Review Article

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Oncolytic viruses & their specific targeting to tumour cells

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Cancer is one of the major causes of death worldwide. In spite of achieving significant successes in medical sciences in the past few decades, the number of deaths due to cancer remains unchecked. The conventional chemotherapy and radiotherapy have limited therapeutic index and a plethora of treatment related side effects. This situation has provided an impetus for search of novel therapeutic strategies that can selectively destroy the tumour cells, leaving the normal cells unharmed. Viral oncotherapy is such a promising treatment modality that offers unique opportunity for tumour targeting. Numerous viruses with inherent anti-cancer activity have been identified and are in different phases of clinical trials. In the era of modern biotechnology and with better understanding of cancer biology and virology, it has become feasible to engineer the oncolytic viruses (OVs) to increase their tumour selectivity and enhance their oncolytic activity. In this review, the mechanisms by which oncolytic viruses kill the tumour cells have been discussed as also the development made in virotherapy for cancer treatment with emphasis on their tumour specific targeting.

Key words Apoptosis - oncolytic viruses - tumour targeting - viral oncotherapy

Introduction

Cancer is one of the leading causes of death worldwide. Despite significant progress made in cancer therapies, mortality rates for most malignancies remain distressingly high. The inhibition of cancer growth and progression is one of the major challenges faced by modern medicine. Cancer results either due to decreased cell death or increased cell birth. In other words, decreased propensity for apoptosis contributes to tumour formation[1]. The classical regimen of cancer therapy (chemotherapy, radiotherapy and immunotherapy) suffers with disadvantages such as narrow therapeutic index that further reduces as tumour evolves drug resistance and severe side-effects[2]. Another major problem of cancer therapy is the incomplete eradication of the invasive primary tumour mass or dissemination of tumour cells leading to recurrence of disease. The current goal for developing new therapies for the treatment of cancer is to design therapeutic agents that have a large therapeutic index (*i.e.* high potency against malignant cells) with little or no toxicities to normal cells. Future treatment modalities need to be more selective and specific, allowing higher and thus effective drug doses to reach at each tumour cell[3]. With the advent of modern biotechnology tools and better understanding of cancer biology and virology, it has become feasible to engineer viruses with increased tumour selectivity and enhanced oncolytic activity. Naturally occurring

lytic viruses have evolved to infect, replicate and lyse cells. It is interesting that the replication cycle of many viruses exploits the same cellular pathways that are altered in cancer cells[4]. Specific targeting of tumour cell can be achieved by taking advantage of the fact that tumour cells have altered microenvironment, display certain tumour specific receptors and modified cellular pathways. Gene therapy and viral oncolysis represent treatment modalities that also offer unique opportunities for tumour targeting.

Viruses and apoptosis

Pathogenesis of certain viruses is mediated by the modulation of apoptotic process (induction or suppression) in the homologous host[5,6]. Induction of apoptosis provides mechanism to evade the host cell immune response as during the process of apoptosis, the progeny virions along with the cellular contents of the cell are packaged into membrane enclosed apoptotic vesicles which are rapidly taken up by the neighbouring cells and thus the infection spreads undetected in the host. Virions enclosed within apoptotic vesicles are also protected from inactivation by host antibodies and proteases[5]. On the other hand, many viral gene products possess the ability to delay or suppress the apoptotic death of host cells. This offers several advantages to the virus since it provides time for replication and assembly of progeny virions which otherwise would severely limit the virus production and reduce or eliminate its spread in the host. Thus most viruses have evolved genes encoding proteins that can effectively mediate the induction or suppression of apoptosis by modulating host cell death pathways in order to facilitate their survival and spread (Table)[7]. Rubella virus induced apoptosis in various cell lines appears to be independent of p53 and Bcl-2 family proteins (B-cell lymphoma 2)[8]. Chicken anaemia virus encoded protein "apoptin" induces p53 independent, Bcl-2 dependent apoptosis in many transformed and tumour cell lines[9,10]. Bovine ephemeral fever virus was found to induce caspase dependent apoptosis in BHK-21 and Vero cells[11]. Li *et al*[12] have reported that Tula hantavirus induces apoptosis in Vero E6 cells through caspase-8 activation. However, the apoptotic pathways and the targets within these pathways which viruses strike, vary between viruses and the type of viral proteins mediating it. Further, it also depends on the cell type[6]. For example, E1A of adenovirus associates with the pRb/p300 family of histone acetyl transferases and induces p53 dependent apoptosis in many cancer cells[13]; while adenovirus early region 4 open reading frame (E4orf4) induced apoptosis is p53 independent in mouse embryo fibroblast derived cells[14]. Further, the same proteins (E4orf4) induce apoptosis in 293T cells through caspase-8/(FADD) pathway[15].

