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Mini review

Development of PEGylated adenovirus vector with targeting ligand

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Abstract

For effective gene therapy, a vector system that transduces the therapeutic gene into target cells efficiently and safely is essential. Adenovirus (Ad) vectors frequently are used for gene therapy research, especially cancer gene therapy, because of their high transduction efficiency. However, broad clinical utility of Ad vectors have not yet been achieved owing to problems related to several properties inherent to Ads. Systemic administration of Ad vectors leads to acute virus accumulation and undesirable transgene expression in the liver, with subsequent inefficient systemic cancer-targeted therapy and pronounced hepatotoxicity. Furthermore, most people have Ad-neutralizing antibodies, which hamper gene expression efficiency. Chemical conjugation of Ad surface with polyethylene glycol (PEG) (PEGylation) is one of the promising strategies to overcome these problems. Furthermore, PEGylation of Ad vectors with targeting ligands on the tip of PEG, which alter the transfection range of Ad vectors will improve the safety and efficiency of Ad gene-delivery vectors. In this review, we describe the molecular biology of Ads and outline this PEGylation approach including our data.

Keywords: Adenovirus vector; PEGylation; Targeting

Contents

1. Introductioon

The number of gene therapy clinical trials has increased every year since 1990, when this technique first was used (Anderson et al., 1990; Xiang, 2007). However, despite encouraging preclinical and early clinical results (Blaese et al., 1995, Cavazzana-Calvo et al., 2000), broad clinical utility of gene therapy has not yet been achieved. The major obstacle to widespread use of gene therapy has been the delayed development of appropriate vectors, and the success of gene therapy depends on the development of vector to transduce the therapeutic gene into target cells efficiently and safely.

Ad vectors continue to be preferred vectors for gene therapy, mainly against various cancers, and studies of gene function because of their many useful features. Several clinical trials

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have demonstrated effective adenoviral cancer gene therapy via intratumoral injection, but successful cancer gene therapy requires treatment of not only the primary tumor but also distant metastases. To treat metastatic cancer, Ad vectors must be administered systemically and their kinetics controlled. However, it is difficult to treat distant metastasis with conventional Ad vectors, because they (and even most capsid-modified Ad vectors) not only distribute in the tumor inefficiently but also accumulate in the liver immediately and induce severe side effects due to unwanted transgene expression after systemic injection (Alemany et al., 2000; Koizumi et al., 2003). Moreover, because many people carry neutralizing antibodies to Ad, administration of high dose of Ad vectors to obtain sufficient therapeutic effects causes negative side effects, thus limiting wide application of Ad vectors (Wohlfart, 1988; Mastrangeli et al., 1996). Chemical conjugation with polyethylene glycol (PEG) (PEGylation) is a promising strategy to overcome these problems. PEGylation has been used frequently in pharmaceutic preparation since the late 1970s (Delgado et al., 1992) to provide a hydrophilic coat to therapeutic molecules to protect them from proteolytic degradation and both humoral and cell-mediated immune responses and to increase the persistence of therapeutic proteins in the blood (Harris and Chess, 2003). Several groups, including our own, have sought to apply the advantageous properties of PEGylation to Ad vectors. In this article, we review the molecular biology of Ads and several approaches of PEGylated Ad (PEG-Ad) vectors.

2. General properties of Ad

Ads are nonenveloped, icosahedral particles 70–100 nm in diameter, with an outer protein shell that surrounds a linear double-stranded adenoviral DNA. The capsid is composed of 240 hexon capsomeres and 12 penton capsomeres with spike-shaped protrusions (fibers) (Rux and Burnett, 2004).

The binding between the C-terminus of fiber, the knob domain, and CAR on target cells is the first step of adenoviral infection (Fig. 1) (Xia et al., 1994). After this binding, interaction between an RGD motif on the surface of the penton base protein and a cell-surface integrin molecule promotes virus uptake by clathrin-dependent receptor-mediated endocytosis (Wickham et al., 1993; Meier et al., 2002). Soon after the viral capsid enters the endosome, the fibers fall off. The decreased pH of the endosome then causes the release of the penton bases from the capsid, and their release causes lysis of the endosome. After entering the cell cytoplasm, Ad virions dock with the nuclear pore complex (Meier and Greber, 2004). Adenoviral DNA then is extruded into the nucleus.

