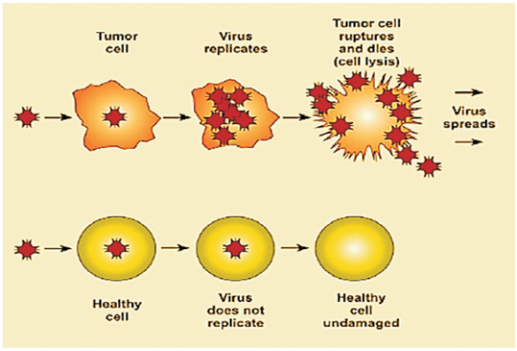

Figure 3: Oncolytic Viruses

Oncolytic viruses and Virotherapy

Viruses that replicate selectively in tumor cells and do not replicate in normal cells are used as agents to fight cancer. This therapeutic approach is known as Virotherapy. Taken as drugs, viruses have some unique properties. They respond to absent molecular targets such as missing IFN or tumor suppressor pathways. Inactivation of oncogenes by conventional drugs is seldom enough to stop cancer because lack of tumor suppressors is central to cancer progression. In addition a conventional drug does not amplify itself and is needed at very high concentrations to reach all tumor cells. Among different oncolytic viruses adenovirus is the most popular. Virotherapy works against cancer by a combination of different mechanisms. A virocentric point of view considers the direct lysis of tumor cells by the oncolytic virus as the most important parameter for efficacy. Immunocentrics consider that the lysis of tumor cells is important as long as it can activate an immune response against the tumor. For virocentric it is important to inhibit the immune response, for Immunocentrics is important to boost it even it will neutralize the virus. Probably a combination of these two mechanisms contributes to Virotherapy with more or less success depending on the architecture and immunogenicity of each tumor. Virotherapy started at the beginning of the twenty century when clinicians noted occasional transient tumor remissions during viral infections of patients. Early Virotherapy involved every newly discovered virus but later only viruses with a natural tropism for tumors were used. After the sixties it was almost abandoned due to lack of clear clinical results. Now the increase virology knowledge and the experience with viruses in cancer gene therapy have prompted a new wave of Virotherapy. Many different viruses are used in Virotherapy. In general terms, RNA viruses replicate in the cytoplasm and they show faster replication cycles than DNA viruses. The tumor-selective replication of RNA viruses is based on their sensitivity to be inactivated by interferon (IFN). IFNs (alpha, beta and gamma) are secreted by infected fibroblasts and T lymphocytes and bind to specific receptors that trigger a virus-resistant phenotype in surrounding cells. The main mediator of this resistance phenotype is the Protein Kinase R (PKR). PKR is an IFN-induced serine/threonine protein kinase that, upon binding to d-RNA produced during virus replication; it phosphorylates the eIF-2-alpha translation factor and leads to shut-off of protein synthesis in infected cells. Besides this antivirus role, IFNs have also antitumor activity. IFNs inhibit cell proliferation by inducing p21 and p202 expression and down regulating c-myc expression; they inhibit caspases and enhance antigen presentation by inducing MHC expression. Tumor cells with a truncated INF-pathway or an enhanced protein translation escape to such antitumor activity of IFNs and are selected. This characteristic defect of the IFN pathway on tumor cells explains the tumor-selective replication of some IFN-sensitive RNA viruses such as Retro-virus, Newcastle Disease Virus and Vesicular Stomatitis Virus. On the other hand, several RNA and DNA viruses express RNAs or proteins that can inhibit IFN function and the deletion of those genes make them tumor-selective. For example, VAI and VA-II RNAs of adenovirus bind to protein Kinase R (PKR) and inhibit the IFN response to adenovirus. Adenoviruses defective in VA-RNAs are blocked by IFN and grow selectively in tumor cells. The deletion of viral genes that counteract IFN has been used also to design oncolytic Herpes Virus (DNA virus) and Influenza Virus (ARN virus). In contrast to ARN viruses, some DNA viruses such as the adenovirus offer the advantage that they reach the nucleus where they must inactivate tumor suppressor genes to take over cell cycle.This trait offers the unique possibility to design defective viruses that are activated only when tumor suppressors are non-functional. Another interesting design applied to DNA viruses is the replacement of essential virus promoters with cellular promoters that are over-activated in tumor cells. Gene therapy, the transfer of genetic material with a therapeutic intention, has been applied to cancer treatment since the early 90s. Except for immunotherapy; the requirement to deliver the therapeutic DNA or to transduce a great number of tumor cells has precluded success. However gene therapy has contributed to the revival of Virotherapy. Gene therapy and irotherapy merge with oncolytic viruses armed with transgenes. Some use oncolytic vectors to spread the anti-tumor genes and others use transgenes to help virus replication or spread. For example transgenes that promote apoptosis can be used to increase virus spread. Clinical trials have progressed from intratumoral injection of single doses to systemic administration of multiple doses and combination with the chemo and radiotherapy. Major development efforts are dedicated to ARN viruses Reo-virus (Reolysin, Oncolytics Biotech Inc.), Seneca Valley Virus (Neotropix), Measles, and Newcastle Disease Virus (Theravir Inc. and Welstat Biologics), and to DNA viruses Herpes Simplex Virus (Biovex Inc.), Vaccine Virus (Jennerex Inc.), and Adenovirus (DNAtrix Inc., ORCA Therapeutics, Berlex Biosciences, Cell Genesys Inc., Shanghai Sunwaybio).As mentioned, Reo-virus and Newcastle Disease Virus replicate preferentially in cells with an inactive IFN pathway. Seneca Valley Virus, a member of a new type of picornaviruses (senecoviruses), has a high selectivity index (the concentration that kills tumor cells compared to the concentration that kills normal cells) based on the interaction with a receptor enriched in tumor cells. Measles Virus also shows a marked tumor-selectivity at the level of cell receptor. With regard to DNA viruses, Herpes Simplex Virus G207 has been designed to replicate selectively in tumor cells by deleting the gene that inactivates PKR and the gene encoding the viral ribonuclotide reductase.A version armed with GMCSF is also in clinical trials. Vaccines virus JX-594 has been rendered tumor selective by deleting the virus Thymidine Kinase gene and it has been also armed with the immune stimulatory gene GMCSF. Adenovirus selectivity has been obtained deleting the virus gene (E1b-55k) that interact with p53 or the E1b domain that interacts with pRB, and by inserting tumor-selective promoters. Reaching all disseminated tumor cells, coping with the antiviral immune response and spreading through the dense stroma of tumors are key to the success of Virotherapy. The genetic or chemical modification of virus capsids and envelopes and the use of tumor-homing cells loaded with oncolytic viruses is an active area of research to improve the targeting of oncolytic viruses to tumor cells. The clinical data of systemic efficacy with different oncolytic viruses suggest that enveloped viruses that are naturally adapted to disseminate in blood (Vaccines, Measles and NDV) reach tumor metastases easily. On the contrary, non-blood borne naked viruses such as adenovirus interact with blood components and target poorly disseminated tumor cells. The immune response barrier may be alleviated using a transient immune suppression with immune suppressors or chemotherapy. However the current trend towards