Apoptosis and cancer

Evasion of apoptosis is fundamental to tumour initiation, progression and maintenance. Despite the fact that many tumour cells have a defect in the decision machinery for apoptosis, these usually retain an intact execution system and hence if provided with an effective apoptotic signal, such tumour cells will die[45]. Unfortunately, various chemotherapeutic agents fail in inducing apoptosis because functional p53 is required for inducting apoptosis in tumour cells[46]. More than 50 per cent of the human tumours, including melanoma, lung cancer or colon carcinoma contain mutated p53 and patients with such tumours have a very low chance of responding to chemotherapy[47]. Likewise, overexpression of the anti-apoptotic proteins Bcl-2 and Bcl-XL or caspase inhibitors negatively influences the chemotherapeutic treatment of a large number of lymphomas[48].

Considerable efforts are currently being made for the development of new anti-cancer therapies, which are based on the induction of apoptosis and are not hampered by the lack of functional p53 and/or overexpression of antiapoptotic genes[49]. Oncolytic virotherapy is a promising form of cancer therapy which employs nature's own agent to find and destroy malignant cells. It has a large therapeutic index but only limited pathogenicity to normal tissue. The anti-tumour activity of oncolytic viruses can be increased by arming these with therapeutic genes[50].

Virotherapy for treatment of cancer

Viruses are nature's nanoparticle with a diameter ranging from 20 to 500 nm[51]. An oncolytic virus (OV) is a virus having the ability to specifically infect and lyse cancer cells, while leaving normal cells unharmed. Here, certain viral genes act as tumour destructive agent, and the viral capsid as a nano-sized nucleic acid delivery vehicle[52]. In general, oncolytic viruses derive their specificity by exploiting cell surface receptor or intracellular aberrations in gene expression that arise in malignancies during tumour development. The greatest advantage that the oncolytic virus offers over chemotherapeutic agent is its ability to be engineered by *in vitro* genetic manipulation in response to preclinical and clinical findings[2]. The first oncolytic virus used in clinical studies was a vaccine strain of

Table. Viruses and their proteins having ability to modulate apoptosis ….

Virus	Virus proteins	Effector/target molecule	Effect on apoptosis	References
Rubella virus	NK	NK	↑	8
CAV	VP3	Bcl-2; Mitochondria	↑	9, 10
Bovine ephimeral fever virus	NK	Caspase	↑	11
Tula hanta virus	NK	Caspase-8	↑	12
Adenovirus	E1A, E4	p53	↑	13
	E4orf4	Caspase-8/FADD	↑	14
	E1B 55K E1B 19K,	p53	↓	15-19
	E3 14.7K,	Caspase-8	↑	
	E3 10.4/14.5K	Caspase-8 and TNF	↑	
African swine fever virus	LMW5-HL	Mitochondria	↓	20
Bovine papilloma virus	E5, E8	NK	↓	21
Baculovirus	p35 IAP	Caspases	↓	22, 23
Cowpox virus	CrmA	Caspases	↓	24
Epstein Barr virus	LMP1	p53	↓	25, 26
	BHRF1	TNF, Fas	↓	
Hepatitis B virus	pX	p53	↓	27
Hepatitis C virus	Core protein	p53	↓	28
Human cytomegalovirus	IE1, IE2	p53, pRb	↓	29
Herpesvirus saimiri	ORF16 product	Bcl2 and Bax	↓	30
Herpes simplexvirus	γ34.5 gene	NK	↓	31
Gamma herpesviruses	γFLIPs	Fas,TRAIL-R	↓	32
Kaposi's Sarcoma virus	KSbcl-2	Bcl2	↓	33
Human Papilloma virus	E6	p53	↓	34
	E7	pRb	↓	35
	E2	p53	↑	36
Myxomavirus	M11L, T2	Bax, Bak	↓	37
Human immuno-deficiency virus/ Simian immuno-deficiency virus	Tat	Fas L, Bcl2	↑	38, 39
SV40	Large T antigen	pRb,p53	↓	40
Vaccinia virus	SPI-2	NK	↓	41
Parvovirus	Non Structural Protein (NS1)	NK	↑	42
PRRSV	p25	NK	↑	43
Newcastle disease virus	HN	TRAIL-R Mitochondria	↑	44