3. Construction of PEGylated Ad vectors

PEG is an uncharged, hydrophilic, nonimmunogenic polymer (Montaguti et al., 1994) that is able to reduce protein–protein interactions between therapeutic compounds, proteins, and cells in vivo. In light of these desirable attributes, PEGylation of the Ad surface is a promising approach for overcoming several limitations of Ad vectors for systemic therapy. Covalent attachment of PEG to the Ad surface is achieved primarily by use of activated PEG – tresyl-monomethoxypolyethylene glycol (TMPEG), succinimidyl succinate-monomethoxypolyethylene glycol (SSPEG), or cyanuric chloride-monomethoxypolyethylene glycol (CCPEG) – which reacts preferentially with the ε-amino terminal of lysine residues on the capsid proteins (O'Riordan et al., 1999, Croyle et al., 2000, 2001). Different types of PEG, such as those that differ in molecular weight or branch type or PEG that contains different active groups in each side chain, can be used in this approach. Furthermore, PEGylation of Ad vectors maintain their viral titer after storage at various temperatures more stably than that of conventional Ad vectors (Croyle et al., 2000). The benefits of PEG itself and the physical

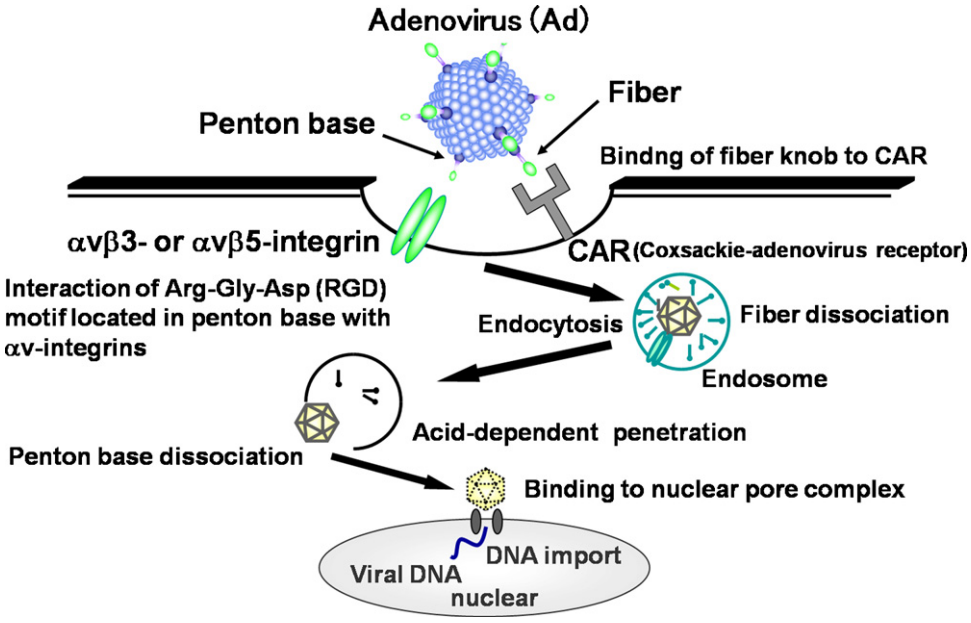

Fig. 1. Binding and entering mechanism of Ad.

stability of PEG-Ad vectors have contributed to the broad development of the PEGylation approach.

4. In vivo properties of PEG-Ad vectors

Because of pharmacokinetic limitations, therapeutic Ad vectors currently are provided mainly through local administration in vivo. Typically when Ad vectors are administered systemically, the virus half-life is less than 2 min, and most of the vectors accumulate in liver immediately (Alemany et al., 2000). To expand the usefulness of systemically administered Ad vectors, their retention time in blood must be increased and they must evade accumulation in the liver. Many groups have reported that PEGylation of liposomes and proteins significantly improves their in vivo kinetics, e.g., by protection from enzymatic degradation and prolongation of circulation time (Inada et al., 1995; Tsutsumi et al., 1996; Zhou et al., 2002; Shibata et al., 2004). These data prompted us and other researchers to evaluate the in vivo kinetics of PEG-Ad vectors after systemic injection to determine whether PEGylation conferred the same benefits on Ad vectors as on liposomes and proteins.