viruses armed with immune-stimulatory genes is contrary to this strategy and assumes that the virus alone will not clear out the tumors. Many tumor types are characterized by small groups of tumor cells surrounded by large areas of tumor-associated fibroblasts and connective tissue. In this environment intratumoral spread is blocked. Expression of hyaluronidases and proteases from oncolytic viruses is being explored to solve this problem. Bystanter effects mediated by Prodrug-activation genes could also enhance the spread of oncolytic viruses. Targeted, selective, potent, and armed gene-Virotherapy vectors designed to face these biodistribution, immune and stroma barriers may become antitumor drugs. The most critical task in oncolytic Virotherapy is ensuring the virus chooses the right cells to destroy. In transduction targeting, the virus protein coat is modified so that it targets cancer cells rather than non-cancerous cells; this has been used especially with adenoviruses. In non-transduction targeting, the virus can enter other cells, but has been genetically modified so that it can only reproduce inside the cancer cells. Cox-2, because its expression is elevated in many cancers, is used in non-transduction targeting to promote the reproduction of these viruses. A double-targeting virus, using both transduction and non-transduction methods to target the virus, is the safest and most effective method for oncolytic Virotherapy.Tailored viruses can also be used for other purposes, like the delivery of suicide genes to cancer cells or inhibition of angiogenesis. The most critical barrier to the widespread use of this cancer treatment is the deactivation of the immune system, which quickly develops ways to destroy tailored viruses [16-20] Figure 4 and Table 1.