NK, not known; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; Bak, Bcl-2 homologous antagonist/killer; HN, haemagglutinin neuraminidase; PRRSV, porcine reproductive and respiratory syndrome virus; TRAIL-R, TNF-related apoptosis-inducing ligand-receptor

rabies virus in 1950s for treatment of patients with melanomatosis, and 8 of 30 patients showed tumour regression[53]. Thereafter, the oncolytic activity of several other viruses was tested in various animal and human models for their anti-cancer potency, tumour specificity and safety[54]. These include West Nile virus strain Egypt 101, mumps, Newcastle disease, measles, autonomous parvo, adeno, reo, vesicular stomatitis, herpes simplex and entero viruses[55]. However, many viruses elicited side effects which ultimately ended the trials and interest in viruses as anti-cancer agents declined during the 1970s. With the advent of modern biotechnology, gene therapy and better understanding of cancer biology, there is resurgence of interest in viral therapy in cancer treatment[44,56]. The field has come a long way from the original pioneering research with Herpes simplex virus type-I to the 1st multiple clinical trials with adenovirus ONYX-015[57]. The world's first oncolytic virus approved by China's State Food & Drug Administration in 2005, was a genetically modified adenovirus-H101 type 5[52], in which E-1B-55 kD and partial E3 genes have been deleted. Though both DNA and RNA viruses have been used in oncolytic virotherapy, DNA viruses are more frequently used as these are more amenable to genetic manipulation[56].

Criteria for selection of oncolytic viruses for cancer therapy

Although viral oncotherapy has great potential for cancer treatment, yet for successful application, the viruses have to meet stringent criteria for safety and efficacy.

Safety: To design a safe viral oncolytic agents, certain criteria should be given due consideration. These include cancer specificity, chances of regaining pathogenecity, possibility of transmission to healthy individual, undesired side effects and pre-existing immunity[58]. The use of non human viruses in viral oncotherapy would help in minimizing these risk factors.

Efficacy: Efficacy of OVs can be enhanced by developing strategies for efficient delivery of viruses and overcoming the host antiviral immune response. Approaches to evade antiviral response include serotype switching *i.e.* administration of different viral serotypes during treatment cycle[59], and polymer coating (modification of amino groups by mixing viral particles with polymers such as poly [N-(2-hydroxypropyl)methacrylamide] (Phpma) bearing reactive 4-nitrophenyl esters on pendent diglycyl side chains) so that antibody cannot recognize the virus particle and use of cellular vehicles[52].

Strategies for tumour targeting

Cancer cells distinguish themselves from their normal counterparts by alterations in cell physiology such as self sufficiency in growth signals, insensitivity to growth inhibition signals, evasion of apoptosis, limitless replication potential, sustained angiogenesis and tissue invasion and metastasis. These alterations make these a generous host for viruses and hence these properties can be utilized for selective replication of OVs in cancer cells. These cancer targeting mechanisms of viruses can be broadly achieved by two general approaches *i.e.* deletion of viral genes required for virus replication in normal cells but dispensable in tumour cells and use of tissue/tumour specific promoters for critical viral genes. The specific tumour targeting can be achieved by targeting various molecular steps/ regulators of cell cycle. Some of these are discussed below:

(i) Pro apoptotic targeting: Many viruses delay apoptosis of infected cells in order to assist their replication. These encode certain proteins which alter the activity of important regulators of programmed cell death such as p53 and pRb[60]. Adenoviral proteins E1A and E1B inactivate pRb and p53 in normal cells, respectively, to delay premature apoptosis[52,61]. A virus having deletion in E1 can be rendered tumour specific. The examples of this type of mutants are *ONYX-15* having mutation in *E1B* (dl 922-947) and *HB101* having deletions in two viral genes- *E1B* and *E3*[62]. Further, certain type of cancer cells express cellular E1A like activity and therefore, E1A deleted adenovirus can efficiently replicate in such cancers, *e.g.* HepG1 and Hep3B[63]. Some viral and non viral proteins are known to induce p53 independent apoptosis in tumour cells[9]. Proteins derived from viruses, *i.e.* chicken anaemia virus derived apoptosis-inducing protein (apoptin), E4orf4 and parvovirus-H1 derived non-structural protein 1 (NS1), torque teno virus derived protein TTV apoptosis inducing protein (TAIP), the human α-lactalbumin made lethal to tumour cells (HAMLET), which is present in human milk or the human cytokines melanoma differentiation-associated gene-7 (mda-7) and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), have the ability to induce tumour-selective apoptosis[64].

(ii) Translational targeting: The response of a cell to virus infection, dsRNA and lipopolysacharide is production of Type I interferon (IFN). This shut downs the protein synthesis in the neighbouring cell, rendering these unfit for virus replication. However, most of the

cancer cells have defective IFN signaling pathways, so one strategy to enhance tumour specificity is to mutate OVs to induce a more potent IFN response[52]. This will minimize the replication of such viruses in normal cells but the cancer cells will remain permissive. Also some viruses block IFN signaling by encoding protein which inhibits the translation of mRNA for IFN, *e.g.* matrix (M) protein of vesicular stomatitis virus (VSV). Such viruses can be rendered tumour specific by mutating the genes encoding the protein inhibiting IFN release. The non pathogenic VSVs can still multiply in tumour cells as these lack ability to produce and respond to IFN. The inherent oncolytic activity of Newcastle disease virus (NDV) was also believed to be derived from defective IFN signaling pathways in tumour cells[65]. However, recent studies suggested that the tumour specificity of NDV is independent of type I IFN response and is due to the tumour cell resistance to apoptosis[66,67].

