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Oncolytic adenoviruses for cancer therapy

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ABSTRACT

The replicative viral reagents represent novel tools for therapeutic approach to neoplastic disease. Killing of target tumor cells by the viral agent is achieved by direct consequence of the viral replication. These oncolytic viruses-viruses that selectively infect or replicate in cancer cells, but spare normal cells-provide an attractive strategy for cancer therapy because these viruses replicate only in cancer cells. The viral replication can result in intratumoral virus spread, triggering cancer-specific cytotoxicity. Especially, adenoviruses have several appealing properties as replicative viral reagents. The adenoviral replication amplifies the infection dose of the virus and helps spread the agent to adjacent tumor cells. In addition, adenoviral infection may generate an antitumoral immune response. Moreover, adenovirus genome can be easily engineered to selectively replicate in cancer cells. Taken together, human replicative adenoviruses are excellent candidates as therapeutic tools against cancer.

Key words : adenoviruses, cancer gene theraphy, oncolytic virus, replicative viral vector

Introduction

Cancer is one of the top causes of death in humans. A number of strategies of such as surgery, radiation, and chemotherapeutic approaches have been developed for the treatment of cancer, however, most of therapeutic treatments are related to significant side effects and the lack of controlled trials. The use of replicative viral reagents represents a novel approach to neoplastic diseases. Regression of tumor cells by the viral agent can be achieved by direct consequence of the viral replication (1). Several oncolytic viruses that selectively infect or replicate in cancer cells, but spare normal cells, have been identified (Fig 1). Some of them are naturally attenuated viral strains that more effectively infect or replicate in cancer cells. Others are genetically modified to mediate oncolytic effects (Table 1). Furthermore, with the advances of molecular biology and understanding the function of viral genes, it has become possible to genetically re-engineer viruses to make them more selectively target tumor cells (2). Among the rest, several biological features as follows have made the adenovirus the most attractive virus for oncolytic virotherapy.

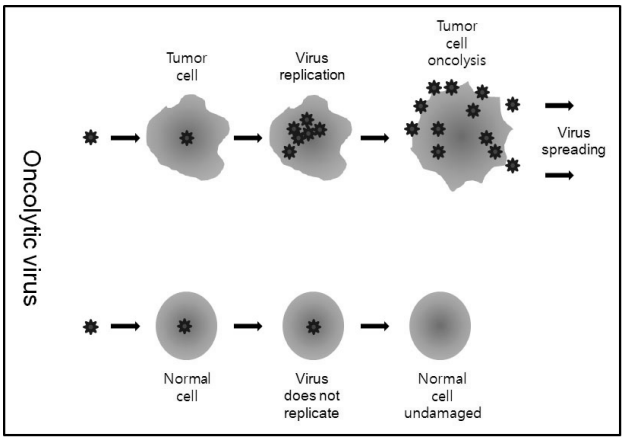

Fig 1. Selective killing of cancer cells with a replication-selective viral agent. Viruses can be engineered to replicate in and to kill tumor cells specifically, leaving healthy cells unharmed. Several strategies are available for designing such agents.

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Table 1. Advantages and disadvantages of different oncolytic virus

Virus	Oncolytic strain appearance	Advantages	Disadvantages
Adenovirus	Laboratory Engineered	Can be easily manipulated genetically; clinical trial experience; good knowledge of viral protein function; associated with relatively mild diseases	Replication cannot be easily shut-off
Poliovirus	Laboratory Engineered	good knowledge of viral gene functions	Cannot be easily manipulated genetically; no clinical trial experience; undesirable viral replication cannot be easily shut-off; associated with fatality or serious diseases
Vesicullostomatitis virus	Naturally appearance	Associated with relatively mild diseases; good knowledge of viral gene functions	Cannot be easily manipulated genetically; no clinical trial experience; undesirable viral replication cannot be easily shut-off
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 gene
Reovirus	Naturally appearance	Associated with relatively mild diseases; good knowledge of viral gene functions	Cannot be easily manipulated genetically; no clinical trial experience; undesirable viral replication cannot be easily shut-off
Vaccinia virus	Laboratory Engineered	Can be easily manipulated genetically; clinical trial experience	undesirable viral replication cannot be easily shut-off; unknown action of many gene; side effects might include potentially fatal or seriously morbid diseases

The adenovirus is a common pathogen in human. Adenoviral replication leads to death in permissive host cells, and amplification of the viral load can be achieved as a result of the viral replication, allowing spread of viral infection to adjacent tissues. Another biological feature is that the adenoviral genome does not integrate into the host cell genome, hence the risk of recombination and mutation in the genome will be low. The genome is also large enough to accommodate the incorporation of foreign genes. The technology to manipulate the viral genome is also readily available so that recombinant adenoviruses can be tumor selective (3). Collectively, oncolytic adenovirus induces a potent anticancer effect and could ultimately demonstrate clinical relevance and therapeutic utility. In this article, we will describe the biology of the adenovirus and the application examples of the virus as oncolytic purposes.