The kinetics involved in the clearance of PEG-Ad vectors from blood initially were reported by Alemany et al. (2000), who clarified that the clearance ratio of PEG-Ad vectors was fourfold lower than that of conventional Ad vectors. Furthermore, Lanciotti et al. showed decreased distribution of PEG-Ad vectors in the liver via intraperitoneal administration compared with conventional Ad vectors (Lanciotti et al., 2003). These studies demonstrate that PEGylation of Ad vectors can alter their in vivo properties and potentially expand the applicability of Ad vectors.

One important finding was that the characteristics of PEG-Ad vectors were significantly affected by the amount of PEG on the surface (i.e., the PEG modification ratio). We constructed PEG-Ad vectors with a wide range of PEG modification ratios and evaluated their in vivo properties after systemic injection into tumor-bearing mice (unpublished data). The results indicate that the half-life of PEG-Ad vectors increased significantly as the PEG modification ratio increased, and PEG-Ad vectors with a modification ratio of approximately 90% (90% of capsid proteins are PEGylated) induced 35-fold higher transgene expression in tumors and lower in the liver after systemic administration, compared with the values for unmodified Ad vectors. We consider that this tumor targeted gene transfer is induced by so-called enhanced permeability and retention (EPR) effect, which is based on the leaky nature of the blood vessels of tumor tissue (Matsumura and Maeda, 1986; Gabizon et al., 1994; Maeda, 2001; Maeda et al., 2001; Greish et al., 2003) and regarded as a 'gold standard' for tumor targeting in the case of PEGylated liposomes or proteins. These findings indicate that systemic injection of PEG-Ad vectors has great potential as an anti-tumor treatment.

5. Ability of PEG-Ad vectors to evade humoral and cellular immune responses

Another potential limiting factor associated with adenoviral gene therapy is that most adults have anti-Ad-neutralizing antibodies, which can limit repeat administration of Ad vectors (Wohlfart, 1988; Mastrangeli et al., 1996). O'Riordan et al. were the first to show that PEGylation of Ad vectors renders them less susceptible to neutralization because of shielding of neutralizing epitopes on the viral surface by PEG chains (O'Riordan et al., 1999). Since then, we and many other groups have reported similar neutralization evasion by PEG-Ad vectors (Romanczuk et al., 1999; Croyle et al., 2001, 2002; Eto et al., 2004; Ogawara et al., 2004; Eto et al., 2005). This evasive ability is unique to PEGylation of Ad vectors—it is not found with other methods such as genetic approaches.

Another limiting factor associated with adenoviral gene therapy is the strong innate and adaptive response against Ad (Alemany and Curiel, 2001). It is well known that macrophages in the liver or spleen efficiently take up Ad particles after intravenous injection, resulting in strong activation and rapid chemokine expression of these cells and subsequent reduced magnitude and duration of transgene expression (Smith et al., 2002; Schiedner et al., 2003). Mok et al. reported that the use of PEG-Ad vectors reduced innate IL-6 responses to 30% of the response to conventional Ad vectors, without reduction of transduction (Mok et al., 2005). This decrease in innate responses paralleled similar reductions in vector uptake by macrophages in vitro and in vivo. In addition, Croyle et al. reported that PEGylation of Ad vectors reduced both humoral and activated cellular immune response, cytotoxic T lymphocytes response (Croyle et al., 2001). Because nonspecific vector uptake by macrophages may be critically involved in the initial activation of both innate and adaptive immune responses, PEGylation, which enables Ad vectors to evade humoral and cellular immune responses, is very useful in clinical applications requiring systemic administration.