HSV-1 can be made tumour selective by mutating the γ_1*34.5* gene (designated as R3616). The product of this gene (ICP34.5) binds with protein phosphatase-1 and inhibits phosphorylation of eukarykotic initiation factor-2 (eIF-2) by activated PKR (ds RNA induced protein kinase). This unphosphorylated eIF-2 cannot inhibit translation of viral transcripts unlike its phosphorylated counterpart. Cancer cells are resistant to the PKR activated inhibition of viral replication due to the high level of Ras activity which inhibits autophosphorylation of PKR. Thus mutant having deleted $\gamma_1$34.5 cannot multiply in normal cells but tumour cells remain permissive[68]. Tumour specificity can be further improved by a second mutation in the UL39 gene (G207, a neuroathenuated, replication competent HSV-1 with deletions in both copies of $\gamma_1$34.5 gene and UL39 gene), encoding the large subunit of the viral ribonucleotide reductase (ICP6). This further compels the virus to multiply in cancer cell with high endogenous ribonucleotide reductase.

(iii) Transcriptional targeting: Oncolytic viruses can be rendered tumour selective by placing essential viral gene under the regulation of tumour specific promoter[69]. However, this technique is limited to DNA viruses (excluding pox viruses). Certain tumour specific gene promoters like human telomerase reverse transcriptase (hTERT) and survivin are active in a variety of tumour types while others are specific for particular tumours, *e.g.* Prostrate specific antigen (PSA) for prostrate, foetoprotein for liver and tyrosinase for skin[3]. Activity of these promoters can be further augmented by binary regulatory system with recombinant Gal 4 fusion protein[52].

Survivin is overexpressed in squamous carcinoma cell which makes it possible to use therapies specifically targeting this gene. Adenoviruses can be made tumour selective by placing essential viral gene (E1) under the control of human E2F-1 promoter[60,61] which is selectively activated in tumour cells with a defect in Rb pathway. Replication-competent adenoviruses that have restricted expression of the *E1A* and *E1B* genes have been produced using prostate-specific promoters, such as the prostate-specific antigen (PSA) promoter, the probasin promoter and combinations of both (*e.g.,* CV706 and CG0787) for prostate carcinomas[70]. Selective expression of E1A has been attempted in specific carcinomas, such as hepatocellular carcinoma (α-foetoprotein promoter) and breast carcinoma (mucin-1 promoter and estrogen-receptor promoter). In addition, the general characteristics of tumour cells, *e.g.* telomerase promoter and hypoxia-inducible factor (HIF) responsive elements have been used to design oncolytic adenoviruses[71]. In case of herpes viruses, transcriptional targeting is achieved by placing the $\gamma_1$34.1 gene under the control of tumour specific promoter[72]. The expression of the essential immediate-early *ICP4* gene under control of the albumin-enhancer-promoter, limits replication of HSV to the liver and to hepatocellular carcinoma[56]. Similarly, the calponin promoter has been used to generate HSV mutants that replicate selectively in malignant human soft tissue and bone tumours. For retroviruses, the U3 gene which promotes enhancer region in the long terminal repeat has been replaced[72].

(iv) Transductional targeting: The fact that tumour cells display high level of tumour specific receptors can be exploited for targeting of oncolytic viruses specifically to cancer cells[73]. For instance, many cancer cells overexpress intra cellular adhesion molecule-1 (ICAM-1) and decay accelerating factor (DAF), the receptors for coxsackievirus A21(CAV_{21})[74]. Another enterovirus, echovirus type 1, gains preferential entry to ovarian cancer cells due to overexpression of the I domain of integrin a2b1[75], and poliovirus infects cells expressing the CD155 receptor, abundant on many human cancer cell types[76]. However, for viruses which utilize receptors that are abundant on normal cells, the mechanisms that govern preferential replication in cancer cells are probably intracellular[55]. For example, despite that mumps viruses use sialic acid as their receptor and alphaviruses use heparin sulphate or ICAM-1, all abundantly expressed also on many normal cells, these viruses show high cancer cell selectivity due to defective interferon (IFN) signaling pathway

in tumour cells[77]. Alternatively, tumour specificity of viruses can be reprogrammed by displaying single chain antibodies or other polypeptide binding ligands on the viral surface.