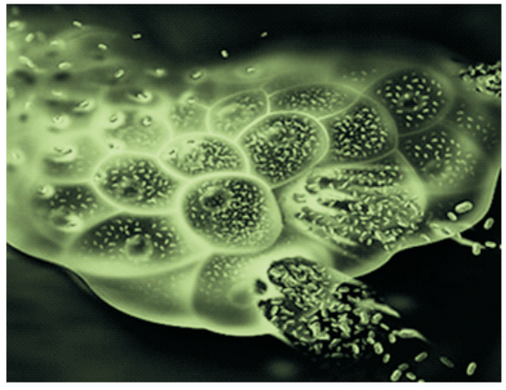

Figure 4: Oncolytic Viruses in Action

Table 1: Advantages and disadvantages of different oncolytic viruses

Virus	Oncolytic strain occurs	Advantages	Disadvantages
HSV1	Laboratory engineered	Can be easily manipulated genetically clinical trial experience; drugs exist to shut off unwanted viral replication.	Side effects include serious or potentially fatal disease unknown action of many HSV1 genes
Adenovirus	Laboratory engineered	Can be manipulated clinical trial experience, good knowledge of viral proton function associated with relatively mild diseases	Replication cannot be easily shut off.
Reo-virus	naturally occurring	Associated with relatively mild diseases, good knowledge of viral gene function	Cannot be easily manipulated genetically, no clinical trials experience
Vaccina Virus	naturally occurring	Associated with relatively mild diseases, clinical trial experience	Undesirable viral replication cannot be easily shutt-off unknown action of many genes,side effects might include potentially fatal or seriously.
Vesicular-stomatitis Virus	naturally occurring	Associated with relatively mild diseases, good knowledge of viral gene function	Cannot be easily manipulated genetically, no clinical trials experience Undesirable viral replication cannot be easily shutt-off unknown action of many genes.
Poliovirus	Laboratory engineered	good knowledge of viral gene function	Cannot be easily manipulated genetically no clinical trials experience Undesirable viral replication cannot be easily shutt-off

Future development of advanced generation adenoviral virotherapeutics agents

The strategy of infectivity enhancement modification by serotype knob switching has allowed dramatic augmentations in gene delivery to tumor targets in preclinical studies, with a specificity that would predict an improved therapeutic index. In particular, the work of Reddy et al. showed that intra-tumoral administration of oncolytic adenoviruses was effective for the treatment of locally confined tumors. As advanced generation adenoviral Virotherapy agents are just entering clinical trials, the gains in therapeutic efficacy through infectivity enhancement strategies should soon become apparent. However, whereas these modifications have shown use to enhance Virotherapy potency based upon CAR-independent tropism, they still are not able to target metastatic disease effectively. In this regard, it may be possible to combine infectivity enhancement with tumor-specific targeting by incorporating high-affinity targeting ligands. This concept of a complex mosaic approach was recently tested by incorporating the RGD motif into HI loop, at the COOH terminus, or both locales of the Ad3 knob, in the context of Ad5/3 chimera fiber, to simultaneously retarget the adenoviral vector to integrins and to the Ad3 attachment receptor CD46. This study showed that complex mosaic modification can function via dual-receptor targeting. The infectivity of an Ad5/Ad3 chimera by insertion of an RGD motif at the COOH terminus of the Ad3 knob. Such strategies to combine the targeting specificities of short peptide ligands together with fiber knob switching of various adenoviral serotypes have the potential for further reduce deleterious side effects and increase the therapeutic index of viro-therapeutics agents in vivo. As their development proceeds, these novel adenoviral Vitrotherapy agents will likely be of universal relevance to a broad spectrum of potential anticancer targets and sites for in vivo gene delivery to patients [21-23] Figure 5.