6. PEG-Ad vectors with targeting ligands

One of the challenges of current gene therapy vector development concerns targeting a therapeutic gene to diseased cells, with the aim of achieving sufficient transgene expression in the affected tissue while minimizing toxicity and expression in other tissues. Attaching targeting ligands to the ends of PEG chains is a promising approach for achieving tissue-specific gene transfer via systemic injection. PEGylation of Ad vectors not only inhibits undesired CAR-mediated gene transfer to normal tissue but also prolongs the retention time in blood (Alemany et al., 2000), which maximizes the likelihood of attachment between Ad vectors and targeted cells. In this approach, peptides, proteins, and antibodies all can be used as targeting ligands, and several studies making use of these approaches have been reported (Lanciotti et al., 2003; Ogawara et al., 2004; Eto et al., 2005) (Fig. 2).

We and another group have developed novel PEG-Ad vectors that have RGD peptides at the ends of the PEG chains (RGD-PEG-Ad) (Ogawara et al., 2004; Eto et al., 2005). RGD is the second mediator of adenoviral cell entry, and its utility as a binding motif in many malignant tumors has been demonstrated by many groups. We reported that transgene expression in both CAR-positive and -negative tumor cells with RGD-PEG-Ad, which binds through integrin, was $\leq$200-fold higher

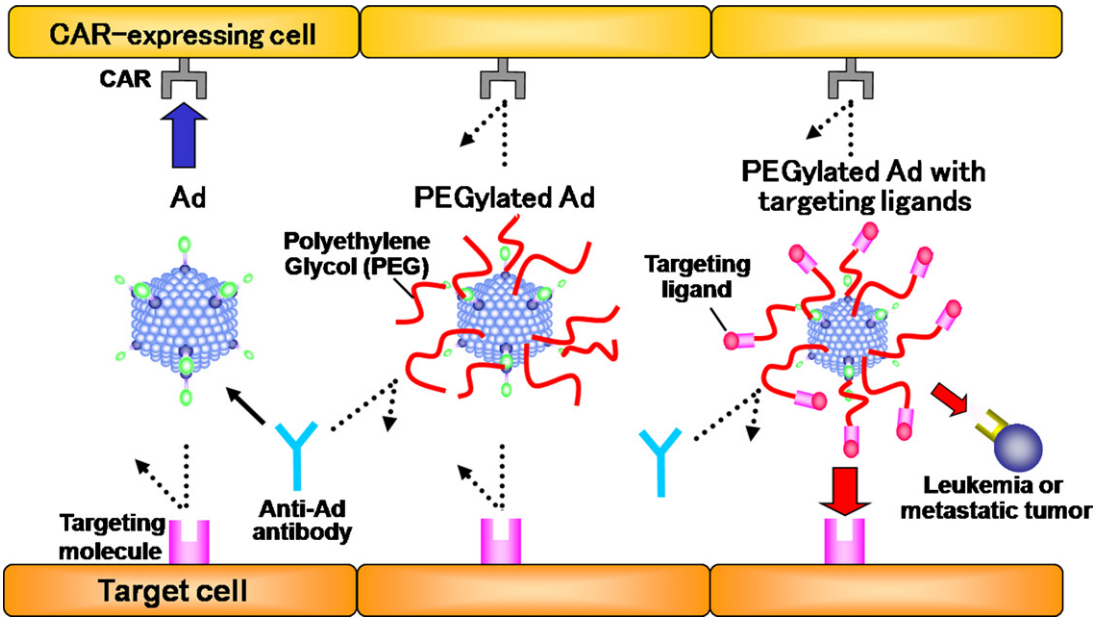

Fig. 2. Characteristics of PEGylated Ad.

than that from conventional PEG-Ad vectors (Eto et al., 2005) (Fig. 3). Moreover, compared with unmodified-Ad, RGD-PEG-Ad showed almost equal and 100-fold higher gene expression in CAR-positive and -negative cells, respectively. In addition, RGD-PEG-Ad retained the antibody evasion ability associated with PEGylation, and in vivo experiments demonstrated efficient transgene expression from RGD-PEG-Ad (Eto et al., 2005). Currently we are using a phage display system to identify potential targeting peptides other than RGD peptide.