(v) Targeting strategies based on tumour microenvironment: To support uncontrolled growth and tissue invasion, tumour cells develop a modified microenvironment such as hypoxia, activation of certain proteases and angiogenesis. This can be harnessed for developing strategies for tumour targeting[69]. A dual regulated oncolytic Ad CNHK500 was developed in which the *E1b* gene is controlled by a hypoxia responsive promoter and the *E1a* gene is controlled by an human telomerase reverse transcriptase (hTERT) promoter[56]. Tumour selectivity might be further improved by incorporation of hypoxia-responsive elements into tumour-specific promoters to exploit the relatively hypoxic conditions within a tumour[78]. Vesicular stomatitis virus (VSV) is known to have an inherent capacity of replication under hypoxic tumour environment. Another approach to develop tumour specific oncolytic viruses is based on utilization of epithelial cell specific promoters to control the expression of important viral genes.

(vi) Targeting tumour using carrier cells as cellular vehicle for oncolytic viruses: Cancer cell secretes a number of chemokines which helps in trafficking of immune cells to tumour. These immune cells can be used as cellular vehicle for efficient delivery of OVs to tumour cells. Other types of cells such as stem cells (mesenchymal, endothelial progenitor cells) have also been developed as cellular vehicles to deliver OVs[79]. Cellular vehicles not only deliver the OVs to tumour site but also help in overcoming the problem of pre-existing antiviral immunity[78]. Specific delivery of HSV-1, adeno virus, VSV, parvovirus, measles virus and vaccinia virus has been achieved by utilizing carrier cells[56,80]. Manipulation of chemokine-chemokine receptor system can be harnessed for tumour targeting. The tumour derived chemokine RANTES (regulated and normal T cell expressed and secreted; also known as CCL5) (CCL5) attracts CD4+ T, CD8+ T and NK cells to tumour site[56]. T cells can be recruited to tumour site by genetic engineering of these cells to express chemokine receptors such as CXR2. Tumour cells can also be used as carriers to deliver an OV. This was first demonstrated in a regional delivery of replication-selective HSV-1 for the intraperitoneal therapy of epithelial ovarian cancer[81]. The tumour carrier cells can be inactivated by gamma-irradiation after infection with the OV. This does not affect the production and release of oncolytic parvovirus[82].

Representative oncolytic viruses

Adenovirus: Adenoviruses were first isolated in 1953 from cultures of human adenoid tissues and were one of the first vector systems to be developed for gene delivery and expression. Adenovirus has emerged as one of the most potential viral therapeutic agent in cancer therapy. In the late 1950s, adenoviruses were reported to induce apoptosis in HeLa cells. Since then, clinical trials have begun on different experimental models both *in vivo* and *in vitro*[83]. Recently, genetically engineered mutants of the virus have been created, which are capable of killing tumour cells, sparing their normal counterparts. The adenoviral genome consists of linear, dsDNA of 26-45 kb. It is a non-enveloped, icosahedral virus with capsid of 65-80 nm diameter. The structural elements of capsid comprise penton, hexon and fibre proteins which play a crucial role in virus entry into the cells. Adenovirus entry into the cells occurs by receptor-mediated endocytosis involving different receptors. The capsid structural elements such as penton recognize and bind with the primary receptor, the coxsackie-adenovirus receptor (CAR) resulting in internalization of the virus and endocytosis by clathrin coated pits and subsequent lysis of the endosomes releases virus particles in the infected cell[84]. In this process, a series of viral proteins co-operate to promote efficient replication of the virus and its release. These major viral proteins include E1A, E1B-55kD, E1B-19kD, E3-11.6 kD and other associated proteins[85]. The two genes particularly, *E1A* and *E1B* (E1B-55kD) have been the targets of modification in order to create tumour-specific viruses. In normal circumstances, the products of these genes act in concert to force the host cell to enter S phase which is a prerequisite for the viral replication process. Thus, deletion of *E1A* will render the virus susceptible to the antiviral mechanisms of the retinoblastoma protein (Rb, a tumour suppressor protein), specifically by blocking the G1 to S transition. On the other hand, deletion of *E1B*, allows p53 to induce apoptosis in infected cells, aborting replication and spread of the virus. Therefore, the productive replication of adenoviral E1-deletion mutants can only take place in cells negative in Rb and p53. Most of the cancer cells fulfill these requirements and hence become selective targets for oncolysis by adenoviral E1-deletion mutants[86].