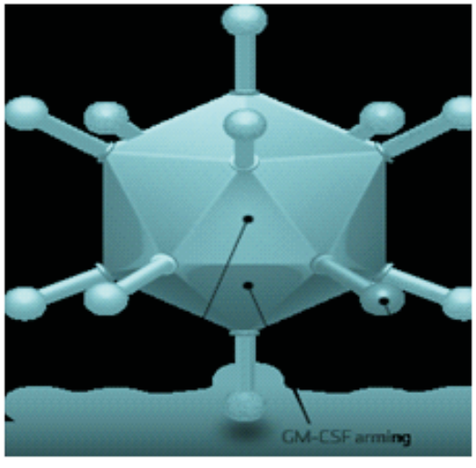

Figure 5: Adenovirus

Advances in OV Technology

Combining OV with standard chemo or radiotherapy

Most tumours have a better response to combination therapies than to a single treatment approach. Clinical trials with ONYX-015 seem to reaffirm this concept. Various preclinical studies have shown that combining chemotherapy with OV administration results in augmented anticancer effects. In a few cases, the rationale for such effects is known. For example, the anticancer and immunosuppressive agent cyclophosphamide facilitates infection of gliomaxenografts by an oncolytic HSV1 by partially inhibiting complement activation against the virus.In another example, treatment with fluoro-deoxy-uridine (FUDR) an inhibitor of cellular thymidylate synthase that is responsible for conversion of DUMP(deoxyuracil monophoshate) and CDP (cytosine diphosphate) to DTTP-stimulates mammalian ribonuclotide reductase activity due to loss of feedback The synergistic effects between OV and some chemotherapies are not always completely understood. For instance, the DNA alkylation agent mitomycin-C acts synergistically with the HSV1 OV 1716 in lung cancer cells, whereas methotrexate, doxorubicin and cisplatin have only additive effects.The synergy was not due to alterations in viral replication, cell-cycle changes or changes in the cellular enzymatic reactions that are responsible for mitomycin-C conversion. Cisplatin also enhanced the effect of another HSV1OV (G207) without inhibiting viral replication, perhaps because of differential mechanisms of action on viral versus cellular DNA. Radiation has also been shown to be effective in combination with OV therapy. Several HSV1 mutants act synergistically with radiation therapy, although in some studies the effect of radiation was primarily additive. The anticancer effects of a combination of an oncolytic adenovirus and radiation were also shown to be more potent than either treatment alone [24].

Improving OV efficacy with cytotoxic genes

OV efficacy can be improved by adding cytotoxic genes to the viral genome, to couple gene-based therapy with viral oncolysis. In this respect, OV might solve one of the problems in gene therapy-the inefficiency of gene delivery. Replication-defective, virus-based, gene-therapy vectors distribute poorly within a tumour mass and therefore fails to distribute such cytotoxic genes efficiently.Experimentally, transgenes delivery by an OV reaches an anatomically larger area of tumour compared with that reached by a replication-defective viral vector. Ganciclovir is a guanosine analogue that is used as a pro-drug to induce suicide of cells transfected with the HSV1 thymidine kinase gene. Including this gene in an HSV1-based OV and combining viral infection of tumour cells with Ganciclovir treatment enhanced its anticancer action in a rat 9L solid brain tumour model, as well as in other models, when compared with OV therapy alone. Similarly, combining a replicating adenovirus that expresses thymidine kinase with Ganciclovir treatment enhanced anticancer activity; however, combining oncolytic viruses that express thymidine kinase with Ganciclovir treatment was not effective in all models.Ganciclovir metabolites are potent inhibitors of both viral and cellular DNA replication. The overall effect of Ganciclovir metabolites (or, for that matter, the effects mediated by a cytotoxic gene other than TK) on viral oncolysis and tumour regression might vary between cells, OVs and anticancer C-DNA strategies. The overall result of Ganciclovir metabolites (or of cytotoxic genes other than TK added to the OV genome) helping or hindering anticancer effects by the replicating OV are complicated and are likely to depend on the replicative ability of the OV, on the presence of a high level of bystander effects and on the mode of action of such metabolites. The Ganciclovir/thymidine inhibition by DTTP. Increased ribonuclotide reductase activity therefore enhances replication of an ul39mutant HSV1. Kinase cytotoxic gene system might provide a fail-safe mechanism that can be added to the OV genome to block unwanted viral replication. C-DNA with more specific anticancer, rather than antiviral, action might therefore provide better choices for addition to the OV genome. For example, the rat cytochrome P450 2B1 gene product metabolizes the pro-drug cyclophosphamide into the active anticancer and immunosuppressive metabolite, phosphoramide mustard. Cyclophosphamide metabolites, however, although causing tumour cytotoxicity, do not eliminate or inhibit viral replicative ability. It is likely that these differential effects might be due to the immunosuppressive properties of the metabolites, which can block the antiviral immune responses. These metabolites also alkylate DNA

