Oncolytic viruses - A Novel therapeutic agents for Head and Neck cancer treatment

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Abstract:

The first oncolytic virus for cancer treatment was approved in 2005 with combination of chemotherapy, was used for the treatment of upper nasal cancers in China. It was done by the Shanghai Sunway Biotech's, they genetically modified adenovirus H101. Oncolytic viruses target particular effected cell and does not harm human normal tissues. Over the past 15 years, new degrees into the molecular mechanisms of viral cytotoxicity have made available the scientific rationale to design more effective oncolytic viruses. New trials using genetically engineered or genetically modified viral strains of viruses, i.e. adenovirus (ONYX-015 and CV706) and herpes simplex virus 1 along with wild-type Newcastle disease virus (PV701) have been promoting, representing these viruses to be relatively non-toxic and tumor specific. This review article discuss about activity of oncolytic viruses in treatment of head and neck cancer. SEPREHVIR (HSV-1716) completed I phase in OSCC of head and neck cancer. It is also tested for phase III trial in head and neck cancer. Oncolytic viruses will continue repeatedly researched because of their therapeutic outcomes, particularly when used in combination with other treatments such as chemotherapy.

Keywords: Oncolytic Virus, head and neck cancer, Oral cancer, Oncolytic virotherapy**.**

Introduction:

Oncolytic viruses came in the frame as a unique platform for the treatment of cancer. Oncolytic viruses may be tumor selective in their wild type or attenuated forms, or may be genetically modified to provide or enhance tumor selectivity (Parato et al, 2005). The potential utility of oncolytic viruses as anticancer therapy is based upon the concept that replication of the virus within the tumor will amplify viral load and enhance anti-tumor potency. Oncolytic viruses are self-replicating viruses which can target and lyse cancer cells specifically (Prestwich RJ et al, 2008). Oncolytic viruses involved in initial cancer therapeutics are non-pathogenic, naturally occurring mutants. Specificity to cancer is determined by tumor-specific genetic mutations that result in aberrant protein expression. Oncolytic virotherapy have a variety of natural occurring and genetically modified viruses. Natural occurring oncolytic viruses are chosen for their low pathogenicity and inherent specificity for tumor cells (Cervantes-Garcia D et al, 2008). Vesicular stomatitis virus, Reovirus, Newcastle disease virus (NDV), Myxoma viruses are natural occurring viruses. Genetically modified viruses are those that are modified to promote tumor specificity, for example through use of tumor-specific promoters and gene deletions, or reduce pathogenicity by serial passage through cell culture (Wong HH et al, 2010). Genetically modified oncolytic viruses are Adenovirus, Herpes simplex virus, Vaccinia virus and Measles virus. This review article discusses about the oncolytic viral agents involved in HNSCC (head and neck cancer) and also discuss the strategies and advancement. The sixth most common cancer in the world and a major cause of morbidity is Squamous Cell Carcinoma (SCC) (Parkin DM et al, 1999). Oncolytic viruses are live viruses that selectively kill cancer cells. Shanghai Sunway Biotech expects to begin marketing H101 in July for treating head and neck cancer. Treatment of oral squamous cell carcinoma has originally relied on classical curative techniques such as surgery, radiation, and chemotherapy or a method with combination of these techniques. Oncolytic viruses do not harm the normal cells, while it destroys the malignant cells.

History of conventional cancer therapies to Oncolytic Viruses:

Here Historical perspective is provided on the numerous approaches that were explored before the modern era of virus engineering through reverse genetics to develop non-pathogenic viruses selectively destructive to human tumor tissue. Implementation of Viruses started for cancer therapy at the end of the 19th century, but contempt numerous of infectious diseases are known to be of viral etiology at that time, yet there was no authentic concept of nature of a virus. Discovery of X-rays by Roentgen in 1898 and their subsequent application in treating cancer, radiation therapy became all the range (Bierman HR et al, 1953). His report was published on X-rays (Bernier J et al, 2004), for radiotherapy that eclipsed surgery as a preferred method of treatment in many cancers, including head and neck cancer (McGurk M et al, 2009). Large doses of radiation given were quantified by the extent of tissue damage to approach where the tumor was irradiated more specifically (Bierman HR et al, 1953). Chemotherapy blossomed seemingly overnight with the introduction of folic acid antagonist aminopterin for treatment of leukemia (Farber S et al 1948). Chemotherapy indeed was an effective modality for leukemia treatment. Understanding of viruses accelerated rapidly in the 1950s and 1960s, in large measure because of the advent of cell and tissue culture systems that allowed *ex vivo* virus propagation (Gey C et al, 1949). It is no coincidence that this was also a time of intensive virotherapy research when the oncolytic properties of numerous viruses were evaluated, first in human tumor cell lines, often implanted in immune-suppressed rodents, and subsequently in humans. During the past fifty years, viruses have been studied with such unparalleled intensity that their biology is now understood more thoroughly than that of any other organism in nature. Their genomes and proteins have been sequenced; their physical structures are known, as are many of the mechanisms where by their genomes are

regulated; their diversity is recognized; their replication cycles and pathogenic strategies have been elucidated; and methods have been developed to manipulate their genome sequences to permit their further refinement as anticancer agents. For more than a hundred years, viruses have been pursued as experimental agents of cancer destruction. Interest in the field has fluctuated during this time, reaching fever pitch in the 1950s and 1960s, followed by near-abandonment in the 1970s and 1980s, and a resurgence of interest in the past two decades, culminating in the first marketing approval of an oncolytic virus, granted by Chinese regulators for the genetically modified oncolytic adenovirus H101 (Garber K, 2006).

Viruses in recent research:

The adenovirus is a non-enveloped, linear, double-stranded DNA virus with a genome size of approximately 30–38 kb. These viruses consist of 60–90 nm non-enveloped particles with icosahedral symmetry. The virus was initially isolated from adenoid cell cultures in the 1950s, and was thus named "adenovirus". Adenoviruses are composed of 52 distinct serotypes, which can be categorized into five subgroups (A to E) based on their ability to agglutinate red blood cells and their oncogenic potential in rodents. Human adenoviruses can transform rodent cells, but are not oncogenic in human cells. Viral particles enter cells slowly through a two-stage mechanism that involves interaction of capsid fiber protein with a variety of cell surface receptors, including major histo-compatibility complex class I molecules and the coxsackie virus and adenovirus receptors (CAR). Internalization is mediated by cellular integrins through a receptor-mediated endocytic process that releases vira+l particles into the cytoplasm.

Herpes Virus are two types of herpes simplex virus, type 1 and type 2 (HSV-1 and HSV-2), both belong to the Herpesviridae family, Alphaherpesvirinae subfamily. Herpes simplex virus type 2 is a common human pathogenic virus and is connected with sexually transmitted diseases. HSV type 1 is also a human pathogen, but is rarely connected with genital area infection. Acute HSV-1 infection generally involves gingivo stomatitis (Lerner AM, 1980). Herpes simplex virus is capable of replicating in a wide variety of tissues, including lymphocytes. As a result of its intracellular locus of replication, it is able to escape anti-HSV antibodies during the replication process. Herpes simplex virus type 1 genomic components controlling pathogenicity have been identified.

(a) Vaccinia Virus is a member of poxvirus family and exists as an enveloped viral particle that contains a linear double-stranded DNA genome. The poxviruses are among the largest mammalian viruses, and the genome consists of a nearly 200 kb genome. The vaccinia genome is regulated by a series of early, early/late, and late viral promoters that control initial uncoating, DNA replication, and reassembly of immature virion particles. The entire life cycle of vaccinia replication occurs in the cytoplasm, and this abrogates concern about insertional mutagenesis when infecting cells with vaccinia. Viral replication is generally rapid, with cell lysis typically complete within 7 hours of infection. Vaccinia also encodes proteins that halt cellular transcription and translation, as well as proteins that interfere with interferon-gamma to help the virus evade immune detection. Vaccinia virus naturally displays tropism towards a wide range of mammalian and non-mammalian cell types, making it an attractive vector for experimental use.

(b) Reovirus (respiratory enteric orphan virus) is a naturally occurring, non-enveloped double-stranded RNA virus that is nonpathogenic in humans. The virus possesses potent oncolytic activity in various human tumor cells. While reovirus binds to ubiquitously expressed sialic acid on mammalian cells and is internalized, reovirus replicates only in tumors with a constitutively activated Ras-pathway (Norman KL et al, 2004).