Human adenovirus biology

All adenoviruses have linear, non-segmented, and double-stranded DNA genomes of 30~38 Kbp. The capsid is nonenveloped and is composed of the structural proteins including hexon and penton, which bind the integrins to allow virus internalization, and fiber, which binds the coxsackie and adenovirus receptor, CAR (3). Adenoviral transcription occurs in three phases: early, intermediate, and late. The first gene that is transcribed in the viral genome is *E1A*, and its product binds with many cellular proteins such as the pRb, p107, and p130 family of proteins. This interaction prevents these cellular proteins from blocking E2F transcription factors, leading to the initiation of DNA replication and the cell cycle (4). The second expressed gene is *E1B*, product of which prevents early death of the host cell, and thus allowing viral replication to occur unimpeded. The E1B-55kD protein binds to p53 and induces its degradation, whereas the E1B-19kD protein functions similarly to the antiapoptotic factor bcl2, thereby preventing the death of the infected cell and allowing viral replication to occur (5). Other proteins in the early phase of the viral replication cycle are encoded by the *E2*, *E3*, and *E4* gene clusters. Among the rest, the *E3* gene is involved in host immune response modulation and cell lysis (6). The viral structural proteins and the proteins necessary for assembly of the virion are encoded by genes expressed during the intermediate and late phases of viral

Table 2. Different types of oncolytic adenovirus.

Name	Basis of tumor-selective propagation	Therapeutic characteristics
Ad wild type	None	Oncolysis
AdD24	E1a deletion abrogates RB binding	Oncolysis
CN706	Regulation of E1a under the PSA promoter	Oncolysis
CN763	Regulation of E1a under the kallikrein 2 promoter	Oncolysis
CN764	Regulation of E1a under the PSA promoter and E1b under the kallikrein 2 promoter	Oncolysis
CV739	Regulation of E1a under rat probasin promoter and E1b under human PSA promoter	Oncolysis
CV787	Regulation of E1a under rat probasin promoter and E1b under human PSA promoter	Oncolysis (enhanced when compared with CV739 due to the presence of E3)
AvE1a041	Regulation of E1a under the AFP promoter	Oncolysis
GT5610+AdHB	Regulation of E1a under the AFP promoter	Oncolysis
DI337	None	Oncolysis (enhanced due to E1b-19kDa deletion)
DI316	The complete deletion of E1a makes this mutant dependent on intrinsic or IL-6-induced E1a-like activity	Oncolysis
DI118	The complete deletion of E1b abrogates p53 binding ; however, E1a-induced apoptosis is not inhibited by E1b-19kDa	Oncolysis
dl1510(Onyx015)	E1b55kda-deletion abrogates p53 binding	Oncolysis
AdTKrc	E1b55kda-deletion abrogates p53 binding	Oncolysis & suicide gene therapy (TK)
Ad-5-CD-Tkrep	E1b55kda-deletion abrogates p53 binding	Oncolysis & suicide gene therapy (CD+TK)
AdvE1AdB-F/K20	E1b55kda-deletion abrogates p53 binding	Oncolysis with enhanced infectivity
AxE1AdB/AdCAhIL	E1b55kda-deletion abrogates p53 binding	Oncolysis & immuno-stimulatory gene therapy

replication. Once viral progeny assembly is complete, new viral particles (>1000/cell) are released by cytolysis.

Anticancer approaches with oncolytic adenoviruses

Two broad approaches are currently being used to engineer tumor-specifically replicating adenoviruses (Table 2). The first is to limit the expression of the E1A gene product to tumor tissues through the use of tumor- or tissue-specific promoters. E1A stimulates S-phase entry and transactivates both viral and cellular genes that are critical for a productive viral infection (7). Tissue-or tumor-specific promoters can replace endogenous viral sequences in order to restrict viral replication to a particular target tissue. For example, the prostate-specific antigen gene (*PSA*) promoter/enhancer element has been inserted upstream of the *E1A* gene. Degree of the viral replication then correlates with the level of PSA expression in a given cell. A second prostate-specific enhancer sequence has subsequently been inserted upstream of the *E1B* region in the CN706 virus. The use of these two prostate-specific enhancer elements to drive separate early gene regions has led to improved selectivity over the first generation virus (8). A similar approach has been pursued by other groups using tissue-specific promoters including hTERT or alpha-feto protein gene promoters to drive E1A expression selectively in specific carcinomas (9, 10).

A second strategy for optimizing tumor selectivity is to delete or mutate gene functions that are critical for efficient viral replication in normal cells but not in tumor cells. Many of the critical regulatory proteins that are inactivated by viral gene products during adenovirus replication are also inactivated during carcinogenesis (11, 12). Because of this convergence, viral gene products that would ordinarily be required to inactivate a cellular protein become superfluous in cancer cells that have already lost the target protein. A strain of adenovirus deleted for the *E1B-55kD* gene, *dl*1520 (ONYX-015) had been developed for the treatment of tumors lacking p53 function (11). Since the E1B-55kD gene product is responsible for p53-binding and inactivation, it was hypothesized that the deletion mutant would be unable to inactivate p53 in normal cells and would thus be unable to replicate efficiently. In contrast, cancer cells lacking func-

tional p53 would be expected to be sensitive to viral replication and subsequent cytolysis. dl1520 has also been tested in phase I and II trials for locally advanced pancreatic carcinoma, ovarian cancer, colorectal carcinoma, non-small cell lung carcinoma, and oral dysphasia, using a variety of systemic or local treatments to administer the virus. However, as a single agent, its potency has been limited (13, 14).

Conclusion

Adenoviruses have many biological features to be employed as specific oncolytic agents. A large number and variety of the oncolytic adenoviruses might become available to clinical trials as single agents or as regimens combined with drugs and/or radiation. These reagents are being developed on the basis of strong scientific rationale, and hence as more is learned about the molecular and cellular mechanisms of oncolytic adenoviruses, recombinant adenoviruses with more selective and increasingly effective activity of oncolysis will be available.

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