A number of conditional replicating mutants have been engineered to target cancer cells that have

mutations or defects in the p53 and Rb pathway. One such mutant of adenovirus, dl1520 (ONYX-015/ CI-1042) has shown promising results as anti-cancer agent in clinical trial conducted in mouse and humans and it was the first engineered replication selective virus to be used in humans[87]. It was proven to be a safe agent in phase I and II trials for the treatment of patients with squamous cell carcinoma of the head and neck[83]. These mutants have deletion of 827 bp in the E1B, an important adenovirus protein which helps in replication of the virus by suppressing the activity of p53[88]. Though these mutants have increased specificity for tumour cells, it is reported that these mutants also replicates in tumour cell line with wild type p53 activity indicating that p53 status may not play an important role in apoptosis and the most likely explanation may be due to late viral RNA export rather than p53 activity[89]. Another mutant dl 922-947 has deletions of 845 bp of the E1A proteins which normally play an essential role in binding and inhibiting the Rb group of proteins. This mutant primarily targets cancer cells defective in the Rb pathway and has shown greater efficacy than the similar mutant AdA-24 which contains a 24 bp deletion in E1A[90]. Studies conducted in mice have demonstrated improved efficacy of these mutants when combined with chemotherapy. Some engineered adenovirus constructs have been developed and many more are in the pipeline in different laboratories worldwide for use as oncolytic agents. Oncolytic adenoviruses have been used in clinical trials for various cancer types such as glioma, ovarian cancer, pancreatic cancer, prostrate cancer and colorecteal cancer[91,92].

Chicken anaemia virus (CAV): CAV is a non enveloped virus which belongs to genus *Gyrovirus* of the family *Circoviridae*. It is one of the smallest known avian viruses with 23-25 nm size. The genome of the virus consists of long circular, single stranded minus-strand DNA of around 2319 bp[93]. It has three partially overlapping open reading frames (ORFs) - ORF-1/C1 (nucleotide position 853-2199), ORF-2/C2 (nucleotide position 380-1027) and ORF-3/C3 (nucleotide position 486-848) which on transcription produces an unspliced polycistronic mRNA of about 2 kb, encoding proteins of 51.6, 24.0 and 13.6 kDa proteins representing VP1, VP2 and VP3 proteins, respectively[94]. The VP3 also known as apoptin is a serine-threonine rich protein of 121 amino acids. Among the major proteins of CAV, VP2 and VP3 are known to induce apoptosis in infected cells. The apoptotic activity of VP2 is much weaker than VP3. Further, VP2 induces apoptosis both in normal and tumour cells but apoptin induces p53-independent apoptosis specifically in tumour cells[95]. Apoptin acts as a multimeric complex and forms super structures upon binding to DNA. In tumour cells, apoptin is phosphorylated and mainly nuclear whereas in normal cells it is unphosphorylated, cytoplasmic, and becomes readily neutralized[62]. Interestingly, apoptin phosphorylation, nuclear translocation, and apoptosis can transiently be induced in normal cells by co-transfecting SV40 large T oncogene, indicating that apoptin recognizes early stages of oncogenic transformation[96]. In cancer cells, apoptin appears to recognize survival signals, which redirect it into cell death impulses. Apoptin targets include DEDAF, Nur77, Nimi, Hippi, PML and the potential drug target anaphase promoting complex (APC1)[62]. The following properties of apoptin makes it a potent candidate for viral oncotherapy - *(i)* selective apoptotic activity against transformed and cancer cells but not in normal diploid ceils, implies that side-effects of apoptin treatment may be minor[97], *(ii)* apoptin senses early stages of oncogenic transformation, *(iii)* apoptin mediated cell death is independent of death receptors, as cells deficient in Fas Associated Death Domain (FADD) or caspase-8, the key regulators of the extrinsic apoptotic pathway, remain sensitive to apoptin[98], *(iv)* apoptin acts independently of p53, the most commonly mutated tumour suppressor gene in cancer, *(v)* its apoptotic activity is stimulated by antiapoptotic Bcl-2 protein[99]. Overexpression of antiapoptotic Bcl-2 and Bcl-XL probably occurs in more than half of all cancers leading to development of therapeutic resistance, and *(vi)* its apoptotic activity is insensitive to BCR-ABL[99], indicating that apoptin can induce apoptosis in cases where present chemotherapeutic agents fail. Further, systemic delivery of apoptin reveals the safety and efficiency of apoptin as an anti-cancer therapeutic agent[100]. Olijslagers *et al*[101] reported that chemotherapeutic agents when combined with apoptin resulted in enhanced cytotoxicity. Higher induction of an effective anti-tumor immune response and tumour regression was found when apoptin was used in combination with interleukin (IL)-18[102]. The anti-neoplastic effect of apoptin has been verified in Rous sarcoma virus induced tumour in chicken[103]. In our laboratory, we have found that the apoptin induced death of HeLa cells is mainly mediated by the intrinsic/ mitochondrial pathway of apoptosis[104].