(c) Newcastle Disease Virus (NDV) is an avian paramyxovirus and exists as an enveloped virus that contains a negative-sense double-stranded RNA genome that forms pleiomorphic particles ranging from 150 nm to 300 nm in size. Compared with other oncolytic viruses, NDV contains a smaller genome slightly larger than 15 kb. NDV enters cells through direct fusion at the plasma membrane or through an endocytic pathway (Cantin C et al, 2007). The virus replicates in the cytoplasm of infected cells and so does not engage in insertional mutagenesis (Kelly EJ et al, 2008). Some NDV strains have been developed to elicit potent oncolytic capacity. The MTH-68/H strain showed beneficial effects in patients with advanced cancer (Csatary LK et al, 2004).

(d) Coxsackie virus is an enterovirus belonging to the Picornaviridae family of non-enveloped viruses containing a linear, positive sense, single-stranded RNA genome. Because RNA viruses replicate in the host cytosol without a DNA phase, insertional mutagenesis is not possible. Coxsackie viruses are divided into two subgroups, A and B, based on pathogenicity in mice. At least 23 serotypes of group A and six serotypes of group B have been described. Coxsackie viruses are considered to be a minor human pathogen. Young children, aged five years and under, are more susceptible to Coxsackie virus A disease, often produced by serotype A16. Infection of individuals occurs mainly via entry through exposed areas, such as the skin and mucosal surfaces (such as hands, feet, mouth, throat, and eyes). However, in most cases, infection is asymptomatic or elicits only mild disease associated with "common cold-like" symptoms (Buckland FE et al,1965; Couch RB et al, 1965; Spickard A et al 1963).

Oncolytic viruses in immunotherapy of oral cancer

Numerous naturally available oncolytic viruses have a preferential tropism for tumor and/or associated endothelial cells. Rests are genetically engineered to alter their biochemical, cellular or organ tropism to cancer. The mechanisms of tumor targeting by oncolytic viruses, which contain selectivity to malignant cells or belongs to endothelial cells with altered signaling pathways of RB/E2F/p16, p53, PKR, EGFR, Ras, anti-apoptosis, hypoxia conditions, or defects in IFN and other cellular innate immune signaling pathways (Norman KL et al, 2004; Balachandran S et al, 2001 and Connor JH et al, 2005). The altered signaling pathways supports cellular environments for particular oncolytic viruses to replicate in cancer cells and belongs to endothelial cells, increasing to straight oncolysis of the infected cells. Viruses show specificity for a cell type, tissue or species, commonly known as viral tropism. Particularly interferons and tumor necrosis factors play an important role in prescription of viral tropism (25 Nemunaitis J et al, 2003 and Park SY et al 2004). Complement system seems to play definite roles, as shown for Newcastle disease virus (Csatary LK et al, 1999). Oncolytic Viruses also represents species specificity even though they broaden their tropism to cancer cells from non-permissive species to many areas. Myxoma virus, a poxvirus former considered rabbit specific, that can replicate in a variety of human tumor cells (Xia ZJ et al, 2004). Bovine herpes virus type 1 is a species specific virus that denied inducing cytopathic effects in human normal cells, yet is capable of infecting and destroying a variety of immortalized and transformed human cell types (Lerner AM, 1980). Human Adenovirus can infect murine cancer cells yet the production of infectious virus progeny is restricted. One reason is the failure of translation of viral mRNAs and this could be overcome partially by expression of L4-100 K in translation (Kelly EJ et al, 2008). An important note that oncolytic virus shows wandering, non-productive infection in non-native organism such as mouse cells. The resulting mode of cell death can differ from oncolysis in human cancer cells particularly in this case. The mode of cell death shows a huge degree to subsequent antitumor immunity. As a result, the antitumor immunity in immune-competent organism models with syngenic tumors might not be authentic to the situation in human cancer patients. Oncolytic viruses mediate multimodal killing of cancer and stromal cells ranging from direct virus-mediated cytotoxicity (Xi S et al, 2003 and Tamatani T et al 2004), cell death due