every 100–150 kb, thereby causing a greater number of cytotoxic DNA crosslink's in the mammalian cell genome than in the much smaller viral genome. Similarly, anticancer effects are enhanced when the pro-drug 5-FC is combined with an HSV1-based UL39-mutant virus that expresses yeast cytosine deaminase.This enzyme converts 5-FC to the active anticancer agent, 5-fluorouracil (5-FU). This enhancement is possibly due to the fact that one of 5-FU metabolites inhibits cellular thymidylate synthase, thereby decreasing DTTP levels. This, in turn, will remove feedback inhibition on mammalian Ribonuclotide reductase levels, so augmenting UL39-mutant HSV1 replication104. Cyokine C-DNAs have also been added to the viral genome. Vectors that express IL-12, IL-4, granulocyte monocyte colony-stimulating factor (GM-CSF), IFN-γ and soluble B7-1 increased antitumour immune responses, whereas IL-10 antagonized the oncolytic effect.The ability to include anticancer C-DNA in OVs represents the potential for achieving multimodal cancer treatment. OVs that contained two anticancer C-DNA that activated two different anticancer pro-drug were more effective in killing tumour cells when only one pro-drug was added. Even tumour cells that were not infected with the OV were killed by the bystander effects of the anticancer gene products. Levels of therapeutic metabolites that were generated within the tumour by the action of the OV were increased compared with levels in the systemic circulation-even when the pro-drug was delivered locally [25]. Attempts at discovering combinations of pro-drug that act synergistically might also facilitate such efforts.

The effects of the immune system

Another relatively controversial topic relates to the effect of the immune response on viral oncolysis. In some studies, immunosuppression has been shown to improve viral oncolysis, whereas in others a robust immune response produces an antitumor vaccination effect that also improves viral therapeutic effects. The actions of the multiple effectors arms of the antiviral immune response might provide an explanation for these discordant findings.The initial infection and propagation phases of the virus within a tumour are met with hyper acute and acute immune responses that probably limit oncolysis. In rats, transient complement and antibody depletion enhance oncolytic effects in the initial phases of the virus–tumour interaction. The innate immune responses against the viral infection might also increase toxicity to the host because of toxic inflammatory effects of complement activation products, and of elevated cytokine levels, such as IL-6 and TNF-α, as shown in a recent clinical trial.As tumours regress, however, and tumour (and viral) antigens are released into the circulation - these antigens can be presented to and activate CD4+ and CD8+ T cells, and lead to the immune destruction of any residual or subsequent tumours. Pharmacological modulation or genetic alteration of OV can be used to activate or inhibit different immune mediators. This approach could be used to aid the initial phases of viral infection and propagation within tumours, and to elicit long-lasting immune responses against residual and recurrent tumours [26-27].