Parvovirus: The genus *Parvovirus* comprised many important pathogens of human and animals that include B19 human parvovirus, feline panleukopenia virus (FPLV), canine parvovirus (CPV), mink enteritis virus

(MEV), *etc*. The cell death in parvovirus infection is mainly due to apoptosis[105]. Members of *Parvoviridae* family like B-19, feline panleukopenia viruses, have an inherent property of oncolysis that could be used for cancer virotherapy[106]. Recent studies have shown that various species of parvovirus can induce apoptosis *in vitro* in different cell types such as haematopoietic cells, lymphocytes and glioblastoma cells[107]. Sol *et al*[108] have reported that B19 human parvovirus induced apoptosis in erythroid cells can be attributed to the expression of non-structural proteins. Non-structural proteins from murine and human parvoviruses promote cell death when expressed in isolation in cell lines[106]. The non-structural proteins (NS1) of B19 human parvovirus, which are highly conserved among parvoviruses, promote apoptosis in erythroid cells through caspase-3 activation[42]. However, its mode of action remains unclear but it appears to be a nuclear protein capable of binding DNA which can alter the synthesis and phosphorylation of a number of cellular proteins. Parvovirus induced apoptotic cell death is one of the key pathogenic mechanisms in causing damage to digestive tract epithelia, lymphoid and haematopoietic tissues in parvovirus-infected cats and dogs[109]. The antineoplastic activity of parvoviruses appears to be linked to loss of genetic stability in tumour cells. Parvovirus DNA replication appears to select against cells bearing mutated or inactivated p53. It has been reported that SV40 virus transformed human fibroblasts were more susceptible to parvovirus infection and lysis than were normal control cells[110].

It is reported that antineoplastic activity of oncolytic viruses is not only by direct killing of the cells but also by modulating the production of immunoregulatory molecules. Evidences suggest that *in vivo* H1 parvovirus infected irradiated tumour cells when administered parentally not only contribute to reduce tumour load by causing direct lysis of neoplastic cells but also by stimulating their recognition as therapeutic vaccine by the immune system[111]. It was also found that infection of human melanoma cells with this virus results in activation of co-cultured dendritic cells and ensuing cross-priming of cytolytic T-cell response[112]. It was also hypothesized that virus can activate tumour cells to reduce immuno-stimulating factors and/or kill these in a way that promote the uptake of tumour antigens[113].

In a monocytic cell line, the apoptotic pathway triggered by H1 virus appears to share at least some steps of the pathway activated by tumour necrosis factor-α (TNF- α), which is accompanied by a rapid and marked downregulation of c-*Myc* expression prior to the appearance of apoptotic signs such as activation of caspase-3, cleavage of poly(ADP)-ribose-polymerase (PARP) and occurrence of apoptotic bodies. This suggests that c-*Myc* or factors regulated in a similar way could play a key role in the signaling of apoptosis induced by parvoviruses[107].

Toolan *et al*[113] first tested the oncolytic potential of H-1PV in two patients diagnosed with advanced osteosarcoma. An estimated dose of 10^9 plaque-forming units (PFU) was administered by intramuscular route. Later, a series of clinical trials were performed using H-1PV in cutaneous metastases from different solid tumors (breast cancers, melanomas, bronchial carcinoma, pancreatic adenocarcinoma and kidney leiomyosarcoma)[114]. Rommelaere *et al*[115] have recently reviewed the oncolytic effect of parvoviruses.

Herpes simplex virus (HSV): HSV is an enveloped, dsDNA virus with 152 kb long genome. The transcription, replication and packaging of HSV take place in the nuclei of infected cells. In cells permissive for this virus, replication cycle is usually completed with in 20 h, releasing thousands of viral progeny on cell lysis. The virus enters into the cell by fusion of the viral envelope proteins with the host cell plasma membrane. The viral protein gC and gB are required for binding of the virus to host cells receptor whereas gD plays a major role in virus entry[116]. The genome of HSV-1 consists of three major gene regions- alpha, beta, and gamma and each of the genes act co-operatively to regulate viral entry, replication and multiplication in host cells. The alpha genes are transcribed in early infection in absence of *de novo* protein synthesis and these products regulate transcription of beta and gamma genes. The beta genes products are important in viral nucleic acid metabolism and are also required for viral DNA replication[117]. The HSV gamma genes have been divided into two subgroups, gamma 1 and gamma 2, which encode different proteins of each type. The gamma 1 genes are expressed in early infection and do not depend on viral DNA synthesis in contrast to gamma 2 genes that are lately expressed and are dependent on viral DNA synthesis and hence can be blocked by inhibitors of viral DNA synthesis. The HSV cycle is usually completed by assembly of virus followed by their release into extracellular space[118].