to anti-angiogenesis and vasculature targeting by oncolytic viruses, to cytotoxic immune effectors induced cytotoxicity. The types of cell death, as classified by morphological and ultra structural changes during cell death, are apoptosis, necrosis, and autophagic cell death. With the exception of apoptosis, all other types of cell death have been considered to be inflammatory and immunogenic. In recent studies by inquirer working on chemotherapy and radiation for cancer therapy have led to new concepts, that apoptotic cell death can be divided into "immunogenic cell death" (ICD) and "non-immunogenic cell death" (NICD) (Xia ZJ et al, 2004). Based on this new classification, apoptotic cell death caused by some oncolytic viruses are ICD. Both immunogenic apoptosis, necrosis, autophagic cell death and pyroptosis of cancer and associated endothelial cells caused by Oncolytic Virus, release and represent danger signals (DAMPs and PAMPs as signal 0) and TAAs (as signal 1) to dendritic cells (DCs) for antitumor and antiviral immune responses. Immunotherapy has been a bright spot in the field of novel therapeutics for cancer in the last few years (Xi S et al,2003; Tamatani T et al,2004 and Perez OD et al 2002).

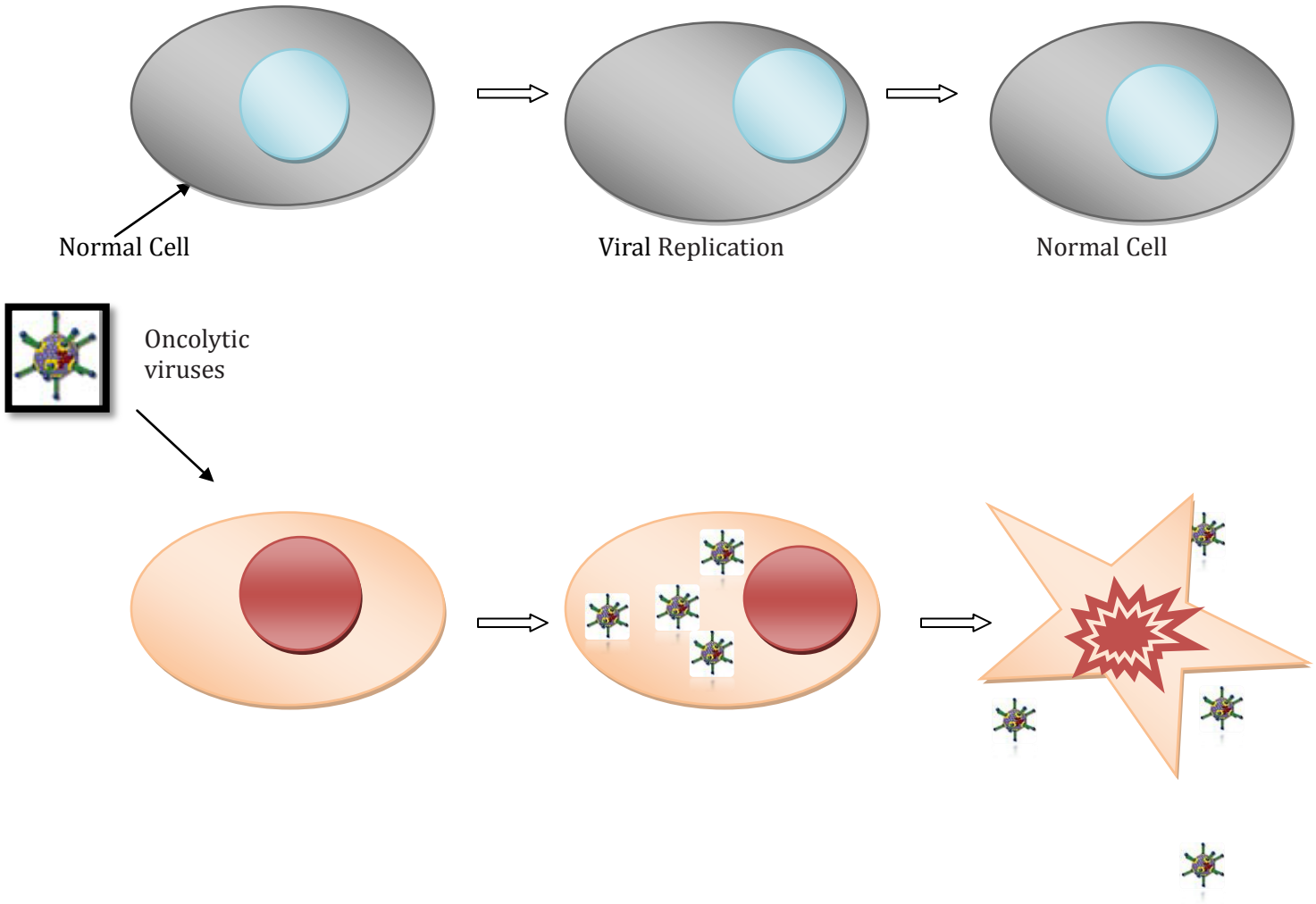

Fig: 1 Oncolysis of Virus (Virotherapy) in Selective cancer specific genetically altered cells.

5. Oncology and molecular based therapy

Molecular-based therapy for oral cancer prevention COX-2 expression in oral mucosa, it is recently demonstrated that COX-2 seems to be selectively up regulated in DNA aneuploid oral dysplastic lesions. COX-2 is upregulated during malignant transition of the oral mucosa and that it is in some manner related to the development of genomic instability (Perez OD et al, 2002). EGFR expression in oral mucosa as recently reviewed by Pomerantz and Grandis, (Han S et al 2004). EGFR specific tyrosine kinase inhibitors (TKIs) are promising targets for drug development in head and neck cancer. A major advantage of TKIs over other classes of EGFR blockers is that TKIs are small molecules given orally. EGFR is over expressed in the vast majority of oral premalignant lesions and oral cancers and correlates with advanced stage and decreased survival (Han S et al, 2004; Pomerantz RG et al, 2003; Ke LD et al, 1998 and Grandis JR et al, 1993).
EGFR family member HER2 (erbB-2) also is overexpressed in oral carcinogenesis. Furthermore it has been found that combination therapy with paclitaxel and PKI166 prolongs survival in an orthotopic preclinical model of tongue cancer by increasing programmed cell death of oral cancer (Grandis JR et al, 1998).