OV delivery with carrier cells

Carrier cells are also being developed that can be infected with OVs and used to deliver them specifically to tumour cells. Human PA1 tera-to-carcinoma cells support replication of an HSV1 OV to a BURST SIZE of 200 infectious units and, when irradiated, to a burst size of 70 infectious units. Ex vivo infected and irradiated PA1 cells, when injected into the peritoneum of mice with ovarian peritoneal disease, have been reported to localize to tumour cells but not to normal mesothelium. Carrier mediated delivery of OV was therefore associated with increased anticancer effects. In another application, growth-arrested (to avoid virus replication and cell lysis) neural precursor cells were infected ex vivo with an oncolytic HSV1. When injected into rat brains with tumours, the neural precursor cells, which can presumably resume cell-cycle progression (allowing for OV replication and release), migrated extensively within tumour and brain cells that were adjacent to the tumour [28].

OV Genome Stability

The genomic stability of OV in culture or in vivo is also an important issue. Viruses that carry deletions in the HSV γ34.5 gene (the gene product, ICP34.5, inhibits PKR) replicate preferentially in cells that have elevated RAS activity. In culture, however, mutants emerge that carry second-site suppressor mutations that suppress the effects of this mutation. Although these mutants retain oncolytic potency, their emergence is a reminder that DNA rearrangements, mutations and recombination's can occur with any OV in vitro and in vivo. This serves as a note of caution to researchers in the field regarding the possible undesirable generation of unwanted viral strains [29] Fig. 6.

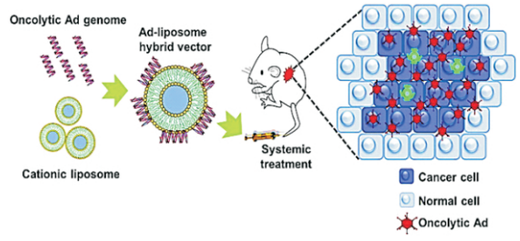

Figure 6: Oncolytic vitrotherapy & genome stability

Applications

Oncolytic vitrotherapy synergism with signalling inhibitors

Rapamycin increases myxoma virus tropism for human tumor cells.Myxoma virus is a rabbit-specific poxvirus pathogen that also exhibits a unique tropism for human tumor cells and is dramatically oncolytic for human cancer xenografts. Most tumor cell lines tested are permissive for myxoma infection in a fashion intimately tied to the activation state of Akt kinase. A host range factor of myxoma virus, M-T5, directly interacts with Akt and mediates myxoma virus tumor cell tropism. mTOR is a regulator of cell growth and metabolism downstream of Akt, and is specifically inhibited by rapamycin. We report that treatment of non-permissive human tumor cell lines that normally restrict myxoma virus replication with rapamycin, dramatically increased virus tropism and spread *in vitro.* This increased myxoma replication is concomitant with global effects on mTOR signalling, specifically an increase in Akt kinase. In contrast to the effects on human cancer cells, rapamycin does not increase myxoma virus replication in rabbit cell lines, or permissive human tumor cell lines with constitutively active Akt [30]. This indicates that rapamycin increases the oncolytic capacity of myxoma virus for human cancer cells by reconfiguring the internal cell signalling environment to one that is optimal for productive virus replication, and suggests a potentially therapeutic synergism between kinase signalling inhibitors and oncolytic poxviruses for cancer treatment [31].

A Telomerase-dependent conditionally replicating adenovirus for selective treatment of cancer

The catalytic component of human telomerase reverse transcriptase (hTERT) is not expressed in most primary somatic human cells, whereas the majority of cancer cells reactivate telomerase by transcriptional up-regulation of hTERT. Several studies demonstrated that the hTERT promoter can be used to restrict gene expression of E1-deleted replication defective adenoviral vectors to telomerase-positive cancer cells. In this study, a conditionally replicating adenovirus (hTERT-Ad) expressing E1A genes under control of a 255-bp hTERT-promoter was constructed. Additionally, an internal ribosomal entry site-enhanced green fluorescent protein cassette was inserted downstream of the E1B locus to monitor viral replication