To specifically target HSV replication to neoplastic cells, a variety of mutants have been designed with functional inactivation of the viral genes that encode for

thymidine kinase, ribonucleotide reductase and infected cell protein 34.5 (ICP34.5)[119]. In quiescent cells, cellular forms of viral ribonucleotide reductase and thymidine kinase are not expressed but are upregulated only during the G1 and S phases of the cell cycle because these generate dNTPs required for DNA synthesis. This limits replication of HSV which is defective in such gene functions to rapidly proliferating cells, such as tumour cells[120]. The neurovirulence factor ICP34.5 has been characterized as an inhibitor of dsRNA dependent protein kinase (PKR). Therefore, PKR-induced shut-off of cellular protein synthesis following infection with HSV is circumvented by ICP34.5. However, Ras activity directly inhibits PKR-mediated effects, and thus the action of ICP34.5 is not required in cell lines that have a constitutively activated Ras signaling pathway[67] and so to reduce the probability of the occurrence of wild-type revertants and to increase the safety margin, some viruses have been created which contain multiple mutations within their genomes[55,121] .

R3616 a deletion mutant of HSV-1 virus, has been constructed that is capable of replicating in cancer cells with high Ras activity[65]. Another mutant, G207 was developed in which mutation was done in the *UL 39* gene which encodes for the large subunit of viral ribonucleotide reductase[122]. These mutants confirm the possibility of any viral or cellular mutation which might specifically eliminate the effects of ICP34.5 deletion and render the virus to replicate preferentially in proliferating cancer cells with high endogenous activity of ribonucleotide reductase. Phase I studies have demonstrated viral replication following direct injection of these mutants into malignant brain tumours, is also safe and there is some evidence of clinical efficacy, indicating this to be a potentially useful adjuvant therapy[119,123].

Newcastle disease virus (NDV): Newcastle disease virus (NDV) also known as avian paramyxovirus-1 belongs to the genus *Avulavirus* of the family *Paramyxoviridae*. The NDV genome is 15,186 nucleotides long single stranded, negative-sense RNA which contains six genes encoding at least eight proteins- six structural (NP, P, M, F, HN, L) and two non-structural (V and W)[124]. NDV binds cells via the haemagglutinin neuraminidase (HN) protein, which attaches to sialic acid-containing host cell receptors[125]. Binding is followed by fusion of viral and cell-surface membranes, a process mediated by F protein. The viral RNA is then released into the cytoplasm and undergoes replication.

NDV is known to cause apoptosis in different cell types including chicken embryo fibroblast (CEF) cells and peripheral blood mononuclear cells[126,127]. The evidence of induction of apoptosis by NDV implies its role in the pathogenesis[44]. A study by Kommers *et al*[128], based on the simultaneous positive staining for apoptosis and viral nucleoprotein in lymphoid tissues suggested that the lymphoid depletion observed in ND may be due to apoptosis mediated by NP. However, in another report, it was revealed that the HN protein alone is capable of upregulating the expression of interferon-alpha and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) in the target cells[129]. In our laboratory, we have demonstrated that HN protein, when expressed alone, causes apoptosis in CEF cells that requires activation of caspases, loss of mitochondrial membrane potential and augmentation of oxidative stress. Our study suggested that at early stage of ND infection, death receptor mediated pathways play role in inducing apoptosis in Vero cells which is shifted to mitochondria mediated pathways in later stage of infection. We also found that for NDV to cause apoptosis of Vero cells, *de novo* synthesis of protein after NDV infection is necessary[130]. Oncolytic potential of NDV and its HN gene on Rous sarcoma virus induced tumour in chicken indicated that NDV has anti-tumour effect which is stimulated when *HN* gene is given along with the intact virus indicating synergistic effect of NDV on tumour regression compared to either NDV or HN alone[15,131]. An attenuated strain of NDV, PV701 was used in phase I clinical trial and it was shown that when administered intravenously, PV701 replicates specifically in tumour cells and causes cytolysis of tumours of epithelial origin (carcinomas including breast, lung, prostate and colon) and of neuroectodermal (melanomas, glioblastmas and neuroblastomas) and mesenchymal origin (sarcomas). In all the cases, survival rate was encouraging with minimal side effects[132]. A strain of oncolytic Newcastle disease virus-NDV-HUJ was found to overcome the antiapoptotic effect of Livin (member of inhibitor of apoptosis protein family) in melanoma cells. The study suggested that NDV-HUJ is a potent inducer of apoptosis that activates caspases, causes cleavage of Livin converting it into the proapoptotic tLivin protein[66]. In future the use of such oncolytic viruses may provide a suitable alternative for management of cancers.

Conclusion and future perspectives

The revolution in molecular and cancer biology and better understanding of viruses and host cell interaction

in the past decade has facilitated the development of rationally designed, targeted cancer therapies. The initial results of clinical trials using OVs are encouraging. There are many OVs currently under development. Tumour specific targeting remains a challenge but pre-clinical data indicate that transcriptional targeting might be achieved using tumour specific promoters. Microarray technology and proteomics will help in identifying novel tumour specific targets. Considering the heterogeneity of tumour in a patient and among patients a combination of OVs along with conventional therapy regimen of cancer such as radiation, chemotherapy, immunotherapy and gene therapy will be useful in combating cancer.

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