Future Perspective:

The oncolytic viruses is used in the field of cancer virotherapy as well as some viruses which display significant oncotropism. Although many of these viruses may never make it to the clinics, they may contribute to virotherapy research in other ways, e.g. by functioning as models or providing insights into the mechanisms of oncolysis.
Cancer is one of life threatening disease of the world. Oncolytic virus is considered one of the mode for treatment of cancer which can be very effective. It is an emerging method to cure the disease or to prevent it. As many technologies are available now a days, it will be great to make use of oncolytic virus in many ways for prevention or treatment of cancer.

Summary:

The sixth most common cancer in the world and a major cause of morbidity is Squamous Cell Carcinoma (SCC). Treatment of oral squamous cell carcinoma has originally relied on classical curative techniques such as surgery, radiation, and chemotherapy or a method with combination of these techniques. Oncolytic viruses are live viruses that selectively kill cancer cells. Oncolytic viruses came in the frame as a unique platform for the treatment of cancer. Oncolytic viruses do not harm the normal cells, while it destroys the malignant cells. Oncolytic viruses may be tumor selective in their wild type or attenuated forms, or may be genetically modified to provide or enhance tumor selectivity. The potential utility of oncolytic viruses as anticancer therapy is based upon the concept that replication of the virus within the tumor will amplify viral load and enhance anti-tumor potency. Oncolytic viruses are self-replicating viruses which can target and lyse cancer cells specifically. Oncolytic virotherapy have a variety of natural occurring and genetically modified viruses. Natural occurring oncolytic viruses are chosen for their low pathogenicity and inherent specificity for tumor cell. Genetically modified viruses are those that are modified to promote tumor specificity, for example through use of tumor-specific promoters and gene deletions, or reduce pathogenicity by serial passage through cell culture (Wong HH et al 2010). Genetically modified oncolytic viruses are Adenovirus, Herpes simplex virus, Vaccinia virus and Measles viruss. Oncolytic viruses mediate multimodal killing of cancer and stromal cells ranging from direct virus-mediated cytotoxicity (Xi S et al, 2003 and Tamatani T et al, 2004), cell death due to anti-

angiogenesis and vasculature targeting by oncolytic viruses, to cytotoxic immune effectors induced cytotoxicity.