The targeting ligand approach for PEG-Ad vectors can be applied to proteins and antibodies as well as peptides. Lanciotti et al., generated PEG-Ad vectors containing fibroblast growth factor 2 (as a targeting ligand) and the heterofunctional PEG tresyl-PEG-maleimide, which reacts preferentially with a reactive sulfhydryl group on the surface of FGF2 (Lanciotti et al., 2003). This vector exhibited ligand-mediated, CAR-independent gene transfer as well as resistance to Ad-neutralizing antibodies. Ogawara et al. reported enhanced transgene delivery to activated vascular endothelial cells by use of PEG-Ad vectors that carried E-selectin-specific antibody on the ends of the PEG chains. Systemic injection of these vectors led to selective targeting of inflamed skin and mediation of local transgene expression in mice with delayed-type hypersensitivity reactions (Ogawara et al., 2006). These experimental approaches likely will be applicable to tumor-targeted therapy by use of PEG-Ad vectors containing targeting ligands.

7. Future prospects

Chemical modification methods involving PEG are advantageous, in that many ligands can be applied to the ends of the PEG chains. Although several groups, including ours, have reported

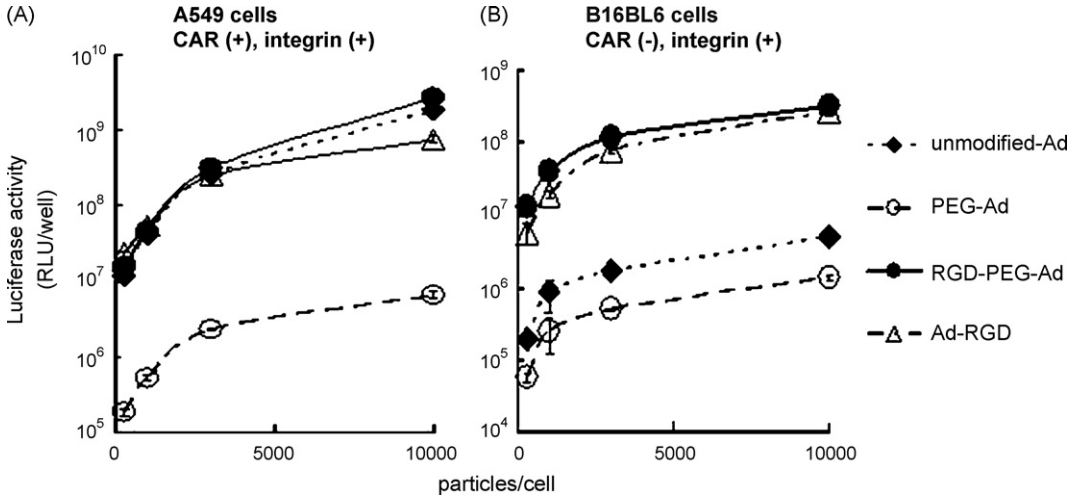

Fig. 3. Transduction efficiency of RGD-PEG-Ad. (A) A549 cells and (B) B16BL6 cells were transduced with 300–10,000 particles/cell of unmodified-Ad, PEG-Ad, RGD-PEG-Ad or Ad-RGD, respectively. Luciferase expression was measured after 24 h. Each point represents the mean ± S.D. ($n = 3$).

the improved pharmacokinetic properties of PEG-Ad vectors lacking targeting ligands (Croyle et al., 2001, 2002; Mok et al., 2005), the pharmacokinetic properties of PEG-Ad vectors with these ligands have not yet been described in detail (e.g., kinetics of PEG-Ad vectors with both ligand targeting and optimized PEG modification ratio for effective tumor targeting). The exact properties of these vectors must be characterized further. In addition, although these Ad vectors reach the vascular endothelial cells of targeted tissue (e.g., tumor tissue), the question of how to transfer PEG-Ad vectors across endothelial cells and into the tissue itself needs to be addressed. Further success with this approach will rely on increased knowledge and improved techniques of chemical modification in regard to pharmaceutic preparations for drug-delivery systems.

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