in vivo. Adenoviral replication of hTERT-Ad and enhancement of enhanced green fluorescent protein expression could be observed in all investigated telomerase-positive tumor cell lines. In contrast, hTERT-Ad infection of telomerase-negative primary human hepatocytes did not result in significant replication. The capability of hTERT-Ad to induce cytopathic effects in tumor cells was comparable with that of adenovirus wild type and significantly higher compared with ONYX-015, regardless of the p53 status of the tumor cells. Single application of low-dose hTERT-Ad to tumor xenografts led to significant inhibition of tumor growth, confirming the potential therapeutic value of conditionally replicative adenoviral vectors. These in vivo experiments also revealed that hTERT-Ad-mediated oncolysis was more efficient than ONYX-015 treatment. These results demonstrate that expression of E1A under transcriptional control of the hTERT promoter is sufficient for effective telomerase-dependent adenovirus replication as a promising perspective for the treatment of the majority of epithelial tumors. According to recent research from the United States, prophylactic alpha interferon treatment increases the therapeutic index of oncolytic vesicularstomatitisvirus (VSV) Vitrotherapy for advanced hepatocellular carcinoma (HCC) in immune-competent rats. "VSV is a negative-strand RNA virus with intrinsic oncolytic specificity due to substantially attenuated antiviral responses in many tumors. We have recently reported that recombinant VSV vector can be used as an effective oncolytic agent to safely treat multifocal HCC in the livers of immune-competent rats via hepatic artery infusion. When administered at doses above the maximum tolerated dose (MTD) [32].

Oncolytic measles viruses for cancer therapy

New strategies using biological agents are being developed to treat cancer. Live viruses are among these new agents. Virotherapy uses replication-competent viral vectors with strong oncolytic properties. With the use of molecular virology techniques, viruses have been genetically engineered to replicate selectively in tumour cells and are under preclinical and clinical investigation at present. Measles virus (MV) is being used for this purpose. Replication-competent attenuated Edmonston B measles vaccine strain (MV-Edm) is non-pathogenic and has potent antitumour activity against several human tumours. The virus is selectively oncolytic in tumour cells, eliciting extensive cell-to-cell fusion and ultimately leading to cell death. Therefore, MV-Edm is a safe and efficient means to kill tumour cells. Further improvements in existing MV vectors may increase tumour selectivity and oncolytic activity. This review discusses the discovery and development of replication-competent oncolytic MV for cancer therapy [33-34].

Conditionally replicative adenovirus for gastrointestinal cancers

The clinical outcome of advanced gastrointestinal (GI) cancers (especially pancreatic and oesophageal cancers) is dismal, despite the advance of conventional therapeutic strategies. Cancer gene therapy is a category of new therapeutics, among which conditionally replicative adenovirus (CRAd) is one promising strategy to overcome existing obstacles of cancer gene therapy. Various CRAds have been developed for GI cancer treatment by taking advantage of the replication biology of adenovirus. Some CRAds have already been tested in clinical trials, but have fallen short of initial expectations. Concerns for clinical applicability include therapeutic potency, replication selectivity and interval end points in clinical trials.In addition, improvement of experimental animal models is needed for a deeper understanding of CRAd biology. Despite these obstacles, CRAds continue to be an exciting area of investigation with great potential for clinical utility. Further virological and oncological research will eventually lead to full realization of the therapeutic potential of CRAds in the field of GI cancers [35-36].

Conclusion

Cells are collected in human cancer patients. Cancer gene therapy is a rapidly maturing field which, without any doubt, will be a part of future cancer therapies. Many of past obstacles and barriers are being actively overcome now. With the advent of genetic engineering and biotechnology, a wide range of viruses are being manipulated and evaluated in various types of cancers. Many clinical trials around the world have had good results with high success rates using oncolytic Virotherapy, and many more clinical trials are in progress with new viral vectors for the treatment of intractable cancers. Significant active research is being done to improve the accessibility, safety and efficacy of oncolytic Virotherapy. Recent advances in molecular biology, and other large-scale genome modification tools, have made it possible that newer oncolytic vectors will be heavily engineered non-viral intracellular parasites, unrecognizable synthetic hybrid vectors, or still yet unforeseen large-scale gene delivery systems.

Conflicts of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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