Conclusion:

Oncolytic viruses are the emerging tools or started to use as a therapeutic agents for anticancer therapy. They have beneficial significant factors to cure cancer by killing these cells, while they do not harm the normal tissues. Because of the recent research into oncolytic viruses at many degrees of experiments, oncolytic viruses have only been approved for a small number of cases. In 2006, the first oncolytic virus treatment was approved with combination of chemotherapy for treatment of upper nasal cancers in China. With, all the positive responses in testing, other intricacy within the body and internal environment have not permitted to oncolytic viral treatments to be as much effective. Consequently, it is for greater selectivity, immune response, and decrement in human tumor that researchers are working and testing continuously to study the oncolytic viruses. Oncolytic viruses will stay repeatedly researched due to their encouraging outcomes, particularly when used in combination with other treatments such as chemotherapy.

References:

Balachandran S, Porosnicu M, Barber GN 2001. Oncolytic activity of vesicular stomatitis virus is effective against tumors exhibiting aberrant p53, Ras, or myc function and involves the induction of apoptosis. *J Virol*; 75(7): 3474-3479.

Bernier J, Hall EJ and Glaccia A 2004. Radiation oncology: a century of achievements. Nat Rev Cancer 4: 737-747.

Bierman HR, Crile DM, Dod KS, Kelly KH, Petrakis NL, White LP et al. 1953. Remissionsin leukemia of childhood following acute infectious disease: staphylococcus and streptococcus, varicella, and feline panleukopenia. Cancer 6: 591-605.

Buckland FE, Bynoe ML, Tyrrell DA 1965. Experiments on the spread of colds. II. Studies in volunteers with coxsackievirus A21. *J Hyg (Lond)*. 63(3):327–343.

Cantin C, Holguera J, Ferreira L, Villar E, Munoz-Barroso I 2007. Newcastle disease virus may enter cells by caveolae-mediated endocytosis. *J Gen Virol*. 2007;88(Pt 2):559–569.

Cervantes-Garcia D, Ortiz-Lopez R, Mayek-Perez N, Rojas-Martinez A 2008. Oncolytic virotherapy. Ann Hepatol; 7(1):34-45.

Connor JH, Lyles DS 2005. Inhibition of host and viral translation during vesicular stomatitis virus infection. eIF2 is responsible for the inhibition of viral but not host translation. *Biol Chem*; 280(14): 13512-13519

Couch RB, Cate TR, Gerone PJ, et al 1965. Production of illness with a small-particle aerosol of coxsackie A21. *J Clin Invest*;44:535–542.

Csatary LK, Bakacs T 1999. Use of Newcastle disease virus vaccine (MTH-68/H) in a patient with high-grade glioblastoma. *JAMA*;281(17): 1588–1589.

Csatary LK, Gosztonyi G, Szeberenyi J, et al 2004. MTH-68/H oncolytic viral treatment in human high-grade gliomas. *J Neurooncol*;67(1–2): 83–93. 48.

Farber, S, Dimond, LK, Mercer, RD, Sylvester, RF Jr. and Wolff, JA 1948. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid (aminopterin). N Engl J Med 238: 787-793.

Garber K 2006. China approves world's first oncolytic virus therapy for cancer treatment. J Natl Cancer Inst 98: 298-300.

Gey C, Coffman W and Kubicek M 1952. Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. Cancer Res 12: 264–265.

Grandis JR, Tweardy DJ 1993. Elevated levels of transforming growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer. Cancer Res;53:3579–84.

Grandis JR, Tweardy DJ, Melhem MF 1998. Asynchronous modulation of transforming growth factor alpha and epidermal growth factor receptor protein expression in progression of premalignant lesions to head and neck squamous cell carcinoma. Clin Cancer Res;4:13–20.

Han S, Roman J 2004. Suppression of prostaglandin E2 receptor subtype EP2 by PPARgamma ligands inhibits human lung carcinoma cell growth. Biochem Biophys Res Commun; 314:1093–9.

Holsinger FC, Doan DD, Jasser SA, Swan EA, Greenberg JS, Schiff BA, Bekele BN, Younes MN, Bucana CD, Fidler IJ, Myers JN 2003. Epidermal growth factor receptor blockade potentiates apoptosis mediated by Paclitaxel and leads to prolonged survival in a murine model of oral cancer. Clin Cancer Res;9:3183–9

Ke LD, Adler-Storthz K, Clayman GL, Yung AW, Chen Z 1998. Differential expression of epidermal growth factor receptor in human head and neck cancers. Head Neck ;20:320–7.

Kelly EJ, Hadac EM, Greiner S, Russell SJ 2008. Engineering microRNA responsiveness to decrease virus pathogenicity. *Nat Med*;14(11): 1278–1283.

Lerner AM 1980. In fections with herpes simplex virus.In: Adams RD, Braunwald E, Petersdorf RG, Wilson JD (eds).Harrison's Principles of Internal Medicine.McG raw-Hill, Inc.: New York, pp 847–852.

McGurk, M and Goodger, NM 2000. Head and neck cancer and its treatment: historical review. Br J Oral Maxillofac Surg **38**: 209–220.

Nemunaitis J, Cunningham C, Tong AW, Post L, Netto G, Paulson AS, et al 2003. Pilot trial of intravenous infusion of a replication-selective adenovirus (ONYX-015) in combination with chemotherapy or IL-2 treatment in refractory cancer patients. Cancer Gene Ther. May; 10(5):341-52

Norman KL, Hirasawa K, Yang AD, Shields MA, Lee PW 2004. Reovirus oncolysis: the Ras/RalGEF/p38 pathway dictates host cell permissiveness to reovirus infection. Proc Natl Acad Sci U S A.;101(30): 11099–11104.

Parkin DM, Pisani P, Ferlay J 1999 Global cancer statistics. CA Cancer JClin 49: 33–64, 1.

Park SY, Seol JW, Lee YJ, et al 2004. IFN-gamma enhances TRAILinduced apoptosis through IRF-1. Eur J Biochem 271: 4222-4228.

Parato,K.A., Senger D., Forsyth P.A.J. and Bell J.C. 2005. Recent progress in the battle between oncolytic viruses and tumours. Nat. Rev. Cancer, 5:965–76.

Perez OD, Kinoshita S, Hitoshi Y, Payan DG, Kitamura T, Nolan GP, et al 2002. Activation of the PKB/AKT pathway by ICAM-2. Immunity. Jan;16(1):51-65.

Pomerantz RG, Grandis JR 2003. The role of epidermal growth factor receptor in head and neck squamous cell carcinoma. Curr Oncol Rep;5:140–6.

Prestwich RJ, Errington F, Harrington KJ, Pandha HS, Selby P, Melcher A 2008. Oncolytic Viruses: Do they have a role in anti-cancer therapy? Clin Med Oncol; 2:83-96

Spickard A, Evans H, Knight V, Johnson K 1963. Acute respiratory disease in normal volunteers associated with Coxsackie A-21 viral infection. III. Response to nasopharyngeal and enteric inoculation. J Clin Invest;42:840–852.

Tamatani T, Azuma M, Ashida Y, Motegi K, Takashima R, Harada K, et al 2004. Enhanced radiosensitization and chemosensitization in NF-kappaB-suppressed human oral cancer cells via the inhibition of gamma-irradiation- and 5-FU-induced production of IL-6 and IL-8. Int J Cancer. Mar 1;108(6):912-21

Wong HH, Lemonine NR, Wang Y 2010. Oncolytic viruses: Overcoming the Obstacles. Viruses; 2(1):78-106.

Xia ZJ, Chang JH, Zhang L, et al 2004. Phase III randomized clinical trial of intratumoral injection of E1B gene-deleted adenovirus (H101) combined with cisplatin-based chemotherapy in treating squamous cell cancer of head and neck or esophagus. Ai Zheng. ;23(12):1666–1670. Chinese.

Xi S, Grandis JR 2003. Gene therapy for the treatment of oral squamous cell carcinoma. J Dent Res. Jan; 82(1):11-6.